RESEARCH PAPER | Pharmaceutical Science | Volume : 6 | Issue : 2 | FEBRUARY 2016 | ISSN - 2249-555X

Effect of Methanolic Extract of Piper Cubeba Linn. Fruits on The Pharmacodynamics of Pioglitazone in Alloxan Induced Diabetic Rats.

KEYWORDS: Pharmacodynamics , Piper cubeba , Pioglitazone,

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ABSTRACT *The present study was aimed to find out the effect of multiple dose (7days) treatment of methanolic extract of Piper cubeba Linn. Fruits (MeOHPCLF) on the hypoglycemic activity of Pioglitazone in diabetic rats. Herb-Drug interaction about Oral antidiabetic drugs is a challenging concept, since the consumption of food and other herbal drugs is not documented in diabetic patients' profile. Albino female wistar rats were divided into 4 groups of 6 animals each and made diabetic by administering Alloxan Monohydrate at a dose of 150mg/kg. Animals of group –II (Extract treated group) were administered orally with the extract at a dose of 400mg/kg for 7 days and Animals of group –IV (Test group) were administered orally with the extract at a dose of 400mg/kg for 7 days followed by fasting overnight on the seventh day and were orally administered with Pioglitazone at a dose of 10mg/kg on 8th day. All the 4 groups were tested with intraperitoneal glucose tolerance test (IPGTT) by administering glucose (2.5%) intraperitoneally and decrease in blood glucose concentration was determined in each group by GOD-POD method. MeOHPCLF enhanced the activity of Pioglitazone and significantly lowered Blood glucose concentration in MeOHPCLF + Pioglitazone treated group .Hence MeOHPCLF enhances the activity of Pioglitazone .*

1. INTRODUCTION

Diabetes mellitus is a common metabolic disorder characterized by hyperglycemia, glycosuria, polyuria and polydipsia induced by Insulin deficiency and insulin resistance[1]. The number of people suffering from this disease is increasing worldwide at an alarming rate .According to Amos about 85-95% of diabetic patients are suffering from Type-2 diabetes which is also known as Non-Insulin Diabetes Mellitus(NIDDM).It is characterized with the predominant insulin resistance through insulin deficiency reduced sensitivity of target tissues to the metabolic effects of Insulin.

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 CYP2C8, CYP3A4 and CYP2C9 to several active and inactive metabolites[1].

Piper cubeba L.is one of the popular medicinal plants extensively used in Indonesia. The fruits are used as spice and for the treatment of Gonorrhea, dysentery, syphilis, abdominal pain, diarrhea, enteritis and asthma. The genus *Piper* belongs to Piperaceae family, widely distributed in the tropical and subtropical regions of the world, and is used medicinally in various ways. Traditional medicines like herbal drugs in primary form or their extracts have been used by many diabetic patients as they assumed to be non-toxic in nature[2] but pharmacologically active constituents such as alkaloids, flavonoids, anthraquinones, lignans etc. found in the herbs or their extract can take part in herb-drug interactions[1].

2. MATERIALS AND METHODS

2.1 Materials

Alloxan monohydrate obtained from Research Lab Fine Chemical industries, Mumbai. Pioglitazone Hcl obtained from Dr.Reddy's Laboratories Pvt.Ltd. *Piper cubeba* Linn. Fruits were purchased and the plant material was identified and authenticated by DR. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati. The authentication number is 1250.

2.2. Animals

Female Wistar rats weighing between 200-250gms were procured and used in the study. They were maintained under standard laboratory conditions at ambient temperature of 25±2°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)*.

2.3 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 1O°c[4].

2.4 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,

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flavonoids, phytosterols, oils and fats, etc.

2.5 Chemical Constituents of the Extract and their Identification.

HPLC analysis

An aliquot of the extract was analyzed by a Waters HPLC system with UV -VIS detection (280 - 600 nm) using a CI8 column with a pre-column of the same material 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 the ratio of 50:50. 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[5].

2.6 Sample collection

Blood samples were collected by tail tip method and blood glucose levels were estimated by GOD-POD method[6].

