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Original Article

Effect of methanolic extract of *Piper cubeba* Linn. fruits on the pharmacokinetics of Pioglitazone in rats

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ABSTRACT

Herb-drug interaction about Oral antidiabetic drugs is a challenging concept. Present work was designed with an objective to investigate any possible pharmacokinetic effect of herbal drug *Piper cubeba* Linn. on Pioglitazone, a commonly used antidiabetic agent. Since the MeOH-soluble fraction of the extract of *Piper cubeba* Linn fruit shows potent CYP3A4 inhibitory activity, it was speculated that the fruit extract may influence the bioavailability of Pioglitazone. In this experiment three groups (I, II & III) of Albino female wistar rats were used for pharmacokinetic study and the plasma levels were determined at various time points (0.5, 1, 2, 4, 8 and 24 h) by HPLC. The extract pretreatment increased $AUC_{0-\infty}$ (bioavailability) of Pioglitazone after oral administration by 72.5% suggesting decreased metabolism, which could be due to inhibition of CYP3A4. In conclusion, add-on preparations containing this fruit extract may increase the bioavailability of Pioglitazone, with increased efficacy and/or adverse events and hence should be cautiously used.

***Key Words*:** *Piper cubeba* Linn, Pioglitazone, CYP3A4. Drug Interactions, Bioavailability.

INTRODUCTION

A food–drug interaction or herb-drug interaction is the consequence of a physical, chemical, or physiologic relationship between a drug and a product consumed either as food or a nutrient or dietary supplement [1]. Such an interaction may manifest clinically as compromised health status due to altered Pharmacokinetics and/or Pharmacodynamics of the drug or dietary substance. Food or dietary/herbal supplements containing bioactive constituents act like "perpetrator" drugs which can either increase systemic "victim" drug exposure leading to increased risk of adverse events and toxicity, or decrease systemic victim drug exposure, leading to therapeutic failure [1].

A lack of an interaction may be due to insufficient concentration(s) of causative ingredients at the enzyme active site or metabolism of causative ingredients to inactive products, or transport of causative ingredients out of target cells (e.g., enterocyte, hepatocyte). Underlying mechanisms by which food exerts such effects generally include physiologic, physicochemical, and/or biochemical processes [2]. Elucidation of these processes in relevant organ systems is essential to resolve issues related to formulation, dosing schedule, and optimal pharmacotherapy. Pioglitazone, a thiazolidinedione derivative decreases insulin resistance via its action at the peroxisome proliferator activated receptor subtype gamma (PPAR-ϒ) and emerged as a novel oral antidiabetic agent in recent past. The pharmacokinetic studies indicate about 80% oral bioavailability of Pioglitazone, and it is suggested that it is metabolized by multiple cytochrome P450 (CYP) isoenzymes, mainly by CYP2C8, CYP3A4 and CYP2C9 to several active and inactive metabolites (quercetin) [3].

The genus *Piper* belongs to the Piperaceae family, widely distributed in the tropical and subtropical regions of the world, and is used medicinally in various ways. *Piper cubeba* Linn. is one of the popular medicinal plants extensively used in Indonesia. The fruits are used as a spice and for the treatment of gonorrhea, dysentery, syphilis, abdominal pain, diarrhea, enteritis, and asthma.

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Economically, Piperaceae plants are important for pepper production in the spice markets. Recently, several reports have demonstrated that on simultaneous administration, some spices, herbs, black teas, and soybean products may cause pharmacokinetic interaction with Western drugs [2-5] the inhibition of cytochrome P450 (CYP) can lead to serious clinical drug interactions when concomitant drugs are metabolized by the same CYP. The fruits of *P. cubeba* were extracted with water, and the water extract was fractionated with EtOH and MeOH to yield EtOH-soluble and MeOH-soluble fractions.

The EtOH-soluble fraction showed inhibitory activity (inhibition: 75% against CYP3A4, 30% against CYP2D6) similar to the MeOH-soluble fraction (inhibition: 84% against CYP3A4, 43% against CYP2D6). CYP3A4 isozymes collectively comprise the largest portion of the liver and small intestinal CYP protein and they are involved in the metabolism of 45–60% of all currently used. Thus, the mechanism-based inhibition of CYP3A4 by natural compounds may have important pharmacokinetic implications. Five methylenedioxyphenyl lignans namely (–)-clusin (1), (–)-dihydroclusin (2), (–)-yatein (3), (–)-hinokinin (4), and (–)-dihydrocubebin (5), were isolated from *Piper cubeba* shows potent and selective inhibitors against cytochrome P450 3A4 (CYP3A4) [4].

