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Influence of Curcumin on Pioglitazone Metabolism and Pk/Pd: Diabetes Mellitus

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Abstract

Background: Curcumin is the principal curcuminoid of the popular Indian spice ingredients commonly known as turmeric. Curcumin is reported to inhibit cytochrome P-450 (CYP) enzymes and its isozymes. As turmeric is being consumed every day in Indian spices, it is essential to determine the potential interaction with drugs metabolised by CYP3A4 system.

Methods and results: The study was conducted to determine the potential influence of curcumin on pharmacokinetics and pharmacodynamics of pioglitazone in normal and diabetic rat models. In first study, three groups (groups 1, 2 and 3; n=6) of rats were taken as non diabetic (normal) groups. Second study, three other groups: (groups 4, 5 and 6) were selected similarly to test the effects on diabetic group after receiving alloxan monohydrate (120 mg/kg). Group 1 and 2; group 4 and 5 received pioglitazone orally (10 mg/kg) and curcumin (60 mg/kg), respectively. Groups 3 and 6 were tested for single dose and multiple dose interaction effects on non diabetic and diabetic rats with curcumin for single day and for eight days, respectively. By the end of curcumin pre- treatment pioglitazone was given on the eighth day. Blood samples (0.8 ml) were collected via retro orbital plexus, at the time intervals of 0, 0.5, 1, 2, 4, 8 and 24 hours and double the volume of sample is replaced with normal saline intra peritoneally to maintain the body fluid in animals and PK and PD parameters were measured. Curcumin significantly increased the area under plasma concentration time curve (AUC) and area under the movement curve (AUMC) of pioglitazone in both normal and diabetic rats. There was a significant decrease in maximum observed plasma concentration (T_{max}) in both normal and diabetic rats.

Conclusion: Curcumin significantly decreased the metabolism of pioglitazone and, the combination has more beneficial effect in diabetes and warrants dose adjustment of pioglitazone in diabetic models.

Keywords: Curcumin; Diabetes mellitus; Alloxan; Pharmacokinetics and pharmacodynamics; Pioglitazone; High Performance Liquid Chromatography (HPLC)

Introduction

Millions of people now a days use herbal medicines along with prescription and non-prescription medications that the natural agents are safer than the conventional synthetic chemo therapeutic agents [1]. Majority of the modern medicine in India are derived from natural sources. Some bioactive ingredients are also present in the prescriptions in United States [2]. The diabetic incidences in India are also increasing with alarming rates. Disease is also increasing not only in the underdeveloped countries but also in developed world and becoming a life style disorder with the high rate of obesity. Diabetes affects approximately 16 million people in the United States and has significant medical, economic, and psychological ramifications. Inadequacies in current approaches for the treatment have led many patients to consider other natural alternatives. Complementary and alternative medicine (CAM) is defined by the National Centre for Complementary and Alternative Medicine as a group of diverse medical and health care systems, practices, and products that are not presently considered to be part of conventional medicine. Despite the limited evidence of safety and efficacy, an estimated 2 to 3.6 million Americans use CAM specifically for diabetes [3,4]. Worldwide, around more than 400 herbs and plant preparations are reported to have beneficial effects in the treatment of diabetes mellitus [5]. Turmeric (*Curcuma Longa*) has been used as a component of Indian ancient Ayurvedic medicine to treat different varieties of ailments including diabetes. Curcumin also reported to have beneficial effects on various diseases, like multiple myeloma, pancreatic cancer, myelodysplastic syndromes, colon cancer, psoriasis, and Alzheimer's disease[6]. Curcuminoids induce glutathione S-transferase and are potent inhibitors of cytochrome P450 3A4 [7-9] and mild inhibitory effect on cytochrome P450 2C8. Pioglitazone is a prescription drug of the class thiazolidinedione (TZD) with hypoglycaemic (antihyperglycemic, antidiabetic) action to treat diabetes. Pioglitazone is metabolised by cytochrome P-450 CYP 3A4 [10]; so, there is more scope for the potential herb-interactions between curcumin and pioglitazone. Pioglitazone can cause fluid retention and peripheral edema as a result, it precipitates congestive heart failure (which worsens with fluid overload in those at risk). It may cause anaemia and mild weight gain is common due to increase in subcutaneous adipose tissue. Patients on pioglitazone had an increased proportion of upper respiratory tract infection, sinusitis, headache, myalgia and tooth problems. Thus in the present study we study the effects of pre treatment of rats either in normal or alloxan induced-diabetic condition in rat model with curcumin on the pharmacokinetics and pharmacodynamics of pioglitazone metabolism as well as the glucose metabolism. We also observed that curcumin can increase the persisted concentration of pioglitazone *in vivo*; particularly, the

