**PHARMACODYNAMIC INTERACTIONS OF PIOGLITAZONE
WITH ATORVASTATIN AND FENOFIBRATE IN RATS**

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ABSTRACT

The present study suggest that Atorvastatin and Fenofibrate decrease the hypoglycemic activity of Pioglitazone by decreasing the bioavailability of Pioglitazone and when compared to Atorvastatin, Fenofibrate in combination with Pioglitazone shows a greater decrease in the hypoglycemic activity of Pioglitazone. The results are statistically significant. Even it may be required to monitor the

blood glucose levels as a precautionary measure, so as to avoid the complications of severe hypoglycemia. Our studies in normal and diabetic rats suggested that drug interaction occurs between Atorvastatin, Pioglitazone and Fenofibrate when they are used concurrently in normal and diseased conditions in rats.

KEYWORDS: Pharmacodynamic, diabetic, Pioglitazone, Atorvastatin, Fenofibrate.

INTRODUCTION

An interaction is said to occur when the effects of one drug are changed by the presence of another drug, herbal medicine, food, drink or environmental chemical agent. The outcome can be harmful if the interaction causes an increase in the toxicity of the drug. For example, there is a considerable increase in risk of severe muscle damage if patients on statins start

taking azole antifungal. It is difficult to estimate the incidence of drug interactions, because published studies have frequently used different criteria for definition, particularly in distinguishing between clinically significant and non-significant interactions. The more drugs a patient takes the greater the likelihood of an adverse reaction will occur. One hospital study found that the rate was 7% in those taking 6 to 10 drugs but 40% in those taking 16 to 20 drugs, which represents a disproportionate increase.

Pharmacokinetic drug–drug interactions (DDIs) are unfavorable clinical events, which are caused by abnormally increased or decreased drug concentrations in the body as a consequence of co-administration of other drug(s)^[1,2] and sometimes its metabolites at the effective sites within the body. The relationship between drug administration and response is divided in to two phases. Pharmacokinetic phase, which related to the body's effect on the drug and. Pharmacodynamic phase, which related to the drug effect on the body.^[3] Patients often receive multiple medications therapy simultaneously, in diseases such as Diabetes, Cancer and AIDS etc, which demand the combination therapy, which works better than an individual drug alone. In other cases, the patient is suffering from several conditions, each of which is being treated with one or more drugs, in this situation there is many potential sites for interaction that exist within the body. An interaction may occur between them by either altered Pharmacokinetics or Pharmacodynamic of one drug by another.^[4]

An interaction is said to occur when the effects of one drug are changed by the presence of another drug, herbal medicine, food, drink or environmental chemical agent. The outcome can be harmful if the interaction causes an increase in the toxicity of the drug.^[5] For example, there is a considerable increase in risk of severe muscle damage if patients on statins start taking azole antifungal. It is difficult to estimate the incidence of drug interactions, because published studies have frequently used different criteria for definition, particularly in distinguishing between clinically significant and non-significant interactions. The more drugs a patient takes the greater the likelihood of an adverse reaction will occur. One hospital study found that the rate was 7% in those taking 6 to 10 drugs but 40% in those taking 16 to 20 drugs, which represents a disproportionate increase.^[6,7,8]

Pharmacokinetic interactions are those that can affect the processes by which drugs are absorbed, distributed, metabolized and excreted the so called ADME interactions. There are marked inter-individual variability's, although these interactions may be expected but there extent cannot be easily predicted.^[9] Such interactions may result in a change in the drug

concentration at the site of action with subsequent decreased efficacy or toxicity. For e.g. Simultaneous usage of warfarin and phenylbutazone may result in the severe hemorrhage due to displacement of warfarin from the plasma protein. This is a harmful pharmacokinetic type of drug-drug interaction. Cimetidine potentiates the effects of sulfonylurea's due to pharmacokinetic type of drug-drug interaction.^[10,11]

Drug absorption is the movement of the drug from its site of administration into the bloodstream. Absorption interactions are changes in a drug's effects caused by food, drink, or medications taken concurrently.^[12] Most of the drugs are given orally and they are observed through the mucous membranes of the gastrointestinal tract and the majority of interactions that occurs within the gut due to reduced absorption rather than increased and it involves by any one of the following mechanisms.^[13] Drug food interaction can affect the total amount of drug absorbed (bioavailability), but most often they only slow absorption. For example, the hypoglycemic effect of glipizide may be delayed slightly if taken with a meal versus 30–60 minutes before a meal, although hemoglobin A1c (A1C) values are unaffected⁶ In addition, components of food may interact. For example, vitamin K intake from green leafy vegetables interacts with warfarin.^[14, 15]

A drug, which is metabolized by a particular iso-enzyme, is a substrate for that enzyme. A drug can be a substrate for several different iso-enzymes or an active metabolite can be a substrate for a different iso-enzyme to the parent drug.^[16, 17]

An inducer is a drug that causes increased activity of a CYP iso-enzyme by causing increased synthesis and therefore an increased amount of the induced enzyme. The metabolic capacity of the iso-enzyme is therefore increased.^[18] The enzyme-inducing agent increases the velocity of the drug metabolic reaction. The process of enzyme induction requires new protein synthesis, so its maximum effect is not reached for 2-3 weeks after starting the enzyme-inducer likewise, the effect may take some weeks to wear off when the enzyme-inducer is stopped. Rifampicin is such a potent enzyme inducer that significant induction occurs in just a few days and takes several weeks to wear off.^[19, 20]

The mechanisms of CYP inhibition can be roughly divided into 2 groups: reversible inhibition and irreversible inhibition, with the former being probably the more common mechanism.^[21] Reversible inhibition can be divided, on a kinetic basis, into competitive, noncompetitive, and uncompetitive inhibition. In competitive inhibition, the inhibitor

competes with the substrate for the same binding site within a CYP enzyme. In noncompetitive inhibition, the inhibitor binds to the same enzyme as does the substrate, but the binding site differs. In uncompetitive inhibition, the inhibitor binds only to an enzyme that forms a complex with the substrate.^[22]

MATERIALS AND METHODS

Induction of Diabetes in experimental rats

Alloxan induced diabetic rats

Rats of either sex weight range 150-250 gm were selected and fasted for 14 hours and water *ad-libitum*. The animals were randomly distributed into different groups. The animals were kept in colony cages at ambient temperature of $28^{\circ} \pm 2^{\circ}$ C and 45 to 55% relative humidity with a 12 hour light/dark cycle. The rats were administered with 120 mg/kg of alloxan intraperitoneally^{4, 5}. After 24 hours, the blood samples were collected and analysed for blood glucose level. It was found that diabetes was induced in about 24 hours. In our experiment, the diabetes was characterised by weight loss and hyperglycaemia. The blood samples were collected and stabilized for three more days. Those animals showing blood glucose levels more than 200 mg/dl were used for antidiabetic study.

EXPERIMENTAL PROTOCOL

Inbred wistar albino rats weighing 160 to 220 g were divided into 8 groups containing 5 rats each. The first set of 4 group's of animals was used for Normal studies and second set of 4 group's of animals was used for Diabetic studies.

Normal rats

The Normal rats were subdivided into four groups as follows; group 1st (control) given vehicle (1% W/V CMC); group 2nd rats given pioglitazone (10mg kg⁻¹, orally in 1% W/V CMC); The treatment was given for seven days. To the 3rd group of normal rats pioglitazone was administered and 30 min later atorvastatin was administered. Later from second day onwards they were treated daily with atorvastatin for six days. During this period the animals had free access to food and water. After 18 h fast on seventh day they were again given the combined treatment with Pioglitazone and 30 min later atorvastatin. To the 4th group of normal rats pioglitazone was administered 30 min later fenofibrate was administered later from second day onwards they were daily treated with fenofibrate for six days. After 18 h fast on seventh day they were again given the combined treatment with Pioglitazone and 30 min later atorvastatin. In all the groups, the blood sample was collected after 1 h drug treatment.

