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EFFECT OF *AZADIRACHTA INDICA* ON THE PHARMACODYNAMICS OF PIOGLITAZONE IN NORMAL AND ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

The objective of the study is to find out the effect of *Azadirachta indica* on pharmacodynamic interactions with concomitant administration with pioglitazone in both normal and diabetic rats. Blood samples were collected from rats by retro orbital puncture at regular intervals of time 0, 1, 2, 3, 4, 6, 8, 10, 12 hr. During the single dose administration of Pioglitazone and *Azadirachta indica* showed reduction of blood glucose levels dose dependently. The pioglitazone (10mg/kg) showed peak glucose reduction at 2hr, *Azadirachta indica* (200mg/kg) at 4hr, the combination at single and multiple dose combinations showed peak glucose reduction at 2hr in both normal and diabetic rats. The insulin levels were also found to be increased according to the blood glucose reduction at peak hours. It indicates that the combination of *Azadirachta indica* and pioglitazone producing the significant synergistic action in producing hypoglycemia and antihyperglycemia. Hence, care must be taken while prescribing the combination in clinical situation.

KEYWORDS: Pioglitazone, Azadirachta Indica, Blood Glucose, Insulin.

1. INTRODUCTION

Broad research on diabetes has resulted in the development of a number of oral hypoglycemic agents including biguanides, sulphonylureas and thiozolidinediones which are available commercially for the management of diabetes. However, these drugs also produce non desirable side effects.[1] In recent years herbal remedies are widely used for the treatment of diabetes and its associated complications due to the presence of wide number of phytochemicals in it. These herbal drugs are complex mixtures of organic chemicals with potential adverse effects of presented phytochemicals. These herbal drugs produce herb drug interactions with severe health hazards in combination with the prescribed allopathic drugs.[2] It has been well established pharmacokinetics and/or Pharmacodynamics can occur through herb drug interaction. Pharmacodynamic interactions affect either the pharmacologic activity/efficacy or the produce/stimulate side effects of a drug without altering its plasma levels. The incidences are happen due to concomitant administration of drugs may effects either at same receptor or might be due to the altering the receptor binding affinity of drugs. The consequent events may lead to agonistic, synergistic or antagonistic interactions, which tends to influence the actual pharmacological actions of drugs.

Azadirachta indica is commonly used in Indian system of medicine to treat diabetes from the time of ancient days.[3] The isolates of *Azadirachta indica* were also reported for antidiabetic activity.[4] It elicits its action by improving glucose utilization in the skeletal muscle and the signaling insulin molecules.[5] The pharmacological actions of *Azadirachta indica* showed ameliorate lesions of pancreatic islets and inhibit action of α-amylase and α-glucosidase activities.[6,7] Previous studies reported the herb drug interaction studies of *Azadirachta indica* with glibenclamide and glimepiride.[8]

2. MATERIALS AND METHODS

Albino rats of either sex obtained from M/s. Mahaveer Enterprises, All animals were maintained on pellet diet supplied by M/s. Rayan Biotechnologies Pvt. Ltd., Hyderabad with 12h/12h light/dark cycle and water ad libitum. Animals were fasted for 18 h before the experiment. The *Azadirachta indica* obtained from Laila impex Pvt Ltd., Vijayawada as gift sample.

2.1 Study in normal rats: A group of six albino rats weighing between 250-300 g were administered with 10mg/ kg body weight Pioglitazone, orally. The same group was administered with 200mg/kg body weight *Azadirachta indica*, orally after a wash out period of one week. The same group was also administered with 200 mg/ kg body weight *Azadirachta indica* 30 min prior to

10mg/ kg body weight Pioglitazone, after a further wash out period of 1 week. Blood samples were withdrawn from retro orbital puncture at 0, 1, 2, 4, 6, 8, 10, and 12h intervals. Blood samples were analyzed for blood glucose levels by GOD/POD method[9] using commercial glucose kits (Span diagnostics) insulin levels were estimated by using ELISA kits.

2.2 Study in diabetic rats: Diabetes was induced by the administration of alloxan monohydrate in two doses 100 mg and 50mg/ kg body weight intraperitoneal for two consecutive days.[10] A group of 6 rats with blood glucose levels above 250 mg/dL was selected for the study. The study similar to the one conducted in normal rats was repeated in diabetic group.