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multiple doses of pretreatment with curcumin prior to pioglitazone. In addition, several other kinetic parameters and dynamics of pioglitazone metabolism were also elevated in the presence of curcumin.

Materials and Method

Animals and diet

All *in vivo* animal experimental protocol conducted in this study were approved (IAEC/13/UCPSc/KU/2011) by the Institutional Animal Ethical Committee, Kakatiya University, Warangal. Female Wistar rats weighing 200-250 g were maintained under standard laboratory conditions as approved by the animal committee. They were fed with standard pellet diet and water *ad libitum*. Animals were fasted for overnight before experiment with water *ad libitum* and during the experiment they were withdrawn from food and water.

Drugs and chemicals

Pioglitazone and rosiglitazone (internal standard) were the kind gift samples from Dr. Reddy's Lab (Hyderabad, India). Curcumin was supplied by Sinthite Pharma, (Kerala, India). Alloxan monohydrate was supplied by Sigma-Aldrich (Bangalore, India). Glucose estimation kits were supplied by Excel diagnostics pvt.ltd (Hyderabad,India). Orthophosphoric acid analytical grade and HPLC grade acetonitrile, methanol and potassium dihydrogen phosphate supplied by Merck (Mumbai, India).

HPLC analysis of pioglitazone

Pioglitazone was estimated by a slightly modified method of an earlier reported reverse phase HPLC method [11,12]. HPLC system consisted of LC-10ATVP solvent delivery module (Shimadzu, Kyoto, Japan), SPD-20AVP variable wavelength programmable UV/VIS spectrophotometric detector, a Class CR-10 Data processor and Reverse Phase C18 column (Wakosil II C-18, 250×4.6 mm, 5 µ porous silica spheres) was used. Rheodyne injection port with a 20 µl sample loop and Hamilton syringe 20 µL was used. Pioglitazone concentration was determined by slight modification of a method reported by Kolte [10,11]. The mobile phase consists of 25 mM Phosphate buffer (PH adjusted to 3 with orthophosphoric acid, acetonitrile and methanol in a ratio of 55:37.5:7.5 (v/v/v)). The mobile phase was degassed and filtered through 0.22 µm membrane filter. The flow rate was 1.2 mL/min and the effluent was monitored at 269 nm. The total run time of the method was set at 10 minutes.

Preparation of test samples

To a volume of 100 µL of test rat serum, 50 µL of rosiglitazone (2.5 µg in methanol) solution as internal standard and 100 µL of acetonitrile were added to precipitate the proteins. The mixture was vortex mixed for 5 minutes after which it was centrifuged at 10000×g for 10 minutes. 20 L of the supernatant was injected onto the HPLC system for analysis.

Limit of detection and limit of quantification

Limit of detection and limit of quantification was studied as described earlier [13]. Three calibration curves were obtained by spiking thrice, the standard dilutions of pioglitazone in serum samples and σ is 0.003251, S is 0.1786 where (σ → Standard deviation of y-intercepts of Calibration Curves; m → Mean of the slopes of calibration curves of pioglitazone). The LOD and LOQ of Pioglitazone from the equations was found to be 0.060072 µg and 0.18204 µg respectively. Hence, the limit of detection and limit of quantification were both found to be within the range of the analyzed levels in serum samples.

