



Effect of *Allium sativum* on the Pharmacodynamics of Pioglitazone in Normal and Alloxan Induced Diabetic Rats

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ABSTRACT

The present study was carried out to study the herb drug interactions between the traditional medicine *Allium sativum* with conventional antidiabetic drug Pioglitazone in normal and diabetic conditions. 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. The Pioglitazone and *Allium sativum* showed dose dependent reduction of blood glucose levels. The optimum blood glucose reduction was obtained at 10mg/kg of pioglitazone and 200mg/kg of *Allium sativum* in normal rats. The blood glucose levels were found to be decreased and insulin levels were increased significantly in both single and multiple dose combinations of Pioglitazone and *Allium sativum* both in normal rats (4hr) as well as in Alloxan induced diabetic rats (3hr) compared with the pioglitazone alone. Since the combination of *Allium sativum* and pioglitazone producing the significant synergistic action in producing hypoglycemia and anti hyperglycemia, care must be taken while prescribing the combination in clinical situation.

Keywords: Pioglitazone, *Allium sativum*, Blood Glucose.

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INTRODUCTION

To meet the primary healthcare needs many of the people across the globe rely upon the traditional systems of medicines, might be due to the growing use of herbal based drugs and its formulation in medical and dietary products. But, care to be taken while using herbal drugs with conventional medicine. Herbal–drug interactions can be characterized as either Pharmacodynamic (PD) or pharmacokinetic (PK) in nature. PD interactions may occur when constituents of herbal products have either synergistic or antagonist activity in relation to a conventional drug (1). There is evidence that the herb drug interactions affect the actual pharmacological property of intended drug by altering its pharmacological activity both in *in vitro* and *in vivo* studies (2). In the course of literature survey; it has been found that a large number of herbs with hypoglycemic activity found to enhance the hypoglycemic activity of co administer antidiabetic drugs by their synergistic effect and also by inhibiting the metabolic enzymes that are involved in the metabolism of co administered antidiabetic drugs in the liver (3).

Now-a-days the treatment with antidiabetic drug therapy becoming very complex and the recommended treatment of combination drug therapy have increased the risk of pharmacokinetic interactions in diabetic patients (4). Although the risk of hypoglycemia with Thiazolidinediones appears negligible but drug interactions may exacerbate adverse effects and safety problems (5). The pharmacokinetic studies indicate about 80% oral bioavailability of pioglitazone; it is metabolized by multiple cytochrome P450 (CYP) isoenzymes, mainly by CYP2C8, CYP3A4 and CYP2C9 to several active and inactive metabolites (6). Pioglitazone is highly bound to plasma proteins, mainly albumin. The protein binding of Pioglitazone was >99%.

It is found that a large number of Indian medicinal plants have been possesses hypoglycemic activity. Among the great number of traditional plants used for diabetes, only a small number of these received scientific evaluation. Garlic, known as *Allium Sativum*, belongs to family Liliaceae and used for food and spice at an ancient time by a different culture. Many authors reported the hypoglycemic and anti hyperglycemic activity of *Allium sativum* (7, 8). Many of the clinical studies also evidenced that the garlic showed significant lipid lowering agent and regulator for high glucose raise (9,10,11).

Hence, in the present study was designed to find out the safety and efficacy of *Allium sativum* when co administered with Pioglitazone both in normal and diabetic rats.

MATERIALS AND METHOD

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 *Allium sativum* obtained from Laila Impex Pvt Ltd., Vijayawada as gift sample.

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 *Allium sativum*, orally after a wash out period of one week. The same group was also administered with 200 mg/ kg body weight *Allium sativum* 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 (12) using commercial glucose kits (Span diagnostics) insulin levels were estimated by using ELISA kits.

Study in diabetic rats

Diabetes was induced by the administration of alloxan monohydrate in two doses 100 mg and 50mg/ kg body weight intra peritoneal for two consecutive days (13). 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.