2.3 Data and Statistical Analysis: Data was expressed as mean ± standard error of mean (SEM). The significance was determined by Two way ANOVA, Bonferroni post test.

3. RESULTS AND DISCUSSION

Thiazolidinediones are frequently used antidiabetic agents alone and in combination with other oral hypoglycemic agents. Pioglitazone belongs to a different chemical class and has a different pharmacological action than the sulfonylureas, metformin, and α-glucosidase inhibitors. Pioglitazone acts as agonist for peroxisome proliferator- activated receptor- gamma (PPARγ). These PPAR receptors are abundantly present in adipose tissue, skeletal muscle, and liver tissues to potentiate insulin action.[11] In type 2 diabetics showed lower blood glucose concentrations, lower plasma insulin levels and decreased insulin resistance with pioglitazone. In addition to its improved insulin sensitivity also showed increased, insulin secretory responses in type 2 diabetics.[12] Pioglitazone is mainly metabolized by CYP2C8, 3A4, and 2C9 in humans and produce both active and inactive metabolites.[13] Most of the antidiabetic drugs and in combinations may cause an increment in its plasma levels thus raise safety concerns. Many reports proven that Aloe barbadensis has been shown to produce a greater anti-hyperglycaemic effect of pioglitazone, Astragalus showed reduced Cmax and increased final velocity (V/F) of pioglitazone[14]; Andrographis paniculata has been shown to inhibit CYP2C19 activity of pioglitazone.[15]

In the present was conducted to know the safety of Pioglitazone in concomitant administration of *Azadirachta indica*. The Pharmacodynamic effect Pioglitazone was tested with aqueous extract of *Azadirachta indica* (AEAI) in normal rats and diabetic rats.

In normal rats, Pioglitazone and AEAI when administered alone produced significant decrease in blood glucose level in a dose dependent manner (fig 1 and fig 2). A dose of 200mg/kg bd.wt found to have optimal reduction in blood glucose levels (about 30%). The insulin levels were found to be enhanced at peak reduction of blood glucose with AEAI at 4hr compared to 0hr (tab 1). In combination, the selected dose of AEAI found to enhance the hypoglycemic activity produced by Pioglitazone with single dose and multiple dose treatments (fig 3). The insulin levels at peak reduction in blood glucose levels were also found to be altered with single and multiple dose treatments (tab 2). This indicates there is an existence of pharmacodynamic interaction between AEAI and Pioglitazone in normal rats. The Pharmacodynamic interaction may be due to their synergistic hypoglycemic effect or due to inhibition of metabolism of ioglitazone as *Azadirachta indica* reported to have inhibitory activity on CYP 3A4.[16]

Alloxan monohydrate is one of the most commonly used inducing agent of diabetes. It acts by inhibiting the oxidative phosphorylation in the beta cells is the primary cause of diabetogenic action of alloxan.[17] Alloxan acts by interfering with some essential enzyme/ enzymes of beta cells; which are also present in liver and kidneys in a specific concentration.

A dose of 200mg/kg bd.wt found to have 28% reduction in blood glucose levels. The insulin levels were found to be enhanced at peak reduction of blood glucose with AEAI at 3hr compared to 0hr. In combination, the selected dose of AEAI found to enhance the antihyperglycemic activity produced by Pioglitazone with single dose and multiple dose treatments (tab 3 & fig 4). *Azadirachta indica* decreases the metabolism of pioglitazone by inhibiting the CYP3A activity in non-diabetic rats and increases its bioavailability. However, the CYP3A content and its activity in liver and intestinal tissues are low in diabetic condition.[18] The insulin levels at peak reduction in blood glucose levels were also found to be altered with single and multiple dose treatments (tab 4). This indicates there is an existence of pharmacodynamic interaction between AEAI and Pioglitazone in diabetic rats.[19] Moreover, type 2 diabetes mellitus is associated with polymorphism of CYP3A4.[20] *Azadirachta indica* produced antagonistic interaction with the glimepiride and glibenclamide upon co-administration.[8] Considering that sulfonylureas have high affinity for proteins coupled with the abundance of tripeptide proteins in *Azadirachta indica*.[21]

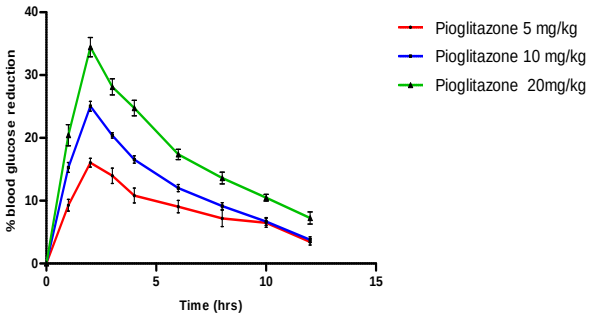

Figure. 1: Comparison of % blood glucose reduction of pioglitazone (5mg/kg, 10mg/kg and 20mg/kg).