Precision and accuracy

Intra and inter-day precision expressed as percentage of standard deviation (%RSD) and accuracy expressed as percentage of relative error (%RE) were obtained from three levels of quality control samples of pioglitazone. The precision and accuracy of the method was established by using quality control samples at low, medium and high concentrations of 0.1, 1 and 10 µg/mL for pioglitazone. All the samples were run in three replicates. Intra-day precision data was obtained by analyzing three sets of quality control samples in a single day, while the inter-day data was obtained by analyzing the quality control samples on three consecutive days of assay. The assay procedure was found to be precise and accurate.

Experimental Design

Pharmacokinetic and pharmacodynamic interaction study in normal rats

Following an overnight fasting, rats were divided into 3 groups each containing 6 rats. First group administered orally with pioglitazone, 10 mg/kg [12] and second group administered orally with curcumin, 60 mg/kg [14] followed by pioglitazone (10 mg/kg PO) for single dose interaction studies. Third group administered orally with curcumin, 60 mg/kg for 7 days and on the 8th day curcumin (60 mg/kg PO) followed by 1 hr pre-dosing with pioglitazone (10 mg/kg PO) for multiple dose interaction studies.

Blood samples (0.8 ml) were collected from retro orbital plexus, and double of the same volume was replaced with normal saline intraperitoneally. The blood is collected into eppendorf tubes at time intervals of 0, 0.5, 1, 2, 4, 8 and 24 hrs of pioglitazone administration in every group. Serum was separated by centrifugation using biofuge 13 (Heraeus instruments, Germany) at 3000 g/15 min and the separated serum was stored at -80°c until further analysis.

Induction of diabetes in rats

Experimental diabetes in rats (200-250 g) was induced by IP injection of alloxan monohydrate 120 mg/kg body weight, freshly dissolved in normal saline to 16 hours over night fasted rats [15,16]. A 20% glucose solution was injected i.p. after 4-6 hrs. The rats were kept for the next 24 hours on 5% oral glucose solution, in their cages to prevent hypoglycaemia [17]. After 72 hours retro orbital blood sampling was done and the serum glucose levels were measured by peroxidise (POD) glucose oxidase (GOD) method [18]. The fasting blood glucose levels of 250 mg/dL and above were considered as diabetic and selected for the study. Control group were given the same volume of normal saline.

Pharmacokinetic and pharmacodynamic interaction study in diabetic rats

Following an overnight fasting, rats were divided into 3 groups: group 4, 5 and 6 each containing 6 rats. Group 4 administered orally with pioglitazone, 10 mg/kg [12] and group 5 administered orally with curcumin, 60 mg/kg [14] followed by pioglitazone (10 mg/kg PO) for single dose interaction studies. Group 6 administered orally with curcumin, 60 mg/kg for 7 days and on the 8th day curcumin (60 mg/kg; POD) followed by 1 hr predating with pioglitazone (10 mg/kg; PO) for multiple dose interaction study. Blood samples (0.8 ml) were collected from retro orbital plexus, and double of the same volume was replaced with normal saline intraperitoneally. The blood is collected into eppendorf tubes at time intervals of 0, 0.5, 1, 2, 4, 8 and 24 hours of pioglitazone administration in every group. Serum was separated

by centrifugation using biofuge 13 (Heraeus instruments, Germany) at 3000 g/15 min and the separated serum was stored at -80°C until further analysis.

Calculations of pharmacokinetic and pharmacodynamic

Non compartmental pharmacokinetic analysis was carried out using Kinetica TM software (version 4.4.1 Thermo Electron Corporation, U.S.A). The following Pharmacokinetic parameters were calculated: C_{max}, T_{max}, $AUC0_{ton}$, AUC_{tot}, $AUMC_{0ton}$, $AUMC_{tot}$, $t_{1/2}$, MRT, Cl, Vd and Vdss.