As both herbal drug and allopathic drug involves same enzymes for metabolism, the present study was carried out with an objective to evaluate any possible herb –drug interaction between Methanolic extract of *Piper cubeba* Linn. fruits – the Perpetrator drug or Precipitant drug and Pioglitazone - the object drug or victim drug.

MATERIALS AND METHODS

Materials: Pioglitazone Hcl obtained from Dr. Reddy's Laboratories Pvt.Ltd. The HPLC grade methanol and ammonium acetate (Merck, Mumbai, India). All other chemicals used were of analytical grade. The drug analysis was carried out using HPLC system (Shimadzu 10AT/Vp, Kyoto, Japan) having Rheodyne injector port (20 µl loop), and UV/VIS detector (SPD 10A Vp). The data interpretation was done with LC-solutions (Shimadzu, Kyoto, Japan) data acquisition software. *Piper cubeba* Linn. fruits were purchased, identified and authenticated by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati. The authentication number is 1250.

Animals: Female Wister rats weighing between 200-250gms were procured and used in the study. They were maintained under standard laboratory conditions at ambient temp of 25 $\pm 2^0$ C with 12-hour light/12-hour dark cycle. They were fed with standard pellet diet and water *ad libitum*. The prior approval for conducting the experiments in rats was obtained from our Institutional Animal Ethics Committee (51/01/C/CPCSEA/2011/09).

Preparation of extract: The shade dried coarsely powdered fruits of *Piper cubeba* Linn. (400gms) was extracted using methanol as solvent by continuous hot extraction process using Soxhlet apparatus. The extraction was continued till the extraction completion. After completion of extraction the extract was concentrated under reduced pressure. That extract was stored in an airtight container in a refrigerator below 10°C [6].

Phytochemical screening: Phytochemical screening of the crude extract was carried out employing standard procedures [4], to reveal the presence of chemical constituents such as alkaloids, glycosides, flavonoids, phytosterols, oils and fats, etc.

Chemical Constituents of the Extract and their Identification.

HPLC analysis: An aliquot of the extract was analyzed by a Shimadzu HPLC system with UV-VIS detection (280 - 600 nm) using a SUPELCOSIL LC-18 (250 × 4.6 mm) column with a pre-column of the same material (both Macherey Nagel; Oensingen, Switzerland) as stationary phase. The mobile phase consisted of two solvent systems {A: 0.1% trifluoroacetic acid (TFA) in water (v/v) and B: 100% acetonitrile in a gradient (0-30 min 90% A, 30-45 min 50% A, 45-46 min 10% A, 46-50 min 10% A)}. The column temperature was kept at 40 °C and the flow rate was set at 1.0 mL/min. The detection was carried out at 280 nm and quantification of the lignan in the extract was performed by the external standard method using cubebin[7], the structure and peaks of pioglitazone is shown in figure 1.

***Sample collection*:** Blood samples were collected by retro-orbital plexus.

Acute Oral Toxicity Study: Acute *oral toxicity* test was carried out according to the OECD guidelines 423. Female wistar albino rats (150-200 gm weight) were used. Rats were kept for overnight fasting prior to drug administration. A total of three animals were used, which received a single oral dose (2000mg/kg body weight) of methanol extract of *Piper cubeba* Linn. fruits. After the

administration of extract, food was withheld for further 3-4 hours. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 hours (with special attention during the first 4 hours), and daily thereafter for a period of 14 days [6].

Selection of dose of the extract: LD50 was done as per OECD guidelines for fixing the dose for biological evaluation. The biological evaluation can be carried out at doses of 200 and 400 mg/kg body weight.

Study design

Pharmacokinetic study normal rats:

Rats were divided into 3 groups (n=6).

Group 1 - (Control) - Rats were orally administered with 0.9%w/v saline

Group 2 - (Standard) - Rats were fasted overnight and were orally administered with Pioglitazone at dose of 10mg/kg.

Group 3 - (Test) - Rats were fed orally with extract at dose of 400mg/kg for seven days followed by fasting overnight on seventh day and were orally administered with Pioglitazone at a dose of 10mg/kg on 8th day.

Preparation of Mobile Phase [2]: Prepared on the day of test and delivered at a rate of 1.2 ml/min. by mixing Phosphate buffer (P^H-3), acetonitrile, methanol in 70:25:25 v/v/v.