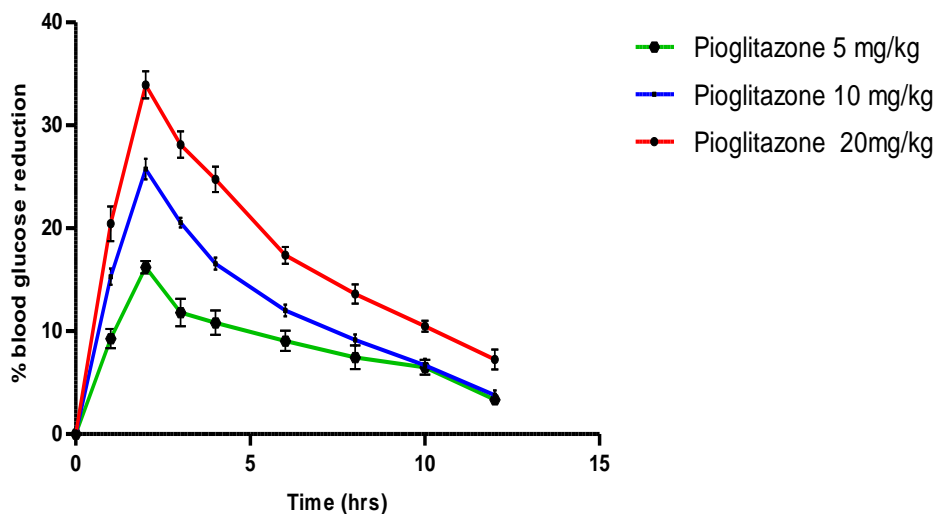
Data and Statistical Analysis:

Data was expressed as mean \pm standard error of mean (SEM). The significance was determined by Two way ANOVA, Bonferroni post test.

RESULTS AND DISCUSSION:

Type 2 diabetes requires lifelong treatment with drugs, diet control and exercise. The type 2 diabetes proceeds through the improvement of insulin sensitivity by lowering insulin resistance in muscle and fat. Thiazolidinediones are generally prescribed in a condition where other medicines have failed to lower blood sugar levels within desired levels. Pioglitazone is a drug of choice when patient requires longer duration of antidiabetic drug therapy with safe and effective treatment in type 2 diabetics (14). These medicines sometimes lower triglycerides and raise HDL cholesterol levels (15). Although the risk of hypoglycemia with thiazolidinediones appears negligible but drug interactions may exacerbate adverse effects and safety problems in some conditions (5).

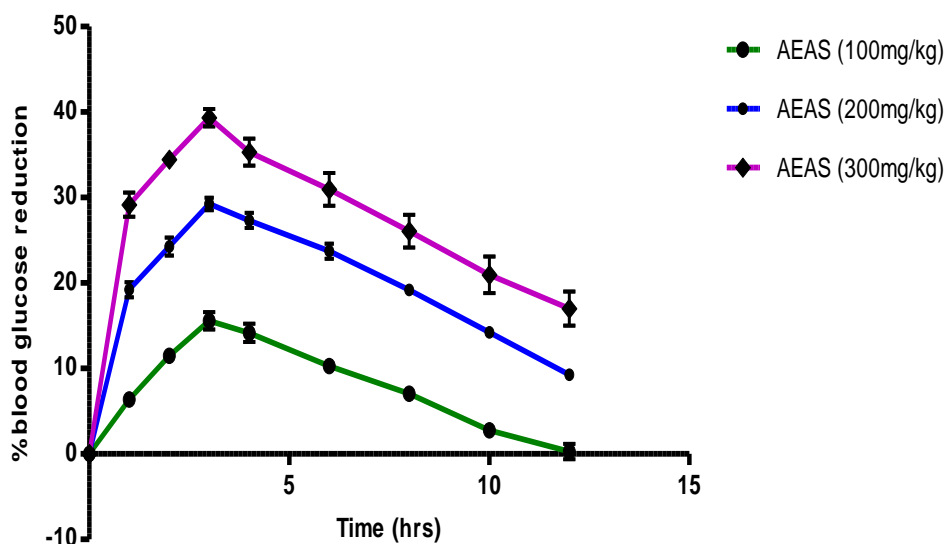
The normal rats were selected for preliminary and quick screening of the drugs and small volumes of blood were collected at regular time intervals for the estimation of blood glucose levels. Dose dependent relationship was observed with 5mg/kg (16.22 ± 0.64), 10 mg/Kg (29.75 ± 1.09), and 20mg/Kg (33.92 ± 1.45) body weight of Pioglitazone in normal rats (graph 1). From these three doses 10 mg/Kg of Pioglitazone was selected for interaction study as it produced optimum blood glucose reduction which is about 25-30% (graph 1).