Mean glucose levels and percentage reduction in blood glucose concentrations were determined for the pharmacodynamic data.

% glucose reduction at t hour = [(Gt – G0) / Gt] ×100

Gt→mean glucose levels at t hour

G0→mean glucose levels at 0 hour

Statistical analysis

The results were expressed as mean ± SD. The difference in between concentration time profiles; in between pharmacokinetic parameters, in between serum glucose levels and difference between the entire range tested were analyzed by one-way ANOVA (Bonferroni post-test). The differences were considered to be significant at $P<0.05$.

Results

Test 1

Pharmacokinetics of pioglitazone and pioglitazone under curcumin pretreatment in normal rats: The pharmacokinetic parameters for pioglitazone were calculated and showed a C_{max} of 4.15 ± 0.78 µg/mL, T_{max} of 2 hours and AUC_{0ton} of 25.28 ± 9.45 µg.hr/mL in normal rats. Increase in AUC, AUMC and MRT; and decrease in clearance was statistically significant in multiple pre-treatment group when compared with control group (Table 1).

Pharmacokinetics of pioglitazone and pioglitazone under curcumin pretreatment in diabetic rats: The pharmacokinetic parameters for pioglitazone were calculated and showed a C_{max} of 4.37 ± 0.14 µg/mL, T_{max} of 2 hrs and AUC_{0ton} of 33.63 ± 10.14 µg.hr/mL in normal rats. The serum concentration levels of pioglitazone in diabetic rats were read out by substituting the peak area ratio values of each sample in the equation obtained from the calibration curve of pioglitazone. The mean values along with standard deviation were calculated for each time point and a concentration versus time curve was obtained by plotting the mean concentration of pioglitazone on x-axis against the time points on y-axis. Increase in AUC and AUMC; and decrease in clearance was statistically significant in multiple pre-treatment group when compared with control group (Tables 1 and 2).

Test 2

Pharmacodynamics effects of curcumin on the pioglitazone in normal rats: The mean blood glucose levels for each time point in normal rats were calculated using glucose oxidase peroxidise method and the percentage glucose reduction at each time point compared to the mean glucose levels at 0 hr was calculated (Tables 3 and 4). The mean glucose levels and percentage glucose reduction was compared in curcumin pre-treatment against control group in normal rats (Figures 1 and 2). Pre-treatment of curcumin was found to have less significant effect on the diabetic control or the percentage reduction of glucose levels in normal rats.

Pharmacodynamics of effects of curcumin on the pioglitazone in diabetic rats: The mean blood glucose levels for each time point in diabetic rats were calculated using glucose oxidase peroxidise method and the percentage glucose reduction at each time point compared to the mean glucose levels at 0 hr was calculated (Tables 2 and 5). The mean glucose levels and percentage glucose reduction was compared in curcumin pretreatment against control group in diabetic rats (Figures 3 and 4). Pretreatment of curcumin was found to decrease the mean blood glucose levels and thus increase the percentage glucose reduction in both single dose and multiple dose exposure, with more statistical significance in multiple dose group $p<0.01$.

Discussion

Diabetes is a chronic metabolic disorder which needs prolonged treatment for maintenance of normal blood glucose levels and to control several complications induced by this disease like retinopathy, nephropathy, peripheral neuropathy, cardiomyopathy and an underlying high oxidant stress. The aim of present study is to determine whether curcumin influences the pharmacokinetics and pharmacodynamics of pioglitazone in normal and diabetic rats or not. In addition, we also check the safety combination effects in same animal models. Several factors that directly or indirectly influence the CYP mediated metabolism are likely to be potential candidates for drug interaction either as a result of induction or inhibition of CYP enzyme [19]. Synthetic drugs or phytochemicals those inhibit CYP