Preparation of stock solutions and working standard solutions [2]

- Standard stock solutions of Pioglitazone of 10 µg/ml.
- Working Standard solutions of 0.5, 1, 4, 8.10,14,28 µg/ml were prepared by diluting standard stock solution in mobile phase and injected into HPLC to know the specific drug peak at specific Retention time.
- HPLC: Shimadzu HPLC system was used with SUPELCOSIL LC-18 column quantified by UV detection at 269 nm at a flow rate of 1.2 ml/min.

Method of extraction of Pioglitazone from Plasma:

Spiked plasma concentration: Blood from Group –I is withdrawn from tail vein in micro centrifuge tubes and plasma is separated by centrifugation at 3000 x g for 15 minutes and stored at -20° c, then in a 1.5 ml Eppendorf tube, 100 µl of plasma were mixed with 50 µl of above working standard solutions and 100 µl Acetonitrile were added to precipitate the proteins. The mixture was vortex mixed for 5 min after which it was centrifuged at 10,000×g for 10 min. 20µl of the supernatant was injected onto the HPLC system for analysis to obtain Area at specific RT for different concentrations and linearity curve is plotted .The areas of peak to specific concentrations at specific RT in Plasma will serve as Plasma Calibrators.

Extraction of Pioglitazone from plasma from the extract and drug treated group: Blood (0.3ml) was withdrawn by retro-orbital plexus and collected in heparinised tubes at 0.5,1,2,4,8 and 24h after oral administration of Pioglitazone. Blood samples were immediately centrifuged at 3000×g for 15 min to obtain plasma, and stored at -20^{0}C until analysis [8]. In brief, to 100 µl of plasma sample, 100 ml of acetonitrile were added to precipitate the proteins. The mixture was vortex mixed for 5 min after which it was centrifuged at 10,000×g for 10 min. 20 µl of the supernatant was injected onto the HPLC system for analysis. The above collected blood samples were also used for determination of glucose by Glucometer [9] or by GOD-POD method [5].

Data analysis: The maximum plasma concentration (C_{max}), time needed to reach the maximum plasma concentration (T_{max}), area under the concentration– time curve ($AUC_{0-\infty}$), mean residence time (MRT), elimination rate constant (K_{el}), clearance and half-life ($T_{½}$) were calculated using non compartmental pharmacokinetic model.

Statistical analysis: All the means are presented with their standard deviation (mean ±S.D). The pharmacokinetic parameters of Pioglitazone groups and extract treated group was compared using paired Student's t-test. $P < 0.05$ was considered statistically significant.

RESULTS

Phytochemical screening: The methanolic extract of *Piper cubeba* Linn. fruits was confirmed to contain, alkaloids, glycosides, flavonoids, phytosterols, oils and fats.

***Acute toxicity study*:** In these studies, it was found that the animals were safe up to a maximum dose of 2000 mg/kg body weight. There were no changes in normal behavior pattern and no signs and symptoms of toxicity and mortality were observed.

***Selection of dose of the extract*:** LD5o was determined as per OECD guidelines for fixing the dose for biological evaluation and the maximum dose of 400 mg/kg body weight was selected.

Chemical constituents of the Extract and their identification by HPLC: Chemical structure of cubebin and the chromatogram of Methanolic

extract of *Piper cubeba* Linn. fruits is shown in Figure 1.

Pharmacokinetic interaction in normal rats: Mean pharmacokinetic parameters of Pioglitazone in Pioglitazone alone and extract treated Pioglitazone groups were shown in Table 1. The pharmacokinetic parameters of Pioglitazone like $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{½}$, K_{el}, Clearance and MRT were altered significantly in rats treated with extract when compared to Pioglitazone alone treated group shown in Table 1. Chromatogram of Pioglitazone in Plasma when spiked at different concentrations and the chromatogram for Pioglitazone at concentration of 10µgm/ml is shown in the Figure 2. Mean serum concentration of Pioglitazone in Standard and Test in normal rats were shown in Figure 3.

DISCUSSION

Thiazolidinediones are used to treat type 2 diabetes especially with Pioglitazone alone or combination with other hypoglycaemic agents, in both animal and human models, which act by enhancing peripheral sensitivity to insulin. Thiazolidinediones are potent antidiabetic compounds [10].The use of complementary therapies for treatment of diabetes is ever increasing, and often remains unnoticed by a physician. Furthermore, now-a-days the antidiabetic pharmacological strategy is becoming increasingly complex, and the recommended global approach of combination herb-drug therapy has increased the risk of pharmacokinetic interactions in diabetic patients [11]. Although the risk of hypoglycaemia with thiazolidinediones appears negligible but herb-drug interactions may exacerbate adverse effects and raise safety concerns.

