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ORIGINAL ARTICLE

Influence of Bosentan on Pharamcokinetics of Pioglitazone and Nateglinide on Experimental animal

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ABSTRACT
A drug interaction is a situation in which a substance affects the activity of a drug, i.e. the effects are increased or decreased, or they produce a new effect that neither produces on its own. Drug interactions are often categorized as pharmacokinetic or pharmacodynamic in nature. It can be either beneficial or detrimental to patients [2]. In such cases it is needed to alter the dose and frequency of administration of one or all drugs, which are to be administered simultaneously. Diabetes is a polygenic disease, amongst many possible side effects and complications, Hypertension is more prone to develop in diabetic patients, so use of anti hypertensive drugs become necessary to co administer with anti diabetic drug therapy. Hence, there may be a great chance of occurring drug-drug interactions while using such multi drug therapy regimen together. Bosentan, a newly launched drug in India, belongs to a class of drug known as Endothelin Receptor Antagonists (ERAs), widely used in the treatment of pulmonary arterial hypertension. In addition, Nateglinide and pioglitazone are antidiabetic agents which have been extensively used. Bosentan (ERAs) is known to metabolize through cytochrome P-450 enzyme system mainly 3A4, and 2C8. These isoenzymes are involves in metabolism of both Nateglinide and Pioglitazone, When these drugs are prescribed concomitantly, there is a possibility of alteration of pharmacokinetics parameters of Nateglinide and pioglitazone by Bosentan, which may affect the hypoglycemia induced by them. Therefore the study to assess the possibility of potential interaction of Nateglinide and pioglitazone with Bosentan is very much needed and the work is highly justifiable.
Keywords: *Bosentan; Nateglinide; Pioglitazone; Drug-drug interaction; hypoglycemic activity; Alloxan Monohydrate; Antidiabetic activity.*

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INTRODUCTION

A change in the pharmacokinetic and pharmacodynamic parameters of any drug substance can result due to the simultaneous administration of another drug resulting into drug-drug interactions [1]. Marked alterations in the effects of some drugs can result from co-administration with another agent. Such interactions can increase the drug effect to the level of toxicity, or they can inhibit the drug effect and deprive the patient of therapeutic benefit. It is known that the incidence of adverse drug reaction to drugs rise from 4.2% when five or fewer drugs are used to 45% when twenty or more drugs are used [2]. This may lead to enhanced or diminished effect of concomitantly used drugs may be useful or harmful.

It is a common practice to prescribe more than one drugs at a time as many as fourteen drugs have been reported to be prescribed at a time in cardiovascular disease. Sometimes it is referred to as therapeutically jungle. This is one of the main causes of drug interactions [3]. A contemporary example of a drug-drug interaction used as an advantage is the co-administration of carbidopa with levodopa in the treatment of Parkinsonism disease, available as Carbidopa/levodopa combination [4].

In diabetes, there is an every possibility of hypertension and other cardiovascular diseases. Having diabetes increases the risk of developing high blood pressure, because it adversely affects the arteries [5]. In such instances, there is a need for co administration of anti hypertensive drugs with oral anti diabetics. In general, most commonly prescribing anti hypertensive drugs are ramipril, hydrochlorothiazide,

amlodipine, nefidipine, atanolol etc., and all above mentioned drugs are having the potent interaction with oral anti diabetics [6].

In addition to these drugs, one of the latest anti hypertensive drugs, that is Bosentan belongs to a class of drug known as Endothelin Receptor Antagonists (ERAs), widely used in the treatment of pulmonary arterial hypertension [7] and has got tendency to interact with other class of drugs like Tadalafiland shown serious interactions [8]. Bosentan-Tadalafil drug interaction has resulted that after 10 days of co administration, Bosentan decreased Tadalafil exposure by 41.5% with minimal and clinically irrelevant differences (<20%) in Bosentan exposure [9]. Similarly antidiabetic agents like Pioglitazone shows detrimental interactions with Gemfibrozil, Ketoconazole, Glucosamine etc, while it shows beneficial interactions with Glyburide and other drugs of Sulfonylurea class. Nateglinide also shows harmful interactions with Rifampicin and Fluconazole, while it shows advantageous interactions with Diclofenac [10].