Pk parameter	In normal rats			In diabetic rats		
	Pioglitazone	PIO+CURC(SDI)	PIO+CURC(MDI)	Pioglitazone	PIO + CURC(SDI)	PIO + CURC(MDI)
C_{max} (µg/mL)	4.15 ± 0.78	4.43 ± 1.14	3.78 ± 0.89	4.37 ± 0.14	4.46 ± 0.36	4.06 ± 1.01
T_{max} (hrs)	2 ± 0	1 ± 0	2 ± 0	2 ± 0	1 ± 0	2 ± 0
$AUC_{0 to n}$ (µg.hr/ mL)	25.28 ± 9.45	28.18 ± 6.16	33.34 ± 9.18*	33.63 ± 7.14	34.65 ± 9.11	41.33 ± 10.38*
AUC_{total} (µg.hr/ mL)	26.86 ± 10.98	30.28 ± 29.07	36.87 ± 21.09**	37.08 ± 8.43	38.20 ± 5.96	46.82 ± 8.59*
$AUMC_{0 to n}$ (µg.hr mL)	154.37 ± 19.23	176.38 ± 21.15*	255.47 ± 17.93**	244.61 ± 31.29	254.71 ± 23.71	337.41 ± 48.05**
$AUMC_{total}$ (µg.hr/ mL)	205.42 ± 32.74	245.03 ± 28.02*	375.80 ± 39.18**	361.81 ± 35.91	374.81 ± 40.92*	529.36 ± 61.43**
$T_{1/2}$ (hrs)	5.63 ± 1.76	6.00 ± 1.09	7.04 ± 2.03	6.89 ± 1.09	6.82 ± 1.10	7.63 ± 0.41
MRT (hrs)	7.64 ± 2.09	8.09 ± 2.79	10.19 ± 1.45*	9.75 ± 1.14	9.81 ± 1.03	11.30 ± 0.78*
Cl (mL/hr)	0.37 ± 0.05	0.33 ± 0.02	0.27 ± 0.03**	0.26 ± 0.03	0.26 ± 0.07	0.21 ± 0.04*
Vd (mL)	3.02 ± 0.29	2.86 ± 0.19	2.75 ± 0.24	2.68 ± 0.45	2.57 ± 0.69	2.35 ± 0.35
Vdss (mL)	2.84 ± 0.17	2.67 ± 0.15	2.76 ± 0.20	2.63 ± 0.39	2.56 ± 0.51	2.41 ± 0.48

Mean +/- SD, ***significant at p<0.001, **significant at p<0.01,*significant at p<0.05 compared to pioglitazone control
SDI: Single Dose Interaction; MDI: Multiple Dose Interaction; PIO: Pioglitazone; CURC: Curcumin

Table 1: Mean Pharmacokinetic parameters of pioglitazone in presence of curcumin (SDI and MDI) in normal rats and in diabetic rats.

Time(hr)	PIO conc (µg/mL)	PIO + CURC(SDI) conc(µg/mL)	PIO + CURC(MDI) conc(µg/mL)
0	0 ± 0	0 ± 0	0 ± 0
0.5	3.32214 ± 0.40012	3.15203 ± 0.22419	3.57120 ± 0.24511*
1	2.76383 ± 0.57146	5.06782 ± 0.92310***	5.91402 ± 0.54653***
2	4.37565 ± 0.74325	5.23959 ± 0.87716***	5.99852 ± 0.39739**
4	3.09569 ± 0.51140	3.43299 ± 0.29897	3.63482 ± 0.44771
8	1.28946 ± 0.61786	0.69315 ± 0.57193	0.11295 ± 0.78430
24	0.36342 ± 0.34822	0.24298 ± 0.48015	0.25619 ± 0.35176

Mean +/- SD, ***significant at p<0.001, **significant at p<0.01, *significant at p<0.05 compared to pioglitazone control;

SDI: Single Dose Interaction; MDI: Multiple Dose Interaction; PIO: Pioglitazone; CURC: Curcumin

Table 2: Mean serum concentration of pioglitazone in presence of curcumin (SDI and MDI) in diabetic rats.