Pioglitazone is an insulin sensitizer acting primarily on Peroxisome proliferator activated receptor subtype gamma type (PPAR-γ) against insulin resistance [4, 9]. Pioglitazone enhances tissue sensitivity to insulin rather than stimulating insulin secretion. As earlier reported the combinative therapy with 4-Hydroxyisoleucine and Pioglitazone proved beneficial than Pioglitazone alone treated group [12] and the risk with the administration of Carica papaya extract with oral hypoglycemics which led to hypoglycaemic condition [6]. Several studies have reported that the lignans are the potent inhibitors of CYP3A4 in vitro, which plays a major role in the metabolism of Pioglitazone [2] and revealed increase in AUC of Pioglitazone.

The present study to evaluate the effect of methanolic extracts of *Piper cubeba* Linn. fruits on the kinetics of Pioglitazone in which the concentration of Pioglitazone in the blood sample was determined by using HPLC technique .For instance the Linearity curve was plotted by spiking the Plasma obtained from rats of group -I with known amount of Pioglitazone which serve as Plasma calibrators. The retention time (RT) for Pioglitazone found at 3.7 minutes as shown in Figure 2which is specific for Pioglitazone extracted by using mobile phase Phosphate buffer (P^H-3), acetonitrile, methanol in 70:25:25 v/v/v at a flow rate of 1.2ml/min and the concentration was determined by the peak area of the chromatogram which are specific to concentration of the drug.

Blood drained from retro-orbital plexus of rats in all the groups at different intervals were processed and injected into HPLC and the concentration of the drug in plasma was determined by comparing with the peak areas of the Plasma calibrators.

All the other Pharmacokinetic parameters were calculated using Non Compartmental Model .The pharmacokinetic parameters of Pioglitazone like $AUC_{0-\infty}$, C_{max}, $T_{1/2}$, and Clearance were altered significantly with treatment of extract (400 mg/kg) in normal rats when compared to Pioglitazone alone treated group as shown in Table 1. As shown in Figure 3 i.e., Plasma drug concentration –time curve plotted between two groups, there is increase in the plasma levels of Pioglitazone in extract pre-treated group which might have decreased the elimination rate constant and increased $t_{1/2}$ to more than 1 hour.

The increase in bioavailability of the extract treated group was by 72.5% over Pioglitazone alone treated group which can be attributed to a decline in hepatic clearance. Marked increase in bioavailability may be because Pioglitazone is metabolized by CYP3A4 and CYP2C8 [2, 5] and MeOHPCLF inhibits CYP3A4 enzymes [4, 13] which clearly indicate that the Herb-Drug interaction i.e., effect of Extract on the Pharmacokinetics of Pioglitazone mediated by metabolic enzymes cytochromes.

Increased bioavailability due to herb drug interaction thus suggests dose adjustment criteria in order to minimize the adverse effects of object drug and sometimes this can be used in a beneficial side to reduce the cost of object drug by decreasing the dose when used with herbs thus supporting Pharmacoeconomics.

CONCLUSION

In conclusion, the extract modestly increased the bioavailability and decreased the oral clearance of

Pioglitazone in rats. The mechanism that underlies the interaction between extract and Pioglitazone probably involves the inhibition of enzyme CYP3A4-catalysed Pioglitazone metabolism by extract. Concomitant administration of extract could thus result in increased plasma concentrations of Pioglitazone with increased efficacy and/or adverse events. Administration of methanolic extract of *Piper cubeba* Linn. fruits with Pioglitazone led to herb drug interaction and augmented the antihyperglycemic activity of Pioglitazone significantly. Thus it is necessary to adjust the dose of Pioglitazone when it is administered with *Piper cubeba* Linn. fruits to minimize the consequences/adverse effects of Pioglitazone.

TABLE 1. MEAN PHARMACOKINETIC PARAMETERS OF PIOGLITAZONE ALONE AND IN PRESENCE OF EXTRACT IN NORMAL RATS.