2.7. 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 [7].

2.8 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.

2.9 Study design

Induction of Diabetes in rats.

Diabetes was induced in rats by the administration of alloxan monohydrate in ice cold normal saline 150mg/kg body weight intraperitoneally. After 72hrs, blood sample was collected from rats by tail vein of all surviving animals and the blood was analyzed for glucose levels. Rats with blood glucose levels of 300mg/dL and above were considered as diabetic and selected for study[7].

Group-I (Diabetic control) – Diabetic rats were orally administered with 0.9% w/v saline.

Group-II (Extract treated group) - Diabetic rats were fed orally with Methanolic extract of *Piper cubeba* Linn. fruits at dose of 400mg/kg for 7 days.

Group-III (Diabetic standard) - Diabetic rats were fasted overnight and were orally administered with Pioglitazone at dose of 10mg/kg.

Group –IV (Diabetic Test) - Diabetic rats were fed orally with Methanolic extract of *Piper cubeba* Linn. fruits at dose of 400mg/kg for 7 days followed by fasting overnight on 7th day and were orally administered with Pioglitazone at a dose of 10 mg/kg on 8th day.

Extract treated group is considered in this experiment to find out if there is Antidiabetic effect associated with the methanolic extract of *Piper cubeba* Linn. fruits.

All the 4 groups were tested with Intraperitoneal glucose tolerance test (IPGTT) by administering glucose(2.5%) intraperitoneally and decrease in blood glucose concentration was determined in each group using GOD-POD method after drug administration at 0 mins,2 hrs,4hrs,6hrs and 8 hrs[3].

2.10 Data analysis

The data obtained was expressed as Mean ± S.D. Results were analyzed statistically by one way ANOVA followed by Tukey-Kramer Multiple Comparisons Test[3].

3. RESULTS

3.1 Phytochemical screening

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

3.2 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.

3.3 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.

3.4 Chemical constituents of the Extract and their identification by HPLC

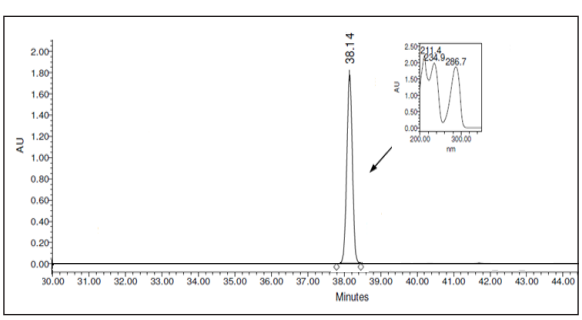

Figure 1. HPLC chromatogram of MeOHPCLF –Peak indicates the presence of Cubebin

3.5 Pharmacodynamic interaction in Diabetic rats.

In a group treated with MeOHPCLF + Pioglitazone, a significant lowering (P<0.001) of blood glucose concentration was seen when compared to Pioglitazone alone treated groups *at* 4 & 6 hours as shown in the Table 1.

Table 1. Blood Glucose Concentration in Diabetic Rats.

Group (Treatment)	Blood Glucose Concentration (mg/dl)					
	Before Glucose administration	After Glucose administration				
		0 hr	2hr	4 hr	6 hr	8 hr
I Normal Saline 0.9%	379.33 ±40.43	459.8 ±25.9	427.5 ±26.6	438.8 ±40.57	458.83 ±38.09	470.33 ±37.43

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II Extract 400mg/kg	362.1 **±43**	455 **±27.7**	430.33 **±34**	434.8 **±39.6**	448.8 **±35.05**	470.83 **±33.97**
III Pioglitazone 10mg/kg	368 **±41.51**	435 ±51.57	391.6 ±51.4*	359.6 ±47.2*	382.33 ±47*	405.66 ±47.56***
IV Extract +Pioglitazone	349 **±28.99**	410.66 ±31.9	353.33 ±31.8	310.6 ±30.7***	328.5 ±30.3***	342.16 ±29.63*

***Values are Mean* ± S.D, n=6**

Statistical analysis by One-way ANOVA followed by Tuckey Kramer multiple comparision test.
- In group III *P values < 0.05 and ***P values < 0.001 when compared to group-I respectively.
- In group IV *P values < 0.05 and ***P values < 0.001 when compared to group-III respectively.