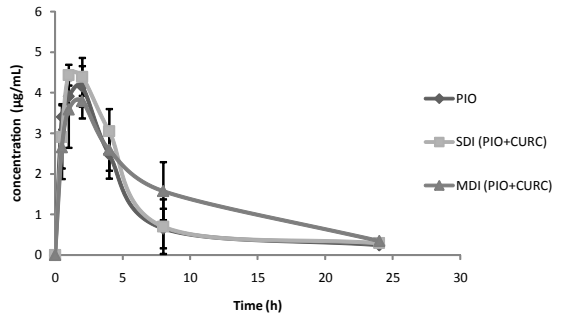


Figure 1: Mean serum concentration of pioglitazone in presence of curcumin (SDI and MDI) in normal rats.

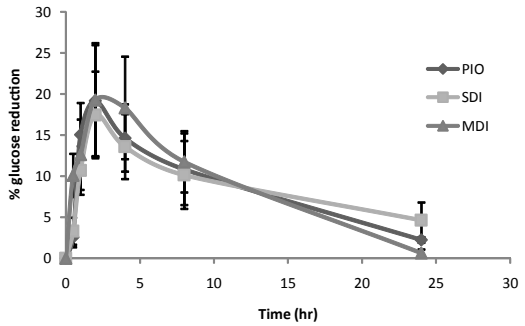
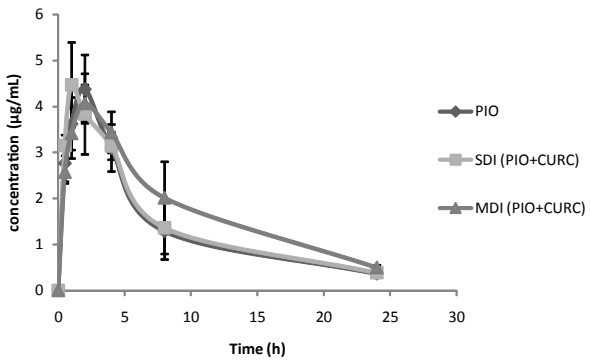


Figure 2: Comparative % glucose reduction in normal rats.



SDI: Single Dose Interaction; MDI: Multiple Dose Interaction; PIO: Pioglitazone; CURC: Curcumin

Figure 3: Mean serum concentration of pioglitazone in presence of curcumin (SDI and MDI) in diabetic rats.

system can significantly increase the plasma concentrations of certain other drugs metabolized by these enzymes and there by enhance their pharmacological and toxicological effect. Induction of CYP enzyme can lower the plasma concentration of concurrently administered drugs and leads to reduced therapeutic benefit [20]. Herbal medicines that modulate intestinal and hepatic CYPs can alter the bioavailability and clearance of co-administered drugs. Pioglitazone is metabolized by CYP 2C8 and CYP3A4 microsomal liver enzymes [20-22]. Curcumin was found to inhibit CYP enzymes and glutathione S-transferases [23-30]. We studied the influence of curcumin on the pharmacokinetic

Time(hr)	PIO - conc (µg/mL)	PIO + CURC(SDI) conc(µg/mL)	PIO + CURC(MDI) conc(µg/mL)
0	0 ± 0	0 ± 0	0 ± 0
0.5	3.90468 ± 0.31409	2.90990 ± 0.77831	1.65410 ± 0.78332
1	3.70254 ± 0.40765	4.73127 ± 0.25601	2.58540 ± 1.44478
2	4.15260 ± 0.50056	3.14920 ± 0.46717	3.68825 ± 0.42379
4	2.48083 ± 0.59884	4.38872 ± 0.54639	1.57314 ± 0.51843
8	0.65313 ± 0.48756	0.51782 ± 0.67227	2.19537 ± 0.71474
24	0.24025 ± 0.22673	0.19371 ± 0.30735	0.24579 ± 0.31364

Mean +/- SD, ***significant at p<0.001,**significant at p<0.01, *significant at p<0.05 compared to pioglitazone control