Graph 1: Comparison of % blood glucose reduction of Pioglitazone 5mg/kg, 10mg/kg and 20mg/kg.

Pioglitazone shown to have significant effect on the glycemic control or the percentage reduction of glucose levels in normal rats and the maximum percentage glucose reduction with Pioglitazone was obtained at 2 hrs in normal rats.

In normal rats, *Allium sativum* (AEAS) when administered alone produced significant decrease in blood glucose level in a dose dependent manner. At doses of 100mg/kg produced 17.76 ± 1.00 ; 200mg/kg showed 28.43 ± 0.32 and 300mg/kg showed 39.18 ± 0.98 percent blood glucose reduction at 3hr (graph 2).



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

A dose of 200mg/kg bd.wt found to have optimal reduction in blood glucose levels (near 30%). The combination of Pioglitazone and *Allium sativum* combinations showed significant synergistic hypoglycemic activity both in single dose and multiple dose treatments 42.24 ± 1.00 and 45.39 ± 0.61 percent blood glucose reduction respectively during 4hr (table 1).

Table 1: Comparison of % blood glucose reduction of Pioglitazone 10mg/kg, AEAS (200mg/kg) and single and multiple dose Combinations in normal rats

| Time (Hrs) | % blood glucose reduction | | | |
|------------|---------------------------|-------------------|-------------------------|-------------------------|
| | Pioglitazone (10mg/kg) | AEAS (200mg/kg) | Combination (SD) | Combination (SD) |
| 0 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 ^{ns} | 0.00±0.00 ^{ns} |
| 1 | 16.39±0.68 | 16.55±0.76 | 21.92±0.78*** | 24.45±0.69*** |
| 2 | 29.75±1.09 | 21.80±0.92 | 33.76±1.05*** | 36.97±0.62*** |
| 3 | 22.77±0.74 | 28.43±0.32 | 39.58±0.87*** | 42.08±0.66*** |
| 4 | 19.57±0.62 | 26.75±0.91 | 42.24±1.00*** | 45.39±0.61*** |
| 6 | 16.38±0.68 | 21.88±0.82 | 38.77±1.21*** | 40.37±0.63*** |
| 8 | 12.40±0.39 | 17.31±0.71 | 34.31±1.03*** | 36.76±0.66*** |
| 10 | 9.39±0.56 | 12.18±0.65 | 30.43±0.89*** | 33.88±0.76*** |
| 12 | 4.61±0.42 | 7.46±0.43 | 26.56±0.88*** | 30.23±1.09*** |

$p > 0.05$ ^{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.

Table 2: Mean Serum insulin (μ IU/mL) with mean serum glucose level (mg/dL) in Pioglitazone, AEAS and single and multiple dose treatment AEAS +Pioglitazone in normal rats.

| Groups | Time (hrs) | Mean serum glucose levels (mg/dL) | Serum Insulin (μ IU/mL) |
|--------------------------|------------|-----------------------------------|------------------------------|
| Pioglitazone | 0hr | 83.33 \pm 0.88 | 8.82 \pm 0.05 |
| | 2hr | 60.67 \pm 0.54 | 10.72 \pm 0.04 |
| AEAS | 3hr | 61.67 \pm 1.81 | 10.52 \pm 0.03 |
| AEAS + Pioglitazone (SD) | 4hr | 49.67 \pm 1.08 | 12.29 \pm 0.04 |
| AEAS + Pioglitazone (MD) | 4hr | 47.83 \pm 1.93 | 12.68 \pm 0.03 |

In combination, the selected dose of AEAS found to enhance the hypoglycemic activity produced by Pioglitazone with single dose and multiple dose treatments. The insulin levels were found to be enhanced at peak reduction of blood glucose with AEAS at 3hr (10.52 \pm 0.03) compared to 0hr (8.82 \pm 0.05). The insulin levels at peak reduction in blood glucose levels were also found to be altered with single dose (12.29 \pm 0.04) and multiple dose (12.29 \pm 0.04) treatments. This indicates there is an existence of pharmacodynamic interaction between AEAS and Pioglitazone in normal rats. The Pharmacodynamic interaction may be due to their synergistic hypoglycemic effect or due to inhibition of metabolism of Pioglitazone as *Allium sativum* reported to have inhibitory activity on CYP 2C9, CYP 3A4 and CYP 2C19 (16). Poonam T., also reported that the synergic activity of *Allium sativum* with widely prescribed antidiabetic drug Glibenclamide (17).