Diabetic rats

The diabetic rats were subdivided into three groups as follows; group 5th (diabetic control) given vehicle (1% W/V CMC); group 6th diabetic rats given pioglitazone (10 mg kg⁻¹, orally in 1% W/V CMC); The treatment was given for seven days. To the 7th group of diabetic rats pioglitazone was administered and 30 min later atorvastatin was administered. Later from second day onwards they were treated daily with atorvastatin for six days. During this period the animals had free access to food and water. After 18 h fast on seventh day they were again given the combined treatment with Pioglitazone and 30 min later atorvastatin. To the 8th group of diabetic rats pioglitazone was administered and 30 min later fenofibrate was administered. Later from second day onwards they were treated daily with fenofibrate for six days. During this period the animals had free access to food and water. After 18 h fast on seventh day they were again given the combined treatment with Pioglitazone and 30 min later fenofibrate. In all the groups, the blood sample was collected after 1 h drug treatment. The plasma was isolated by centrifugation of blood for 10 min at 3000 rpm and subjected to glucose (Trinder, 1969) estimation.

GROUPING OF ANIMALS

The animal were divided into eight groups of 5 animals, each group separation was as follows.

Table: Grouping of animals.

Animals	Groups	Treatment	Dosage & route
Normal Rats	Group I	VEHICLE	Normal rats were treated with vehicle (1% W/V CMC) by oral route.
	Group II	PIOGLITAZONE	Normal rats were treated with pioglitazone (10 mg kg ⁻¹ , orally in 1% W/V CMC) by oral route.
	Group III	PIO+ATR	Normal rats were treated with pioglitazone and 30 min later atorvastatin was administered by oral route.
	Group IV	PIO+FEN	Normal rats were treated with pioglitazone and 30 min later fenofibrate was administered by oral route.
Diabetic Rats	Group V	VEHICLE	Diabetic rats were treated with vehicle (1% W/V CMC) by oral route
	Group VI	PIOGLITAZONE	Diabetic rats were treated with pioglitazone (10 mg kg ⁻¹ , orally in 1% W/V CMC) by oral route.
	Group VII	PIO +ATR	Diabetic rats were treated with pioglitazone and 30 min later atorvastatin was administered by oral route.
	Group VIII	PIO+FEN	Diabetic rats were treated with pioglitazone and 30 min later fenofibrate was administered by oral route.

Mortality and general condition of the animals were observed daily throughout the whole experiment lasting 4 weeks. Body weights were recorded 2 times per week during the treatment and until the end of experiment.

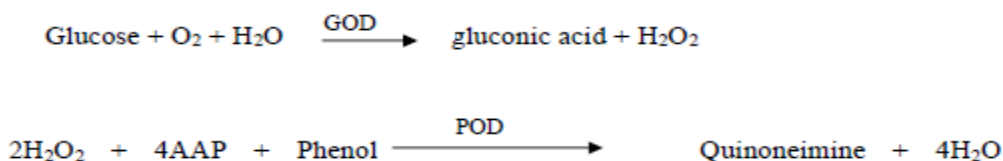
ESTIMATION OF BIOCHEMICAL PARAMETERS.

Estimation of blood glucose by GOD-POD method.

The method is intended for *in vitro* quantitative determination of glucose in serum/ plasma or cerebrospinal fluid and utilizes two enzymes glucose oxidase (GOD) and peroxidase (POD) along with the chromogen L-amino antipyrine and phenol. Older methods like Nelson-Somogyi and end point *o*-toluidine were based on reducing properties of glucose. But these methods do not measure the true glucose because of interferences of other reducing sugars. Subsequently other chemical and enzymatic methods were developed to overcome this problem. The GOD/POD method is one such method developed by Trinder in 1964. This is simple, single stepped, rapid, more reliable and having acceptable precision. Therefore this method is used in our study for glucose estimation in plasma. No interferences were found with creatinine, fructose, galactose, reduced glutathione, ascorbic acid and xylose. Even haemoglobin or bilirubin upto 10 mg % does not affect the test.

Principle

Glucose is oxidized by glucose oxidase (GOD) to produce gluconate and hydrogen peroxide. The hydrogen peroxide is then oxidatively coupled with 4 amino- antipyrine (4-AAP) and phenol. The intensity of the colored complex (quinoneimine) is proportional to the glucose concentration in the sample and can be measured photometrically at 505 nm (500-540nm)



Kit contents

1. Enzyme reagent -- 2 vials
2. Buffer solution – 2 x 500 ml
2. Glucose standard (3mg/dl) -- 2 x 5ml vial

Working reagent Preparation

Dissolve one vial of enzyme reagent in 500ml of buffer solution. Mix immediately.

Reagent storage and stability

1. The reagents are stored at 2 - 8⁰C protected from light.
2. The reagent bottle is closed immediately after use.
3. Avoid contamination of the reagents during use.
4. The reagents are stable for 60 days when stored in dark at 2-8⁰C.

Specimen collection and handling

1. Blood is collected in Eppendroffs tubes containing EDTA (200 IU/ml of blood).
2. Plasma should be separated as early as possible.
3. In separated and non haemolysed plasma, the glucose concentration is generally stable for 3 hours at room temperature and up to 72 hours at 2–8°C.

Procedure

After collection of blood sample into a micro centrifugation tube contains an anticoagulant and it was centrifuged at 3000rpm for 15min then the plasma glucose was estimated by making following dilutions.

Table: Summary of GOD-POD methods working procedure.

	blank	Standard	Test
Working reagent	1.0ml	1.0ml	1.0ml
Glucose standard (3mg/dl)	----	10µl	----
Plasma	----	----	10µl

Mix well. Incubate at 37⁰ C for 10 minutes or 15 minutes at RT. Read absorbance of Standard(S) and Test (T) against Blank (B) at 505 nm.

CALCULATIONS

Glucose concentration in mg/dl = Abs of T/ Abs of S x 3

The final color is stable for 1 hour at R.T (32⁰C)

RESULTS AND DISCUSSION

Oral Glucose Tolerance Test (OGTT): OGTT was carried out to confirm the induction of type 2 diabetes. Rats, after 12 hr of fasting were given orally, a glucose challenge of 2g/Kg body weight. Blood glucose was determined by the above mentioned method at 0, 30, 60,120 and 180 min after glucose challenge. A plot of Blood glucose level versus time obtained was analyzed for impairment in glucose tolerance, to confirm the induction/extent of diabetes.

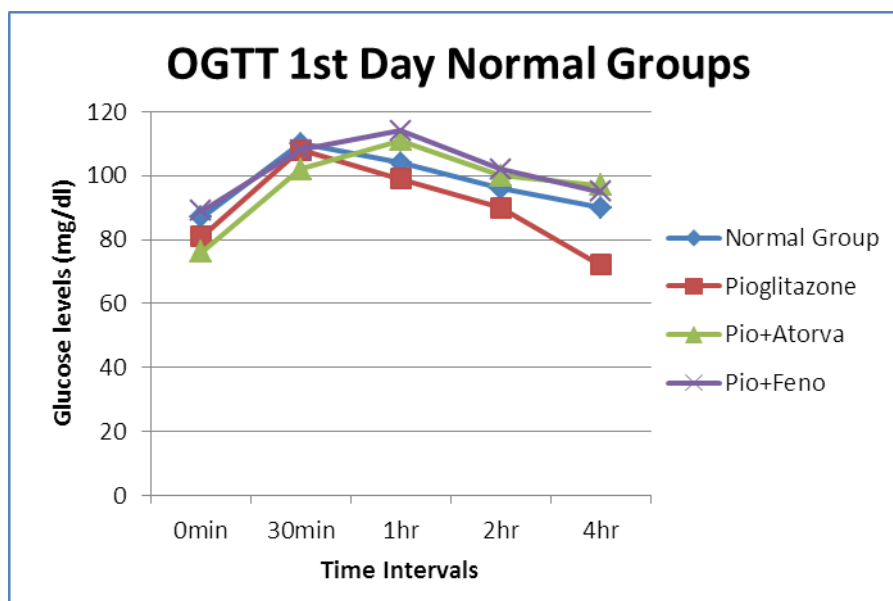
Blood glucose levels (mg/dl) on 1st day.**Table 1: OGTT 1st day of Non Diabetic Rats.**

	Normal Group	Pioglitazone	Pio+Atorva	Pio+Feno
0min	87±1.5	81±1.2	76±1.6	89±1.3
30min	110±1.6	108±2	102±1.2	108±1.2
1hr	104±0.5	99±1.4	111±1	114±2
2hr	96±1	90±0.5	100±1	102±2
4hr	90±2.3*	72±1*	97±2*	95±1.09

Values are expressed as mean ± S.E.M; n=6, *P<0.1 significant, **P<0.01 more significant.