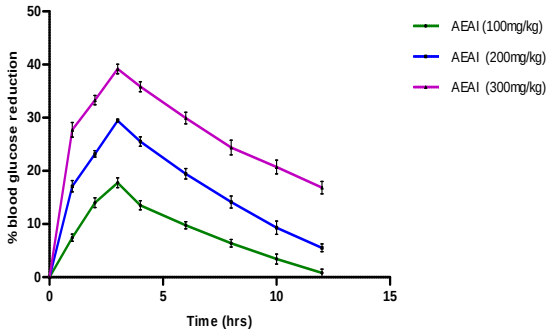

Figure. 2: Comparison of % blood glucose reduction of AEAI (100mg/kg, 200mg/kg and 300mg/kg).

Table 1: Comparison of blood glucose of Pioglitazone 10mg/kg, AEAI (200mg/kg) and single and multiple dose Combinations.

Time (Hrs)	Blood glucose (mg/dL)			
	Pioglitazone (10mg/kg)	AEAI (200mg/kg)	Combination (SD)	Combination (MD)
0	82.17±1.18	84.00±2.17	76.83±1.40 ^{ns}	80.83±2.12 ^{ns}
1	69.50±0.62	71.33±1.97	65.83±1.43 ^{ns}	63.67±1.01 ^{ns}
2	**60.67±0.83**	66.83±1.95	**52.00±1.30***	**51.33±1.12***
3	64.50±0.68	63.33±1.99	54.50±1.29*	55.33±1.12*
4	67.50±0.62	**62.83±2.16**	57.83±1.21*	58.00±1.23*
6	70.33±0.92	66.50±1.78	60.50±1.22*	61.17±1.53*
8	73.50±1.01	70.00±1.62	63.33±1.15*	64.17±1.73*
10	76.17±1.04	73.67±1.62	66.00±0.89*	67.33±1.62*
12	79.67±1.01	77.50±2.07	70.50±0.79*	71.50±1.89*

$p>0.05$ ^{ns,} $p<0.001$* Significance followed by two way ANOVA followed by Bonferroni post test when compared with Pioglitazone (10mg/kg) group.

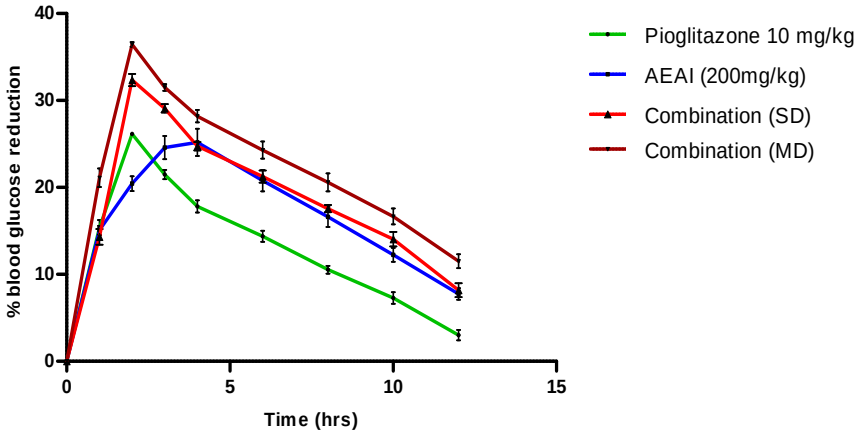

Figure 3: Effect of Pioglitazone, *Azadirachta indica* and their combinations (single dose and multiple dose) on percent blood glucose levels in normal rats.

Table. 2: Mean Serum insulin (µIU/mL) with mean serum glucose level (mg/dL) in Pioglitazone, AEAI and single and multiple dose treatment AEAI +Pioglitazone.