There is a controversy regarding the type of diabetes produced by alloxan and some reports indicate it to be IDDM model (type I) (18). But many articles mentioned it to be type II model including the recent review (19). Our results with the dosage schedule of alloxan employed, produced diabetes in rats, which responded in a dose dependent manner to antidiabetic drugs indicating that alloxan produced type II diabetes. Further it was observed that the rats returned back to normal from diabetic condition in 2-3 months. Majority of the articles in the literature mentioned that the diabetes with alloxan in rats was reversible (20) supporting our results. If it were producing type I, reversal back to normal was not possible. Hence alloxan may produce type I or type II depending on the dose and dosage schedule. Our results support the precipitation of type-II with the dose administered.

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

| Time (Hrs) | % blood glucose reduction | | | |
|------------|---------------------------|-------------------|--------------------------|--------------------------|
| | Pioglitazone (10mg/kg) | AEAS (200mg/kg) | Combination (SD) | Combination (MD) |
| 0 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 ^{ns} | 0.00±0.00 ^{ns} |
| 1 | 26.09±0.92 | 20.45±0.85 | 26.13±1.39 ^{ns} | 28.19±1.15 ^{ns} |
| 2 | 38.76±1.05 | 25.11±0.71 | 36.51±1.10 ^{ns} | 38.96±0.96 ^{ns} |
| 3 | 32.27±0.78 | 30.52±0.63 | 44.83±0.75*** | 47.08±1.14*** |
| 4 | 27.02±0.66 | 26.77±0.79 | 38.54±1.30*** | 41.11±0.95*** |
| 6 | 22.44±0.54 | 21.68±0.64 | 31.22±1.15*** | 34.27±0.95*** |
| 8 | 18.29±0.40 | 19.56±0.79 | 25.47±1.76*** | 28.88±1.25*** |
| 10 | 14.72±0.40 | 17.14±0.88 | 19.48±1.54** | 22.88±0.91*** |
| 12 | 10.92±0.42 | 13.99±1.23 | 15.20±1.32** | 19.48±0.63*** |

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.

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

| Groups | Time (hrs) | Mean serum glucose levels (mg/dL) | Serum Insulin (µIU/mL) |
|--------------------------|------------|-----------------------------------|------------------------|
| Pioglitazone | 0hr | 253.50 ±4.90 | 6.49±0.03 |
| | 2hr | 155.33± 4.82 | 7.76±0.03 |
| AEAS | 3hr | 191.67± 2.96 | 7.05±0.04 |
| AEAS + Pioglitazone (SD) | 3hr | 147.00± 4.88 | 8.14±0.03 |
| AEAS + Pioglitazone (MD) | 3hr | 142.00 ±6.50 | 8.18±0.03 |

The Pioglitazone (10mg/kg) showed 38.76±1.05 percent blood glucose reduction at 2 hr and a dose of 200mg/kg Pioglitazone showed 30.52±0.63 percent reduction of blood glucose levels at 3hr. The single and multiple dose combinations of both Pioglitazone and AEAS showed significant reduction of 44.83±0.75 and 47.08±1.14 during 3hr respectively (table 3). The insulin levels were found to be enhanced at peak reduction of blood glucose with AEAS at 3hr (7.05±0.04) compared to 0hr (6.49±0.03). In combination, the selected dose of AEAS found to enhance the anti hyperglycemic activity produced by Pioglitazone with single dose (8.14±0.03) and multiple dose (8.18±0.03) treatments. The insulin levels at peak reduction in blood glucose levels were also found to be altered with single and multiple dose treatments. This indicates there is an existence of pharmacodynamic interaction between AEAS and Pioglitazone in diabetic rats. The Pharmacodynamic interaction may be due to their synergistic anti hyperglycemic effect or due to inhibition

of metabolism of Pioglitazone as *Allium sativum* reported to have inhibitory activity on CYP 2C9, CYP 3A4 and CYP 2C19 (John S., 2003).

CONCLUSIONS:

Since the combination of *Allium sativum* and pioglitazone producing the significant synergistic action in producing hypoglycemia and anti hyperglycemia care must be taken while prescribing the combination in clinical situation.

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