One way ANOVA followed by Dunnet Multiple comparison test using Graph pad INSTANT version 5.

From the above data obtained we consider that the normal group blood glucose levels were compared with Pioglitazone group it is showing positive result i.e. at 0hr the normal glucose level was 87mg/dl and Pioglitazone group showed only 81mg/dl similarly at final interval at 4hr after glucose load the normal group showed 90mg/dl and the Pioglitazone group showed a vast decrease i.e. 72mg/dl. Similarly when we compared the groups like Pioglitazone with Atorvastatin and Pioglitazone with Fenofibrate compared with normal group showed a little variation i.e. 97mg/dl and 95mg/dl which are almost similar with normal group.

**Figure 1: OGTT 1st Day of Non Diabetic Rats Graph-I.**

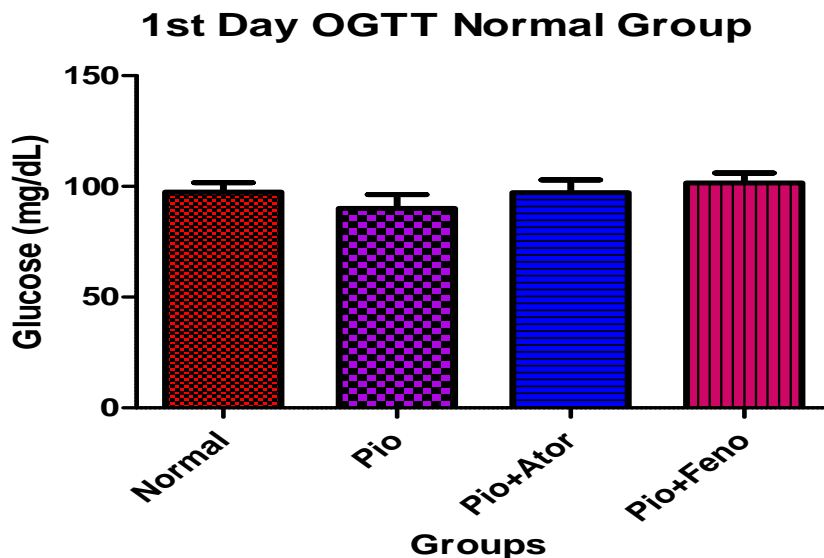


Figure 2: OGTT 1st Day of Non Diabetic Rats Graph-II.

Blood glucose levels (mg/dl) on 7th day.

Table 2: OGTT 7th day of Non Diabetic Rats.

	Normal Group	Pioglitazone	Pio+Atorva	Pio+Feno
0min	82±1	96±1	72±2	87±1.5
30min	98±.5	120±1.5	102±1	107±1
1hr	95±1.3	106±1.3	97±0.5	94±2
2hr	89±1.5	98±1.6	90±1	89±0.6
4hr	78±2*	84±2*	71±2*	87±0.4*

Values are expressed as mean ± S.E.M; n=6, *P<0.1 significant, **P<0.01 more significant.

One way ANOVA followed by Dunnet Multiple comparison test using Graph pad INSTANT version 5.

From the above data obtained we consider that the normal group blood glucose levels were compared with Pioglitazone group it is showing positive result i.e. at 0hr the normal glucose level was 82mg/dl and Pioglitazone group showed only 96mg/dl similarly at final interval at 4hr after glucose load the normal group showed 78mg/dl and the Pioglitazone group showed decrease in blood glucose level i.e. 84mg/dl. Similarly when we compared the groups like Pioglitazone with Atorvastatin and Pioglitazone with Fenofibrate compared with normal group showed a little variation i.e. 88mg/dl and 82mg/dl respectively.

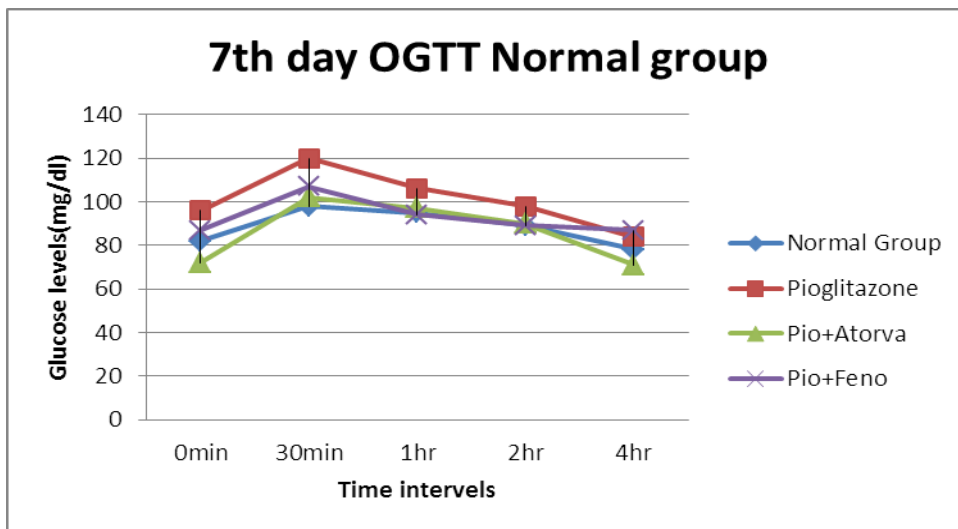


Figure 3: OGTT 7th Day of Non Diabetic Rats Graph-I.

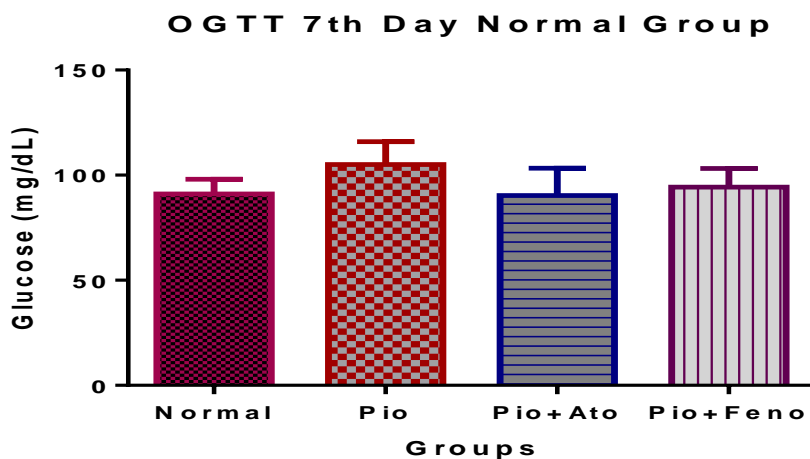


Figure 4: OGTT 7th Day of Non Diabetic Rats Graph-II

Blood glucose levels (mg/dl) on 14th day.

Table 3: OGTT 14th Day of non Diabetic Rats.

	Normal Group	Pioglitazone	Pio+Atorva	Pio+Feno
0min	72±2	79±1.5	68±1.4	82±1.6
30min	99±1.5	105±0.5	92±1.3	102±1.4
1hr	90±0.4	97±0.6	87±0.5	97±1.3
2hr	86±1*	90±1.6	82±0.6*	81±2.4
4hr	70±1.3**	74±1.2*	65±0.4**	80±1.2*

Values are expressed as mean ± S.E.M; n=6, *P<0.1 significant, **P<0.01 more significant.

One way ANOVA followed by Dunnet Multiple comparison test using Graph pad INSTANT version 5.

From the above data obtained we consider that the normal group blood glucose levels were compared with Pioglitazone group it is showing positive result i.e. at 0hr the normal glucose level was 72mg/dl and Pioglitazone group showed 79mg/dl similarly at final interval at 4hr after glucose load the normal group showed 70mg/dl and the Pioglitazone group showed decrease in blood glucose level i.e. 74mg/dl. Similarly the groups like Pioglitazone with Atorvastatin and Pioglitazone with Fenofibrate at 0hr shows 68 and 82mg/dl respectively. Whereas at 4thhr showed a slight decrease in glucose levels i.e. 65 and 81mg/dl respectively.