Groups	Time (hrs)	Mean serum glucose levels (mg/dL)	Serum Insulin (µIU/mL)
Pioglitazone	0hr	82.17 ±1.18	9.18±0.02
	2hr	60.67± 0.83	11.26±0.03
AEAI	4hr	62.83± 2.16	10.77±0.04
AEAI + Pioglitazone (SD)	2hr	52.00± 1.30	11.93±0.04
AEAI + Pioglitazone (MD)	2hr	51.33 ±1.12	12.15±0.03

Table. 3: Comparison of blood glucose of Pioglitazone 10mg/kg, AEAI (200mg/kg) and single and multiple dose Combinations in alloxan induced diabetic rats.

Time (Hrs)	Blood glucose (mg/dL)			
	Pioglitazone (10mg/kg)	**AEAI (200mg/kg)**	**Combination (SD)**	**Combination (MD)**
0	259.50±5.58	271.67±5.37	261.50±6.45 ^{ns}	258.33±6.88 ^{ns}
1	195.83±6.17	216.67±4.99	173.83±3.10***	163.17±4.42***
2	**160.83±4.94**	206.17±5.20	**150.83±3.58****	**141.67±4.34*****
3	175.83±5.86	193.83±5.89	165.67±5.37**	158.17±5.06***
4	189.33±5.38	**188.83±5.25**	180.83±4.85*	172.67±5.27***
6	199.83±4.67	201.83±5.34	192.83±5.06 ^{ns}	186.17±6.46***
8	213.00±5.46	212.50±4.89	204.17±5.44*	198.17±5.84***
10	220.17±4.82	222.83±5.77	218.17±5.40 ^{ns}	214.17±6.36 ^{ns}
12	229.00±4.87	231.17±5.66	226.17±3.58 ^{ns}	224.83±4.05 ^{ns}

$p>0.05$ ^{ns,} $p<0.05$*, $p<0.01$**, $p<0.001$*** Significance followed by two way ANOVA followed by Bonferroni post test when compared with Pioglitazone (10mg/kg) group.

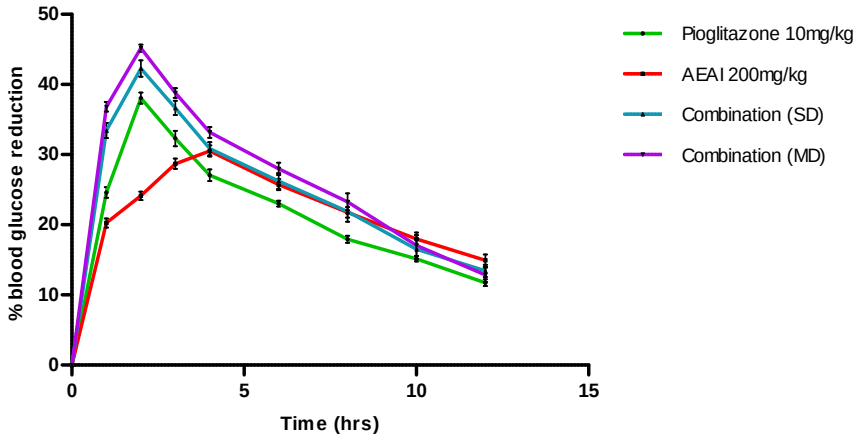

Figure. 4: Comparison of % blood glucose reduction of Pioglitazone 10mg/kg, AEAI (200mg/kg) and single and multiple dose Combinations in alloxan induced diabetic rats.

Table. 4: Mean Serum insulin (µIU/mL) with mean serum glucose level (mg/dL) in Pioglitazone, AEAI and single and multiple dose treatment AEAI +Pioglitazone.

Groups	Time (hrs)	Mean serum glucose levels (mg/dL)	Serum Insulin (µIU/mL)
Pioglitazone	0hr	259.50 ±5.58	6.36±0.02
	3hr	175.83± 5.86	7.20±0.03
AEAI	3hr	193.83 ±5.89	6.67±0.03
AEAI + Pioglitazone (SD)	2hr	150.83± 3.58	7.76±0.02
AEAI + Pioglitazone (MD)	2hr	141.67 ±4.34	7.84±0.02

CONCLUSIONS

The combined use of Azadirachta *indica* and pioglitazone significantly produce synergistic hypoglycemia and antihyperglycemia. Hence care must be taken while prescribing the combination in clinical situation.

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