Pharmacokinetic Parameters	**Pioglitazone**	**Extract+Pioglitazone**
AUC $_{(0-24)}$ (µg-h/ml)	80.6±4.6	136.4±7.6***
$AUC_{0-\infty}$(µg-h/ml)	82.45±5.12	142.23±5.85***
C_{max} (µg/ml)	12.03±0.55	16.73±2.27*
T_{max}(h)	2±0	2±0 ^{ns}
Clearance(ml/h)	0.4±0.006	0.02±0.004**
$T_{½}$(h)	3.51±0.41	4.75±0.58**
MRT(h)	6.16±0.4	6.88±0.5 ^{ns}
K_{el}(ml/h)	0.2±0.03	0.14±0.02**

Mean ±SD (n=6);

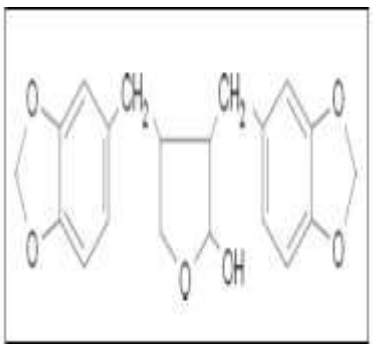
* Significant at P<0.005 compared to Pioglitazone control.

** Significant at P<0.001 compared to Pioglitazone control.

*** Significant at P<0.0001 compared to Pioglitazone control.

ns non-significant

Data analysis was done by using statistical Program software Prism Graph pad and the significance was determined by students paired‘t’ test.



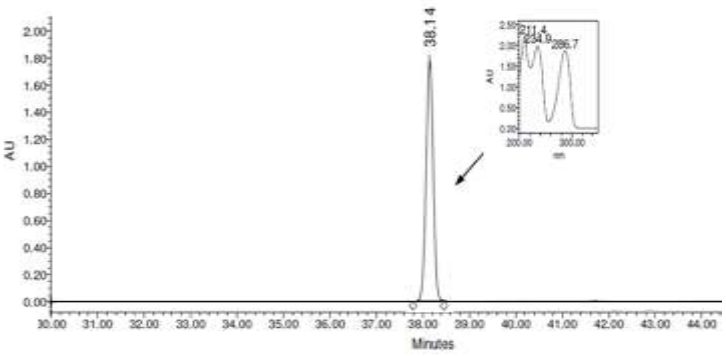


Figure 1. Chemical structure of cubebin & HPLC chromatogram of MeOHPCLF - Peak indicates the presence of cubebin.

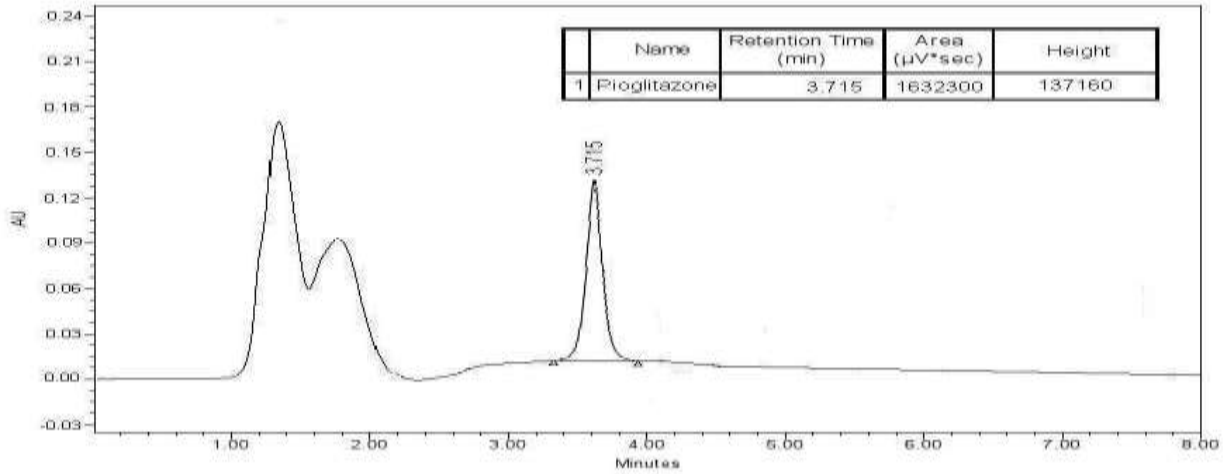


Figure 2.Chromotogram of Pioglitazone in Plasma when spiked at a concentration of 10µgm/ml.