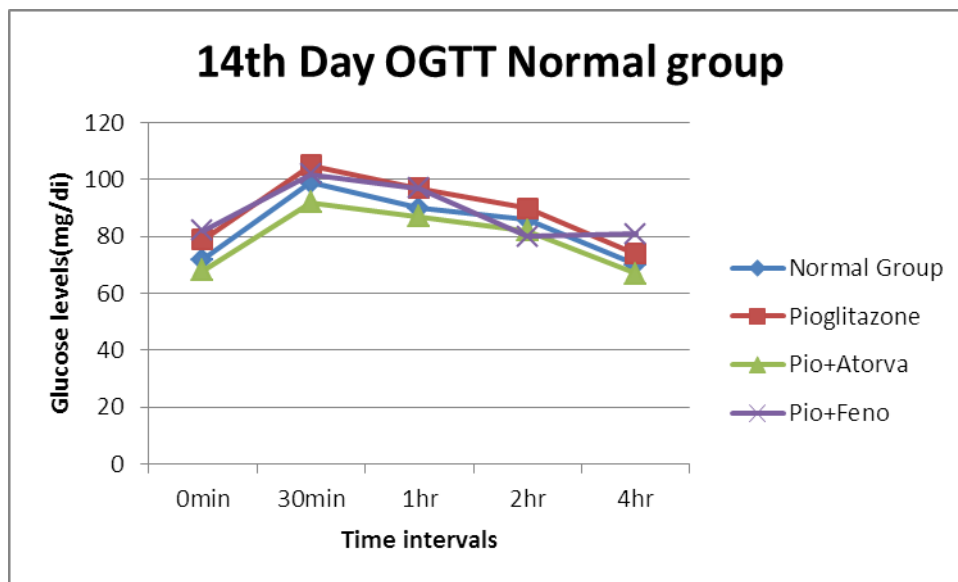


Figure 5: OGTT 14th Day of non Diabetic Rats Graph-I.

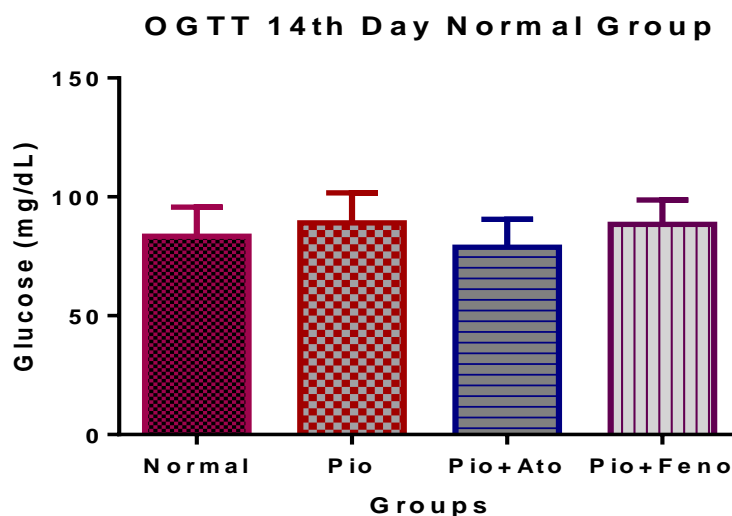


Figure 6: OGTT 14th Day of non Diabetic Rats Graph-II.

Blood glucose levels (mg/dl) on 21st day.

Table 4: OGTT 21st Day of non Diabetic Rats.

	Normal Group	Pioglitazone	Pio+Atorva	Pio+Feno
0min	80±0.5	78±1.3	84±1.5	76±1.3
30min	94±1	102±2	107±2	100±2
1hr	90±2	93±1	101±1.3	96±2
2hr	84±1.6*	82±0.5	95±0.5*	91±1.2
4hr	76±1.3**	74±0.2*	82±2**	75±1*

Values are expressed as mean ± S.E.M; n=6, *P<0.1 significant, **P<0.01 more significant.

One way ANOVA followed by Dunnet Multiple comparison test using Graph pad INSTANT version 5.

From the above data obtained we consider that the normal group blood glucose levels were compared with Pioglitazone group it is showing positive result i.e. at 0hr the normal glucose level was 80mg/dl and pioglitazone group showed 78mg/dl similarly at final interval at 4hr after glucose load the normal group showed 76mg/dl and the pioglitazone group showed decrease in blood glucose level i.e. 74mg/dl. Similarly the groups like pioglitazone with atorvastatin and pioglitazone with fenofibrate at 0hr shows 84mg/dl and 76mg/dl respectively. Whereas at 4thhr showed a slight decrease in glucose levels i.e. 82 and 75mg/dl respectively.

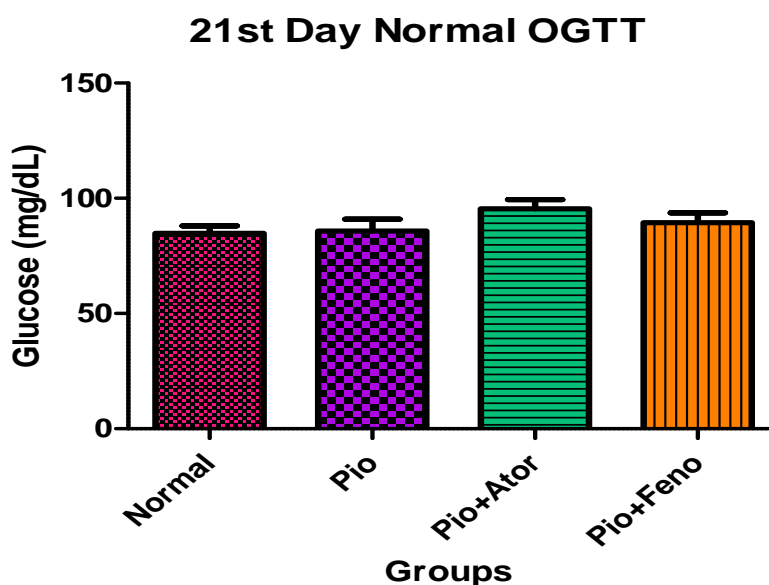


Figure 8: OGTT 21st Day of Diabetic Rats Graph-II.

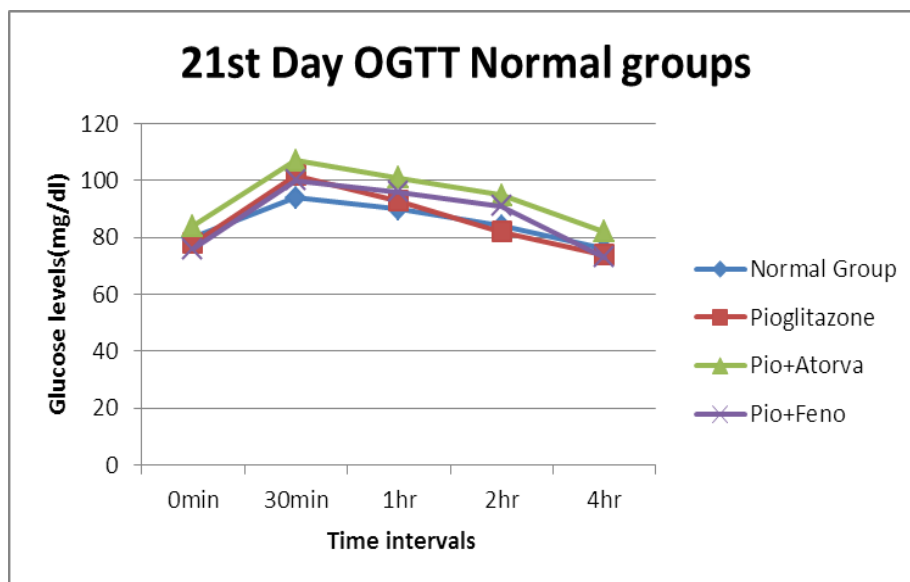


Figure 7: OGTT 21st Day of Non Diabetic Rats Graph-I.