However from the above mentioned drug interactions it has been confirmed that drug interactions studies with Bosentan and anti-diabetic agents like Nateglinide and Pioglitazone have not been conducted and reported.

Hence, the present study is planned to understand the possible drug – drug interaction between Nateglinide and Pioglitazone along with Bosentan.

MATERIAL AND METHODS

The studies were carried out in the Department of Pharmacology in our institution which is duly licensed by the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments in Animals). The study protocols were approved according to current regulations of CPCSEA by the Institution Animal Ethics Committee for studies in experimental animals.

Animals:

All the animals (rats) used in the study were procured from Laxmi Biotec, Ale Phata, Pune. Registration number ARACOP/IAEC/20/4 and were housed under standard husbandry conditions in the institutional animal house. Hence the same may be considered as source of animal procurement in the subsequent sections. A total of 50 rats (either sex) were selected for the current study.

Common Experimental Techniques

Method for oral administration: [11]

Oral feeding administration was done by oral feeding needle (purchased from Space Labs, Nashik) and 1 ml glass syringe. An 18 gauge needle was suitably covered with flexible polythene tubing, where the edge was made blunt; the needle was fixed to the 1ml tuberculin syringe. The rat was held firmly in left hand, the tubing was moistened with glycerine and inserted right into the esophagus and gently pressing plunger for drug administration, and this was followed by 0.2ml of distilled water to ensure administration of correct dose of the drug.

Method for blood sampling: [12, 13, 14]

The rat was anesthetized by anesthetic ether in anesthetic chamber. After small anesthesia rat was taken up from anesthetic chamber. Now put animal on operation table and tail is squeezed with Xylene to dilate the vein and cut the tip of tail and blood is collected in the epindroff tubes containing pinch of anticoagulant mixture (sodium fluoride and potassium oxalate in 1:3 ratio).

Estimation of blood glucose

Enzyme, GOD-POD endpoint colorimetry [15]

The GOD/POD method is one such evolved method by Trinder in 1964. This method is simple, single stepped, rapid, reliable and acceptable precision. Hence in the present study this method has beenAdopted [16].

Effect of Bosentan pre-treatment on the hypoglycemic activity of Nateglinide and Pioglitazone in healthy albino rats:

In the first part of this study, the hypoglycemic effect of Nateglinide and Pioglitazone was established in healthy albino rats. In the next part of this experiment, the effect of dose of Bosentan 10mg/kg per day for one week on the hypoglycemic activity of Nateglinide and Pioglitazone was carried out in the same animals.

EXPERIMENTAL PROCEDURE

Healthy Albino Rats:

Six albino rats of either sex weighing between 150-180 gm were selected for the study. The animals were randomly distributed into four groups (I, II, III, and IV); each group was consisting of 6 animals. They were marked suitably for ready identification. The animals were housed in colony cages under standard husbandry conditions. On the previous day of experimentation, the food was withdrawn 18-hrs advance.

However water was allowed *ad libitum*. The fasting was continued till the completion of the experiment. On next day, the blood samples were withdrawn from tail vein (0.5 ml, each) for determination of basal glucose concentration. Thereafter, the animals of first group were administrated with suspension of Nateglinide 50 mg/kg trough oral route. Blood sample were withdrawn from the tail vein at intervals of at 0, 0.5, 1, 2, 4, 6, 8, 12, 18 and 24 hours and analyzed for blood glucose concentration by GOD/POD method. Animals of second group were administrated with suspension of Pioglitazone 0.3 mg/kg trough oral route. Blood sample were withdrawn from the tail vein at intervals of at 0, 0.5, 1, 2, 4, 6, 8, 12, 18 and 24 hours and analyzed for blood glucose concentration by GOD/POD method. Animals of third group were administrated with suspension of Bosentan 10mg/kg trough oral route for 7 days, on 7th day, 6 hours after the dose of Bosentan in 2% acacia suspension of administration; the animals were fasted for 18 hours. This fasting was continued till the end of experiments. However water supplied *ad libitum.* On 8th day 1 hour after the dose administration, after 60 min of respective treatment, Nateglinide 15 mg/kg, p.o. was administered to the same animals. Thereafter the blood samples (0.5 ml, each) were collected at 0, 0.5, 1, 2, 4, 6, 8, 12, 18 and 24 hours and analyzed for the determining the glucose concentration using GOD/POD method.