SDI: Single Dose Interaction; MDI: Multiple Dose Interaction; PIO: Pioglitazone; CURC: Curcumin

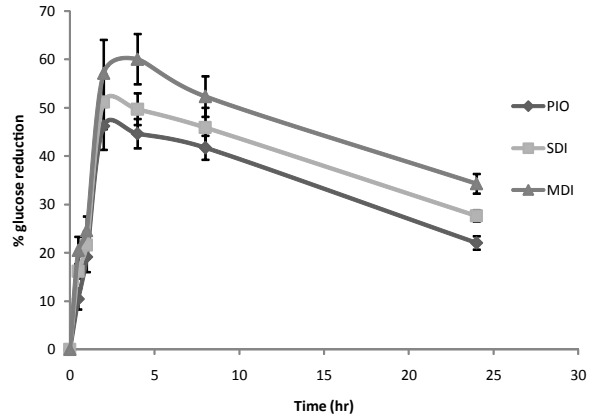
Table 3: Mean serum concentration of pioglitazone and pioglitazone in presence of curcumin (SDI and MDI) in normal rats.

Time(hr)	PIO conc (µg/mL)	PIO + CURC(SDI) conc(µg/mL)	PIO + CURC(MDI) conc(µg/mL)
0	0 ± 0	0 ± 0	0 ± 0
0.5	3.32214 ± 0.40012	3.15203 ± 0.22419	2.57120 ± 0.24511
1	2.76383 ± 0.57146	5.06782 ± 0.92310	2.71402 ± 0.54653
2	4.37565 ± 0.74325	1.23959 ± 0.87716	3.16852 ± 0.39739
4	3.09569 ± 0.51140	3.43299 ± 0.29897	2.63482 ± 0.44771
8	1.28946 ± 0.61786	0.69315 ± 0.57193	2.11295 ± 0.78430
24	0.36342 ± 0.34822	0.24298 ± 0.48015	0.25619 ± 0.35176

Mean +/- SD, ***significant at p<0.001, **significant at p<0.01, *significant at p<0.05 compared to pioglitazone control

SDI: Single Dose Interaction; MDI: Multiple Dose Interaction; PIO: Pioglitazone; CURC: Curcumin

Table 4: Mean serum concentration of pioglitazone in presence of curcumin (SDI and MDI) in diabetic rats.



SDI: Single Dose Interaction; MDI: Multiple Dose Interaction; PIO: Pioglitazone; CURC: Curcumin

Figure 4: Comparative % glucose reduction in diabetic rats after pretreatment with curcumin single and multiple doses.

Time (hr)	PIO		PIO + CURC (SDI)		PIO + CURC (MDI)	
	Mean glucose level (mg/dL)	% glucose reduction	Mean glucose level (mg/dL)	% glucose reduction	Mean glucose level (mg/dL)	% glucose reduction
0	276.19 ± 12.45	0 ± 0	274.47 ± 29.08	0 ± 0	253.16 ± 16.42	0 ± 0
0.5	247.36 ± 21.08	10.43 ± 2.18	230.14 ± 16.15	16.15 ± 1.49*	201.37 ± 21.19	20.45 ± 2.83**
1	223.34 ± 10.12	19.13 ± 3.15	215.15 ± 8.11	21.61 ± 2.83	190.92 ± 14.50	24.58 ± 2.91*
2	148.5 ± 6.08	46.23 ± 4.96	133.98 ± 13.08*	51.18 ± 5.64*	108.62 ± 12.10*	57.09 ± 6.93**
4	152.94 ± 8.13	44.62 ± 3.03	138.09 ± 8.90*	49.68 ± 3.28*	101.17 ± 11.39*	60.03 ± 5.20**
8	161.01 ± 2.14	41.70 ± 2.48	148.44 ± 6.97	45.91 ± 4.05	120.77 ± 7.05	52.29 ± 4.19**
24	215.37 ± 18.72	22.02 ± 1.39	198.65 ± 19.18*	27.62 ± 1.18*	166.43 ± 14.30	34.25 ± 2.03**