Oral Glucose Tolerance Test (OGTT) on 1st day: Diabetic Rats.

Table 5: OGTT 1st Day of Diabetic Rats.

	Diabetic Control	Pioglitazone	Pio+Atorva	Pio+Feno
0min	267±0.5	272±1.5	259±1.2	278±1
30min	288±0.25	298±0.5	286±2	292±1.5
1hr	296±1.3	282±1	290±1.7	306±1.25
2hr	304±1.2*	277±2	282±1.25*	290±1
4hr	312±2*	266±1*	255±0.5*	277±0.5*

Values are expressed as mean ± S.E.M; n=6, *P<0.1 significant, **P<0.01 more significant.

One way ANOVA followed by Dunnet Multiple comparison test using Graph pad INSTANT version 5.

From the above data obtained we consider that the diabetic control group blood glucose levels were compared with Pioglitazone group it is showing positive result i.e. at 0hr the control group glucose level was 267mg/dl and pioglitazone group showed 272mg/dl similarly at final interval at 4hr after glucose load the diabetic control group showed 312mg/dl and the pioglitazone group showed decrease in blood glucose level i.e. 266mg/dl. Similarly the groups like pioglitazone with atorvastatin and pioglitazone with fenofibrate at 0hr shows 255mg/dl and 277mg/dl respectively. Whereas at 4thhr showed a slight decrease in glucose levels i.e. 258 and 279mg/dl respectively.

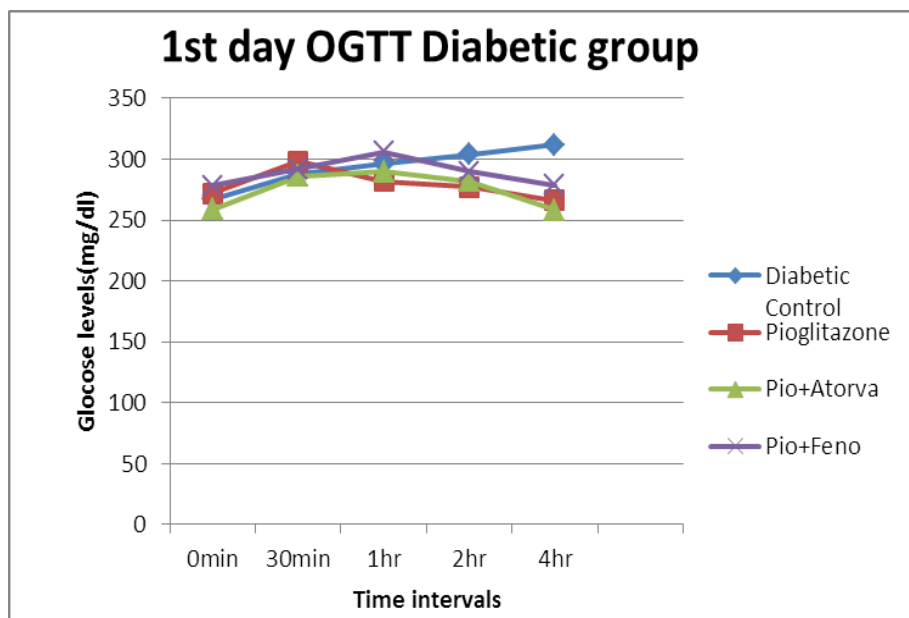


Figure 9: OGTT 1st Day of Diabetic Rats Graph-I.

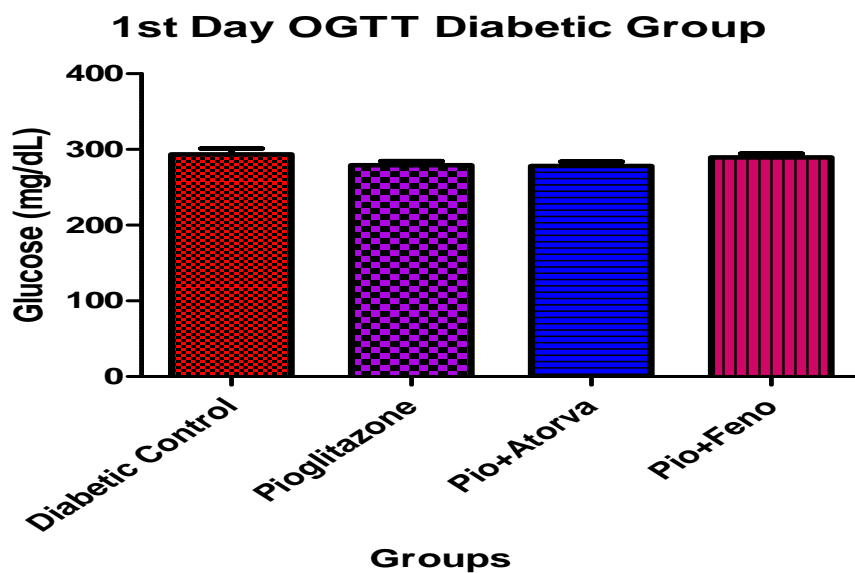


Figure 10: OGTT 1st Day of Diabetic Rats Graph-II.

Oral glucose tolerance test (OGTT) on 7th Day: Diabetic rats.

Table 6: OGTT 7th Day of Diabetic Rats.

	Diabetic Control	Pioglitazone	Pio+Atorva	Pio+Feno
0min	284±1	236±0.25	242±1	228±2
30min	301±0.5	268±0.6	268±1.2	257±1.6
1hr	312±0.3	250±1.4	276±2	269±1.5
2hr	324±1.2*	241±2*	270±1.5*	261±0.5*
4hr	346±1.5**	228±1.6*	239±0.2**	227±0.3*

From the above data obtained we consider that the diabetic control group blood glucose levels were compared with Pioglitazone group it is showing positive result i.e. at 0hr the diabetic control group glucose level was 284mg/dl and Pioglitazone group showed 236mg/dl similarly at final interval at 4hr after glucose load the diabetic control group showed 346mg/dl and the Pioglitazone group showed decrease in blood glucose level i.e. 228mg/dl. Similarly the groups like Pioglitazone with Atorvastatin and Pioglitazone with Fenofibrate at 0hr shows 242mg/dl and 228mg/dl respectively. Whereas at 4thhr showed a slight decrease in glucose levels i.e. 239 and 227mg/dl respectively.