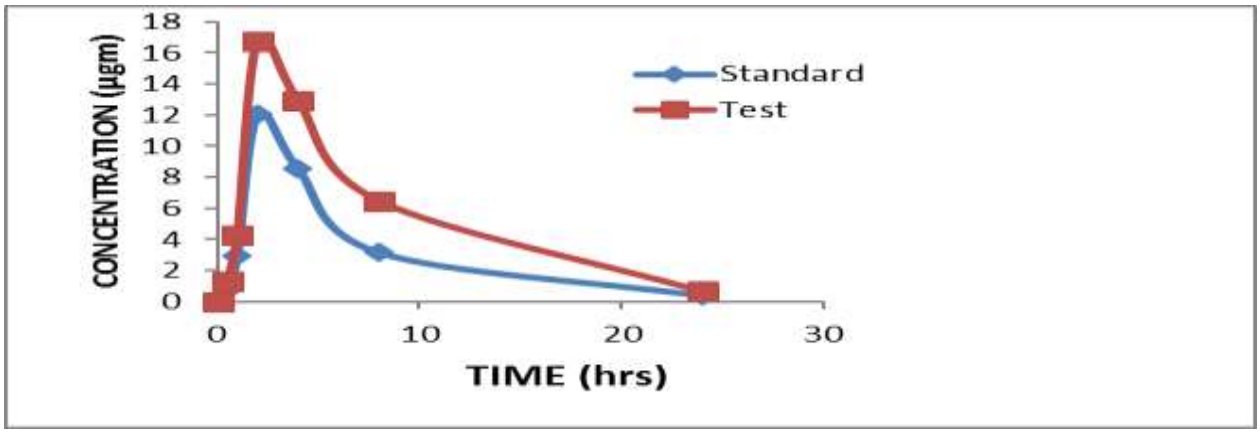


Figure 3. Plasma concentrations time curves of Pioglitazone following its oral administration at 10mg/kg (standard) and Extract (400 mg/kg) pre-treated rats (Test).

REFERENCES

1. Santos C. A., & Boullata J. I. An approach to evaluating drug–nutrient interactions. Pharmacotherapy 2005; 25: 1789–1800.
2. Fleisher D et al. Drug, meal and formulation interactions influencing drug absorption after oral administration. Clinical implications. Clin Pharmacokinet 1999; 36: 233–54.
3. Sudhir N. Umathe et al. Quercetin pretreatment increases the bioavailability of Pioglitazone in rats: Involvement of CYP3A inhibition. Biochemical Pharmacology 2008; 75: 1670-76.
4. Tepy Usia et al. Potent CYP3A4 inhibitory constituents of *Piper cubeba*. Journal of Natural products 2005; 68 (1): 64-8.
5. Sunday Oyedemi et al. Antidiabetic Activities of Aqueous Stem Bark Extract of *Strychnoshenningsii* Gilg in Streptozotocin-nicotinamide Type 2 Diabetic Rats. Iranian Journal of Pharmaceutical Research 2012; 11 (1): 221-28.
6. Md.Parvez et al. Screening of *Piper cubeba* Linn. Fruits for Anti-ulcer activity. International Journal of Pharm Tech Research 2010; 2 (2): 1128-32.
7. Von Jianying Yam aus et al. The search for bioactive compounds in tropical plants to target hormone imbalance associated diseases. Singapore Basel 2007; 47.
8. Simonson DC et al. Mechanism of improvement in glucose metabolism after chronic glyburide therapy. Diabetes 1984; 33: 838-45.
9. Gayasuddin Mouid Md et al. Effect of ethanolic extract of *Piper cubeba* Linn. Fruits on activity of pioglitazone. International Journal of Pharmacy & Industrial Research 2011; 1 (4): 312-14.
10. Prasad Neerati, Keerthana Chenna. Effects of ritonavir on the pharmacokinetics and pharmacodynamics of pioglitazone in normal and diabetic rats. Journal of Pharmacy Research 2012; 5(2): 958-62.
11. Zimmet PZ. Challenges in diabetes epidemiology: from West to the rest. Diabetes Care 1992; 15: 232-52.
12. S.L Bodhankar et al. Combinative therapeutic approach for better blood sugar level control in Alloxan diabetic Mice. Int.J.Diabetes & Metabolism 2006; 14: 104-05.
13. DK Suresh et al. A study on effect of Amiodarone on the pharmacokinetics of pioglitazone in diabetic rats. International Journal of Pharmaceutical Research and Development 2012; 4 (5): 111-15.