Animals of fourth group were administrated with suspension of Bosentan10 mg/kg trough oral route for 7 days, on 7th day, 6 hours after the second dose of Bosentan in 2% acacia suspension of administration; the animals were fasted for 18 hours. This fasting was continued till the end of experiments. However water supplied *ad libitum.* On 8th day 1 hour after the dose administration, after 60 min of respective treatment, Pioglitazone 0.3 mg/kg, p.o. was administered to the same animals. Thereafter the blood samples (0.5 ml, each) were collected at 0, 0.5, 1, 2,4, 6, 8, 12, 18 and 24 hours and analyzed for the determining the glucose concentration using GOD/POD method. The percentage reduction in blood glucose levels at time„t" was calculated by using the following equation.

$$\%\ Blood\ sugar\ reduction\ at\ time\quad 't' = \frac{A-B}{A}\ X\ 100$$

Where, A = Initial blood glucose level before drug administration.
B = Blood glucose levels at time „t" after the drug administration.

Diabetic Rat:

However, it is not clear whether Bosentan has got any influence on antidiabetic drugs in the pathophysiological conditions like diabetes mellitus in various species of animals. Hence, in the present study, we have planned to use diabetic rats as experimental animals to clarify this aspect. In the earlier part of this study it was observed that pre-treatment with Bosentan at dose (10 mg/kg) has significant influence at the peak hypoglycemia induced by Nateglinide and Pioglitazone. Therefore the dose of Bosentan was selected for the study to verify the interactions in diabetic albino rats.

Various methods for induction of experimental diabetes[21]:

In experimental animals permanent diabetes can be produced by:

- Repeated injection of pituitary extract.
- Total pancreatectomy.
- Injection of insulin antibodies.
- Selective destruction of the beta cells by single injection of alloxan or closely allied compounds such as alloxantin, dialuric acid which are possibly converted to alloxan can also induce permanent diabetes.
- Streptozotocin destroys cells of the islet of Langerhans (in rats when givenby I.P. in the dose of 35 to 55 mg which is dissolved in ice cold saline solution.
- Streptozotocin induced beta cell in the pancrease is due to the alkylation of DNA thereby producing hyperglycemia. Streptozotocin (50mg/kg i,p) induced diabetes in rats was selected for the present study.

Induction of diabetes:

Rats of either sex weighing between 150–250 gm were selected and fasted for 18 hours and water *ad-libitum*. The animals were randomly distributed into different groups. The animals were kept in colony cages at standard husbandry condition. The rats were administered with 150 mg/kg of Alloxan intraperitonially. After 48 hours, the blood samples were collected and analyzed for blood glucose level. It was found that diabetes was induced in about 48 hours. In our experiment the diabetes was characterized by weight loss and hyperglycemia. The blood samples were collected and analyzed for seven more days for stabilization of blood glucose levels. These animals were further used for our antidiabetic study.

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Experimental procedure

Diabetic rats (blood glucose level more than 250mg/dl after 48hrs of Alloxan injection) of either sex were divided in to two groups (groups I and II) of 6 animals and they were marked conveniently. The animals were randomly distributed into different groups. The animals were kept in colony cages at standard husbandry condition. The animals were fasted for 18 hrs before commencing the experiment. During this period the rats were supplied with water *ad libitum*. Fasting was continued throughout the experiment. The blood samples were collected for fasting blood glucose estimation. In the first part of this anti-diabetic study, the animals in group I, II and III received suspension of Nateglinide 50 mg/kg, Pioglitazone 0.3 mg/kg and Bosentan 10 mg/kg respectively through oral route. Blood samples were collected at pre-determined time intervals and blood glucose levels were estimated by GOD/POD method. In the next part of this experiment, all the animals in group I and II were treated with Bosentan at dose 10 mg/kg. On the 7th day, 6 hrs after administration of Bosentan, the rats were fasted for 18hrs. On the 8th day, Bosentan 15 mg/kg was administered orally to all the animals in group I and II. 60 minutes later, Nateglinide 50 mg/kg and Pioglitazone 0.3 mg/kg was administered to group I and II respectively. Blood samples were collected thereafter at different time intervals at 0, 0.5, 1, 2, 4, 6, 8, 12, 18 and 24 hours and were analyzed by GOD/POD method. Blood glucose levels were expressed as mg/dl of blood.