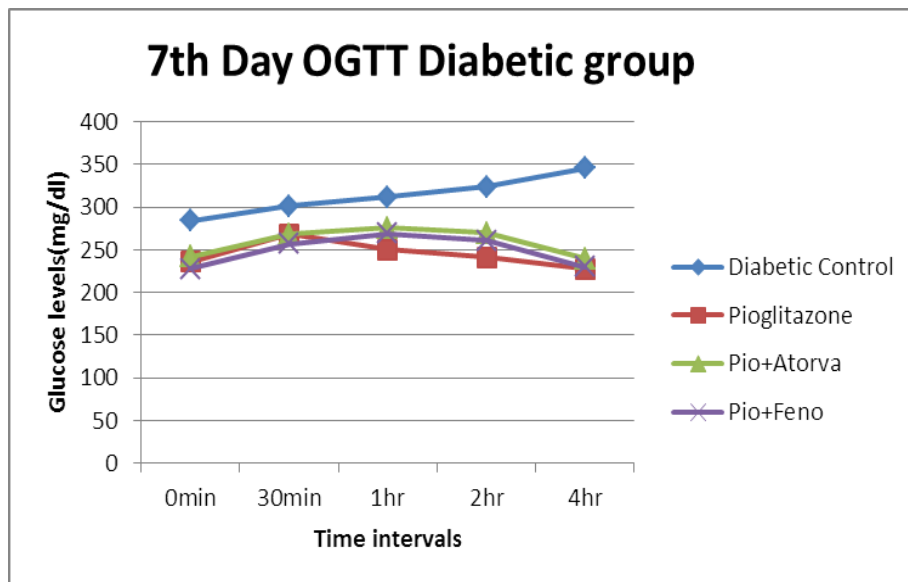


Figure 11: OGTT 7th Day of Diabetic Rats Graph-I

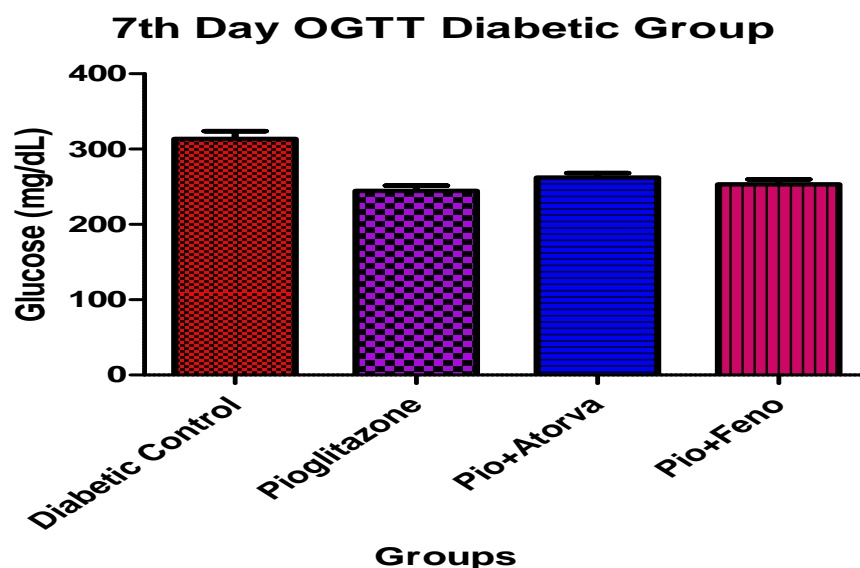


Figure 12: OGTT 7th Day of Diabetic Rats Graph-II

One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. Different? (P < 0.05)	Yes
Number of groups	4
F	15.77
R square	0.7472

Oral Glucose Tolerance Test (OGTT) on 14th day: Diabetic Rats

Table 7: OGTT 14th Day of Diabetic Rats.

	Diabetic Control	Pioglitazone	Pio+Atorva	Pio+Feno
0min	278±1.4	176±1.65	192±0.32	186±0.5
30min	292±1	202±0.6	228±0.1	208±0.25
1hr	308±2	184±0.45	234±1	216±0.5
2hr	317±1.3*	171±0.35*	219±1.2*	210±1.5*
4hr	326±0.1**	162±0.3*	189±1.5**	185±2*

From the above data obtained we consider that the diabetic control group blood glucose levels were compared with Pioglitazone group it is showing positive result i.e. at 0hr the diabetic control group glucose level was 278mg/dl and Pioglitazone group showed 176mg/dl similarly at final interval at 4hr after glucose load the diabetic control group showed 326mg/dl and the Pioglitazone group showed decrease in blood glucose level i.e. 162mg/dl. Similarly the groups like Pioglitazone with Atorvastatin and Pioglitazone with Fenofibrate at 0hr shows 192mg/dl and 186mg/dl respectively. Whereas at 4thhr showed a slight decrease in glucose levels i.e. 189 and 185mg/dl respectively.

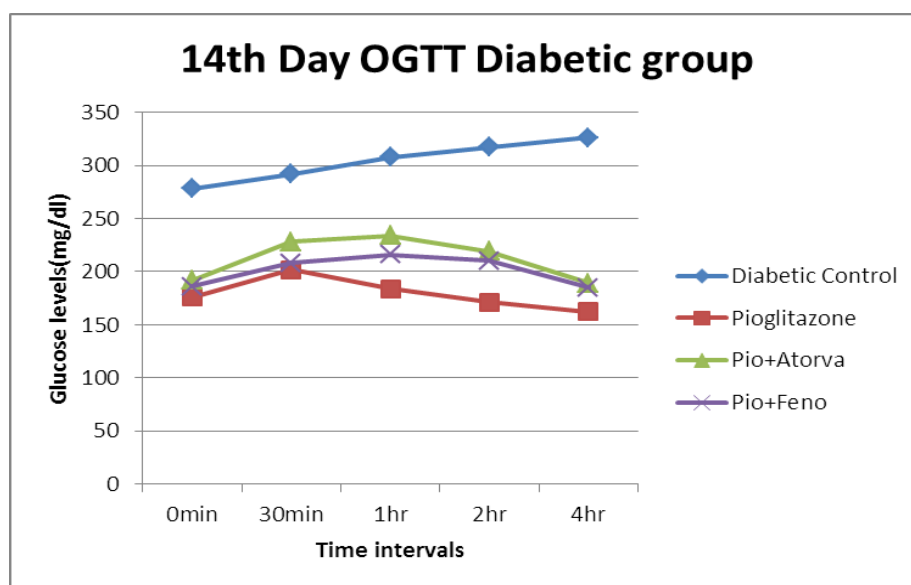


Figure 13: OGTT 14th Day of Diabetic Rats Graph-II.

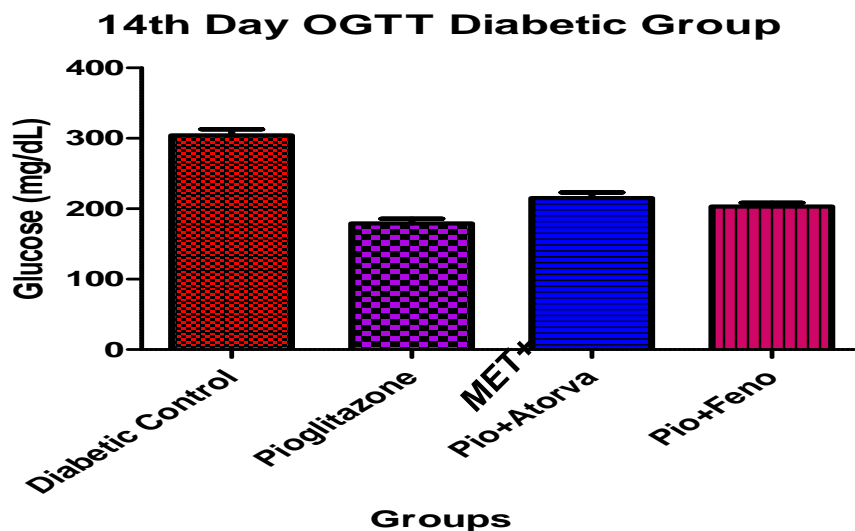


Figure 14: OGTT 14th Day of Diabetic Rats Graph-II.

One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	4
F	56.68
R square	0.9140

Oral Glucose Tolerance Test (OGTT) on 21st day: Diabetic Rats

Table 8: OGTT 21st Day of Diabetic Rats

	Diabetic Control	Pioglitazone	Pio+Atorva	Pio+Feno
0min	287±0.3	140±2	162±2	171±2
30min	301±0.4	168±2	188±1	190±1.4
1hr	313±0.75	160±1.3	190±1	201±06
2hr	320±0.25*	142±1.7*	179±0.5*	188±0.35*
4hr	337±0.5**	116±1.5*	155±0.4**	169±0.6*

Values are expressed as mean ± S.E.M; n=6, *P<0.1 significant, **P<0.01 more significant.

One way ANOVA followed by Dunnet Multiple comparison test using Graph pad INSTANT version 5.

From the above data obtained we consider that the diabetic control group blood glucose levels were compared with Pioglitazone group it is showing positive result i.e. at 0hr the diabetic control group glucose level was 287mg/dl and Pioglitazone group showed 140mg/dl similarly at final interval at 4hr after glucose load the diabetic control group showed

337mg/dl and the Pioglitazone group showed decrease in blood glucose level i.e. 116mg/dl. Similarly the groups like Pioglitazone with Atorvastatin and Pioglitazone with Fenofibrate at 0hr shows 162mg/dl and 171mg/dl respectively. Whereas at 4thhr showed a slight decrease in glucose levels i.e. 155 and 169mg/dl respectively.