Group (Treatment)	Blood Glucose Concentration (mg/dl)						
	Before glucose administration	After glucose administration					**Mean±S.D**
		0hr	2hr	4hr	6hr	8hr	
III Pioglitazone Alone	2.98	5.99	8.38	18.04	16.67	13.74	1 0.96±6.09
IV Extract + Pioglitazone	7.99	10.69	17.34	29.2	28.3	27.2	20.12±9.41

Table 2.Mean Percentage Blood Glucose reduction in Diabetic Rats

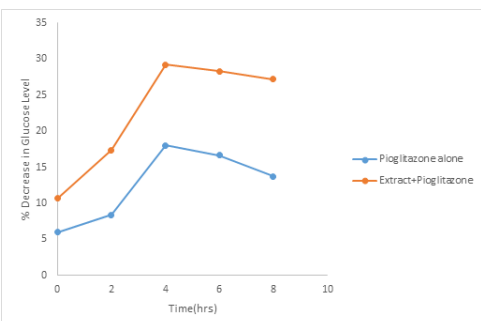

Figure 3. Mean percentage blood glucose reduction in diabetic rats after oral administration of Pioglitazone and Pioglitazone with extract.

4. DISCUSSION

Thiazolidinediones are used to treat type 2 diabetes especially with Pioglitazone alone or combination with other hypoglycemic agents, in both animal and human models, which act by enhancing peripheral sensitivity to insulin. Thiazolidinediones are potent antidiabetic compounds.

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. Although the risk of hypoglycemia with thiazolidinediones appear negligible but herb-drug interactions may also exacerbate adverse effects and raise safety concerns.

Pioglitazone is an insulin sensitizer acting primarily on Peroxisome Proliferator Activated Receptor subtype gamma type (PPAR-ϒ) against insulin resistance[8,9]. Pioglitazone enhances tissue sensitivity to insulin rather than stimulating insulin secretion. Alloxan decreases the insulin sensitivity and decreases the glucose uptake of cells.

The blood glucose concentration of MeOHPCLF treated group was found to be almost similar to that of Normal Saline group, this clearly rule out the antidiabetic activity of MeOHPCLF.

The blood glucose concentration of Me 0HPCLF + Pioglitazone treated group when compared to Pioglitazone alone treated group has shown significant reduction in blood glucose level which clearly indicates that the decrease in blood glucose level was due to synergistic effect of MeOHPCLF on Pioglitazone. Significant Mean percentage blood glucose reduction in diabetic rats after oral administration of Pioglitazone and Pioglitazone with extract is shown in Figure 3.As earlier reported the combinative therapy with 4- Hydroxyisoleucine and Pioglitazone proved beneficial than Pioglitazone alone treated group and the risk of administration of Carica papaya extract with oral hypoglycemic which led to Hypoglycemic condition but the present studies has not shown any hypoglycemic condition but show antihyperglycemic action. Enhanced antihyperglycemic action of MeOHPCLF treated Pioglitazone group over Pioglitazone alone treated group may be due to metabolic inhibition of Pioglitazone in intestine by the MeOHPCLF as Pioglitazone is metabolized by CYP3A4 and CYP2C8[10,11] and MeOHPCLF inhibits CYP3A4enzymes ,thus leading to Herb-Drug Interaction.

5. CONCLUSION

The mechanism that underlies the interaction between the Methanolic extract of *Piper cubeba* Linn. fruits and Pioglitazone probably involves the inhibition of CYP3-catalysed Pioglitazone metabolism extract. Concomitant administration of extract could thus result in increased plasma concentrations of Pioglitazone with increased efficacy and 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* fruits to minimize the adverse effects of Pioglitazone.

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