Mean +/- SD; ***significant at p<0.001;**significant at p<0.01; significant at p<0.05 compared to pioglitazone control
SDI: Single Dose Interaction; MDI: Multiple Dose Interaction; PIO: Pioglitazone; CURC: Curcumin

Table 5: Mean blood glucose changes in diabetic rats after oral administration of pioglitazone, curcumin and their combination (SDI and MDI) in diabetic rats.

and pharmacodynamics of pioglitazone in rats model of diabetes and compared with normal rats. The pharmacokinetic parameters were found to alter in curcumin pretreatment, but we observed statistically more significant in multiple dose pretreatment. In normal rats, pretreatment of curcumin resulted in a significant increase in AUC_{tot} by 37% ($p<0.001$) and $AUMC_{tot}$ by 82% ($p<0.001$). In diabetic rats, pretreatment of curcumin resulted in an increase in AUC_{tot} by 26% ($p<0.05$) and $AUMC_{tot}$ by 46% ($p<0.001$). Surprisingly, there was a decrease in T_{max} from 2 hrs to 1 hr only in single dose treatment groups, in both normal and diabetic rats may be due to slow absorption and slow metabolism. Pre-treatment of curcumin was found to have no significant effect on the glycemic control or the percentage reduction of glucose levels in normal rats and the maximum percentage glucose reduction were obtained at 2 hrs in all groups of normal rats. Whereas, in diabetic rats, it was found to significantly decrease the mean blood glucose levels and thus significantly increase the percentage of glucose reduction. In diabetic rats, maximum percentage glucose reduction was obtained at 2 hrs in control and single dose pre-treatment groups (46.23 ± 4.96% and 51.18 ± 5.64 respectively) and at 4 hrs in multiple dose pre-treatment groups (60.03 ± 5.20). Percentage of glucose reduction was much significant ($p<0.01$) in multiple dose than in single dose pre-treatment group ($p<0.05$).

The decrease in T_{max} of pioglitazone only in single dose interaction and an increase in AUC and AUMC in curcumin pre-treatment suggests, an inhibitory influence of curcumin on intestinal metabolism of pioglitazone as curcumin has poor bioavailability [31-34] it was allowed for transient but more exposure to intestinal region; and an inhibitory influence on hepatic metabolism as a compound with poor absorption would require long term exposure in order to show significant influence on hepatic metabolism. Influence of curcumin being effective in improving pharmacodynamics (glycemic control) only in diabetic rats indicates that the alteration might be partly because of improved pharmacokinetics of pioglitazone and partly because of anti-hyperglycemic activity of curcumin.

There was a very significant ($p<0.001$) influence on the percentage of glucose reduction in diabetic rats under multiple dose treatment but no significant ($p>0.05$) influence in normal rats. Thus, the improved pharmacokinetic parameters of pioglitazone was more observed in the multiple dose treatment groups, and the improvement of pharmacodynamics was significant in only diabetic rats under multiple dose treatment, thus showing the significance of influence of curcumin in multiple dose exposure under diabetic condition.

Conclusion

The results of increased pioglitazone levels as a result of its metabolic inhibition under curcumin exposure suggests an interaction which may be due to decreased metabolism of pioglitazone as a result of CYP3A4 and CYP2C8 inhibition. Since, the alterations are more pronounced in multiple dose treatment groups, it indicates the significance of long term exposure of curcumin in diabetic condition being controlled by pioglitazone in rats, and thus it may apply to diabetic patients under pioglitazone treatment. Further investigations needed in the simultaneous atherosclerotic [35] and alloxan drug induction of diabetes in the mice to confirm and further validate these studies. Hence, the combination has a beneficial effect in diabetic condition, but special concern has to be observed in diabetic patients with cardiovascular complications in view of the side effects of pioglitazone. Hence the present investigation warrants further studies to find out the relevance of this interaction in human beings and postulates the exact mechanism involved.

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