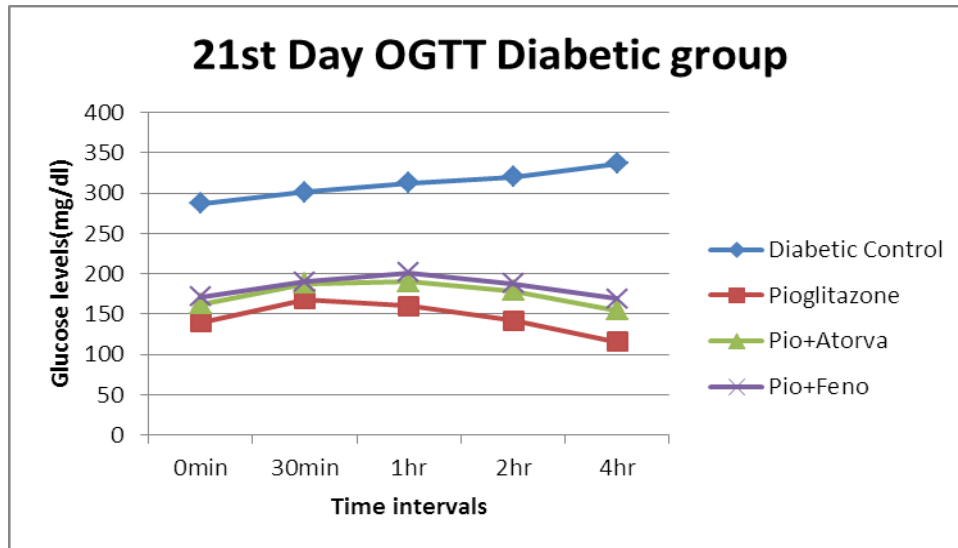


Figure 15: OGTT 21st Day of Diabetic Rats Graph-I.

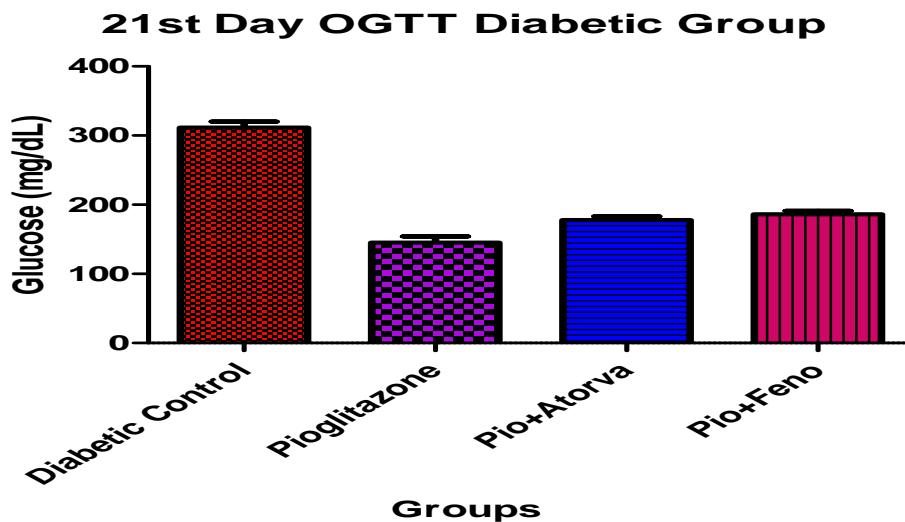
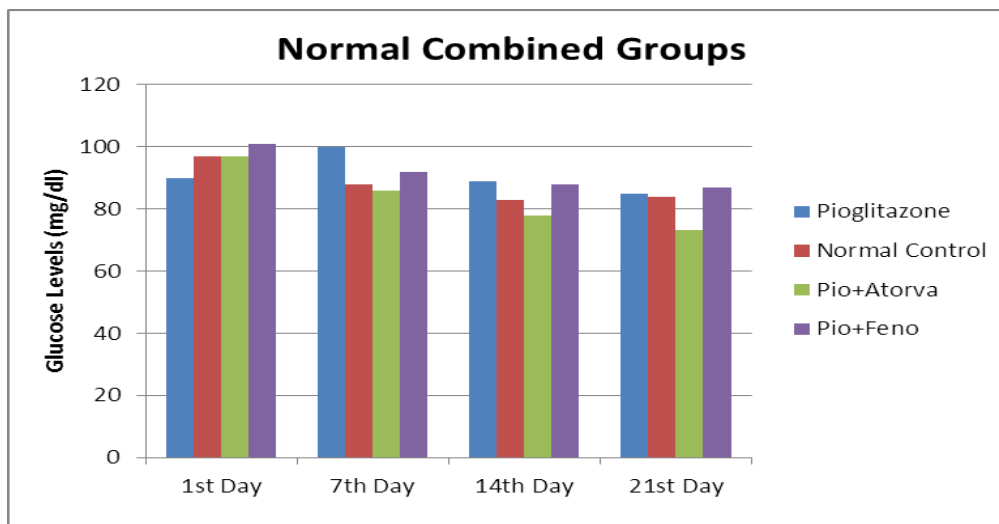


Figure 16: OGTT 21st Day of Diabetic Rats Graph-II.

One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. Different? (P < 0.05)	Yes
Number of groups	4
F	103.4
R square	0.9510

COMBINED COMPARISON.**Table 9: comparison of glucose levels in different groups in different days in normal group rats.**

	Normal Control	Pioglitazone	Pio+Atorva	Pio+Feno
1 st Day	97±1.2	90±2.1	97±1.02	101±2.2
7 th Day	88±2.2	100±2.2	86±1.4	92±2.3
14 th Day	83±1.4	89±1.8*	78±1.6*	88±1.1
21 st Day	84±1.6*	85±1.1**	73±1.9**	87±1.2*

**Figure 17: Comparison of glucose levels in different groups in different days in normal group rats.****Table 10: Comparison of glucose levels in different groups in different days in diseased group rats.**

	Diabetic Control	Pioglitazone	Pio+Atorva	Pio+Feno
1 st Day	293±1.2	279±2.3	274±1.9	288±2.4
7 th Day	313±2.1	244±1.1	258±1.2	248±2.1
14 th Day	311±1.4	179±1.7*	212±1.1*	201±1.3
21 st Day	304±1.9*	145±1.4**	174±3.1**	183±1.6*

From the above data we consider that the diabetic control group showed increased blood glucose levels from 1st day to 21st day. Pioglitazone group shows 279mg/dl on 1st day and 145 mg/dl on 21st day. i.e. there is decrease in blood glucose levels. In the same way Pio+Atorva and Pio+Feno shows 274 and 288mg/dl on 1st day and 174 and 183mg/dl respectively on 21st day. That means pioglitazone decrease blood glucose levels and in Pio+Atorva group as the atorvastatin drug decreases the antidiabetic activity of pioglitazone it shows slight increase in glucose levels compared to pioglitazone. In the same way Pio+Feno group shows a greater increase in glucose levels compared to Pio+Feno group animals.

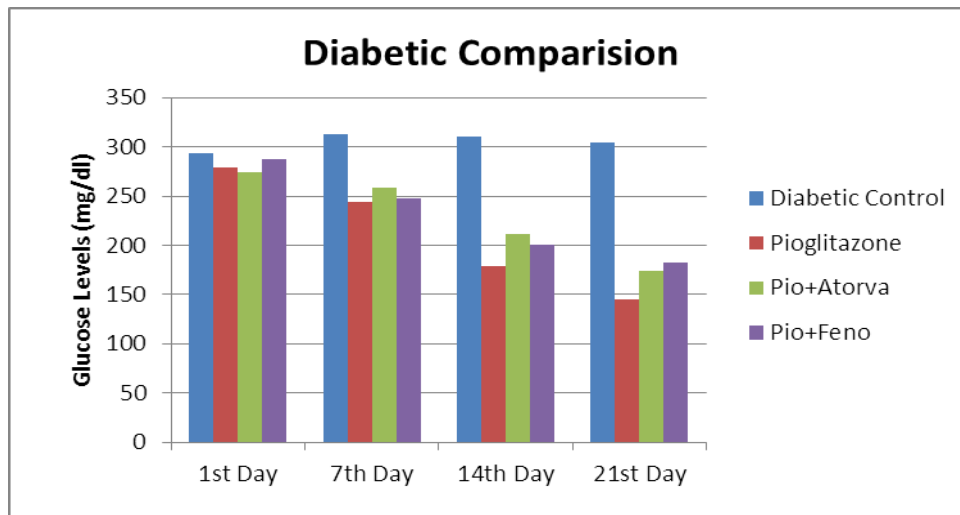


Figure 18: Comparison of glucose levels in different groups in different days in diseased group rats.

BODY WEIGHT AND SERUM LIPID PROFILES.

The body weight was slightly increased in the normal control rats, whereas in the diabetic rats there was a significant reduction in body weight is due to poor glycemic control and impaired carbohydrate metabolism. Pioglitazone and in combination of Pioglitazone + Atorvastatin and in combination of Pioglitazone + Fenofibrate treatment significantly prevented this reduction in the body weight of animals in these groups. Although there is a marginal reduction in the body weight of animals in these groups compared to initial body weights it fell short of statistical significant. However the reduction in the body weight was significant when compared to the final weight of normal control rats weight in Both Non Diabetic (ND) and Diabetic (D) Rats.

Table 11: Effect of Pioglitazone and the combination of Pio+Atorva and Pio+Feno on body weights.

Groups	Body weight		
	Initial (0 day)	7th day	14th day
Normal control	183.3±0.4	192.0±7.6	196±6.3
Pioglitazone(ND)	194.2±2.4	182±0.3*	168±0.5**
Pioglitazone+ Atorvastatin(ND)	189.3±0.9	154±0.84*	137±0.9**
Pioglitazone+ Fenofibrate(ND)	186 ±2.3	154±0.84*	137±0.9*
Diabetic control	182.1±0.2	174 ±0.50	168±4.8
Pioglitazone(D)	185.6±0.6	167±0.40	156±8.4*
Pioglitazone + Atorvastatin(D)	187.2±0.5	174±9.2*	153±9.8**
Pioglitazone+ Fenofibrate(D)	186 ±2.3	175±0.84*	167±0.9*

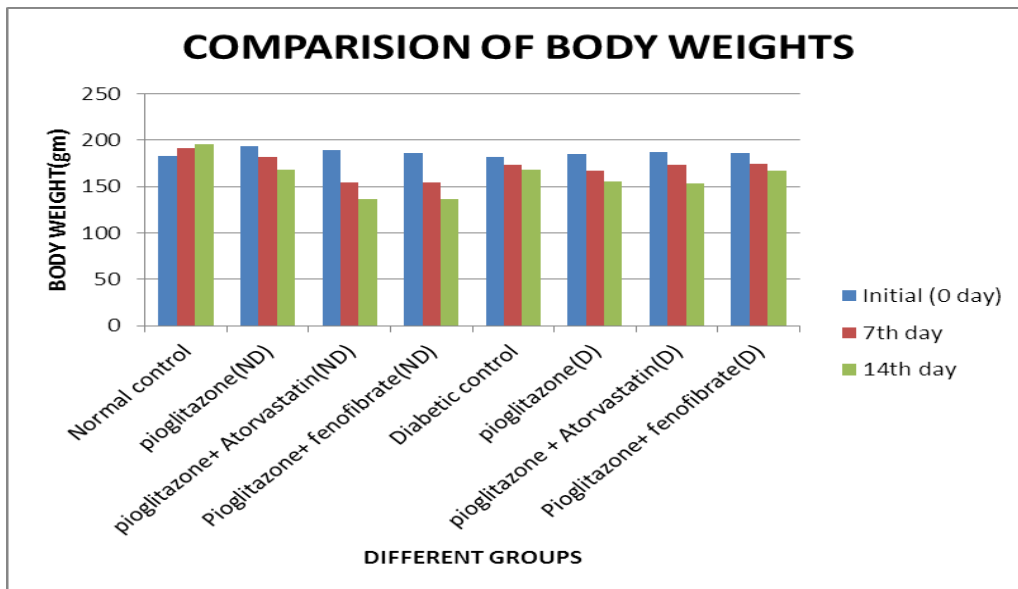


Figure 19: Comparison of body weights (gm) in different groups at different days.

LIPID PROFILE

Table 12: Effect of Pioglitazone and the combination of Pio+Atorva and Pio+Feno on lipid levels.

Groups	Serum lipid profile (mg/dl)		
	TG	TC	HDL
Normal control	103±10.2	144.0±5.2	61.2±5.2
Pioglitazone(ND)	159.2±8.4	164.3±3.5	50.2±4.8
Pioglitazone+ Atorvastatin(ND)	110.7±2.5	138.6±5.3	53.8±4.5
Pioglitazone + Fenofibrate (ND)	122±2.2	153±3.1	55±2.1
Diabetic control	172.3±11.0	211.5±6.3	36.03±7.3
Pioglitazone (D)	161.5±11.3	186.7±5.4	50.8±5.4
Pioglitazone+Atorvastatin (D)	111.3±10.2	141.3±5.2	54.3±7.9
Pioglitazone + Fenofibrate	124±3.5	165±2.1	57±3.4

Diabetic rats showed increase in serum glucose levels than control. Serum glucose levels showed a reversal near to control values by treatment with Pioglitazone. Whereas treatment with combination of Pioglitazone + Atorvastatin decreased the serum glucose concentration lower than metformin treatment. Diabetic rats showed increase in the serum levels of TG, TC and decrease HDL level when compared to control. Atorvastatin decreased the lipid profile near to normal control, which is statistically significant. Pioglitazone also slightly altered the lipid profile. On the other hand the treatment with combination of Pioglitazone + Atorvastatin on diabetic rats decreased serum TG, TC and LDL levels and increased HDL levels than Atorvastatin treatment, which is statistically significant.

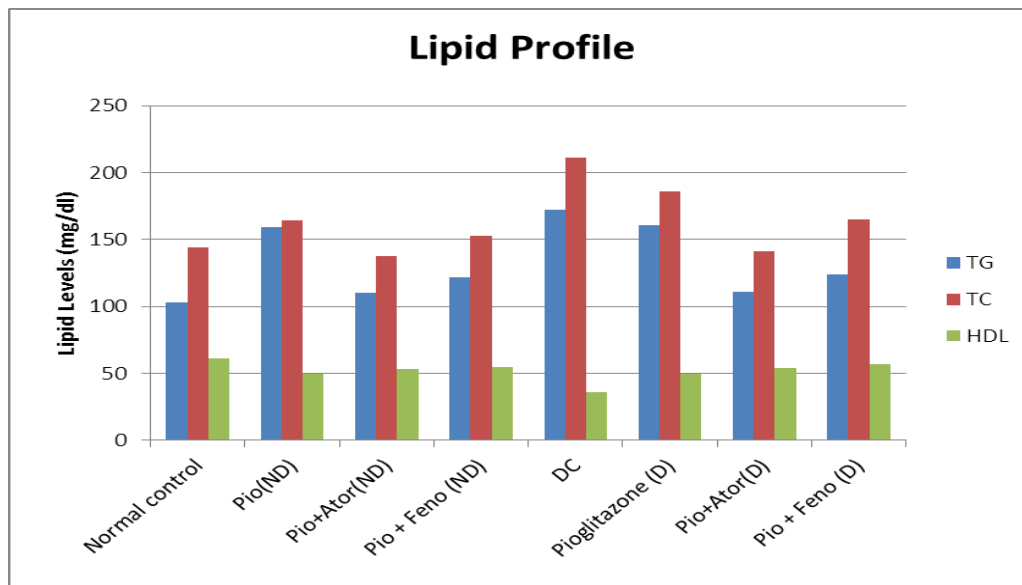


Figure 20: Comparison of lipid levels in different groups at different days.

CONCLUSION

The present study results suggest that Atorvastatin and Fenofibrate decrease the hypoglycemic activity of Pioglitazone by decreasing the bioavailability of Pioglitazone and when compared to Atorvastatin, Fenofibrate in combination with Pioglitazone shows a greater decrease in the hypoglycemic activity of Pioglitazone. The results are statistically significant.

Even it may be required to monitor the blood glucose levels as a precautionary measure, so as to avoid the complications of severe hypoglycemia.

Our studies in normal and diabetic rats suggested that drug interaction occurs between Atorvastatin, Pioglitazone and Fenofibrate when they are used concurrently in normal and pathophysiological conditions of diabetes mellitus.

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