Then the antidiabetic activity of Nateglinide and Pioglitazone at time "t"was calculated and the % blood glucose reduction at various time intervals were calculated before and after Bosentan treatment.

$$\%\ Blood\ sugar\ reduction\ at\ time\ \ 't' = \frac{A-B}{A}\ X\ 100$$

Where, A = Initial blood glucose level before drug administration.

B = Blood glucose levels at time „t" after the drug administration.

RESULTS

Effect of Bosentan Pre-Treatment on Hypoglycemic Effect of Pioglitazone and Nateglinide on Healthy Albino Rats:

In this study Bosentan pretreatment, i.e. 10 mg/kg has not significantly altered the onset of hypoglycemia (23.67 2.08% reduction before treatment to 19.57 1.21% reduction after treatment) at 2nd hr, but peak hypoglycemia was effected significantly (48.74 2.68% reduction before treatment to 40.86 0.86% reduction after treatment, P< 0.001) at 6th hr of pioglitazone. However duration of hypoglycemia was 16 hours before treatment and reduced to 12 hours only after treatment (25.58 2.08% reduction before treatment and 24.48 1.21% reduction after treatment, P<0.001).

The results of these findings are compiled in table No.1 and 2 and graphically depicted in figures No. 1. In this study Bosentan pre-treatment, i.e. 10 mg/kg has not significantly altered the onset of hypoglycemia (24.70 1.93% reduction before treatment to 21.30 1.21% reduction after treatment) at 1sthr but peak hypoglycemia was reduced significantly (49.80 2.54% reduction before treatment to 41.27 1.30% reduction after treatment, P< 0.0001) at 5th hr of Nateglinide, While duration of hypoglycemia was 11 hours before treatment and reduced to 7 hours only after treatment (23.64 3.72% reduction before treatment to 23.66 6.86% reduction after treatment, P< 0.001).

These findings are recorded in table No.5 and 6 and graphically shown in figure No. 2

Table No 1: Blood Glucose Levels with Pioglitazone (0.3 mg/kg) in healthy Albino Rats before and after Bosentan (10 mg/kg) treatment

Sr. No.	Time in Hrs	Blood Glucose Levels (mg%) with Pioglitazone (MEAN± SEM)	Blood Glucose Levels (mg%) with Pioglitazone + Bosentan (MEAN± SEM)
1	0	99.51±3.68	103.2±0.8
2	30	93.69±3.69	96.81±0.9
3	1	85.76±3.57	90.83±1.1
4	2	75.99±3.72	82.94±1.1
5	4	68.30±3.08	74.89±1.2
6	6	59.87±2.49	67.22±1.6
7	8	50.60±1.66	58.95±1.0
8	12	61.97±1.45	64.48±1.3
9	18	73.73±1.53	69.90±1.3
10	24	83.99±2.23	74.52±1.9

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Table No 2: Percentage Blood Glucose Levels with Pioglitazone in healthy Albino Rats before and after Bosentan

Sr. No.	Time in Hrs	Blood Glucose Levels (mg%) with Pioglitazone (MEAN± SEM)	Blood Glucose Levels (mg%) with Pioglitazone + Bosentan (MEAN± SEM)
1	0	-	-
2	30	5.89±0.44	6.14±0.76
3	1	13.84±1.28	11.93±1.21**
4	2	23.67±2.08	19.57±1.21***
5	4	31.37±1.59	27.41±0.97***
6	6	39.65±2.66	34.86±1.30***
7	8	48.74±2.68	40.86±0.86**
8	12	37.32±2.59	24.48±1.21**
9	18	25.58±2.08	12.91±1.29*
10	24	13.20±2.91	11.70±2.18

** Highly significant at<0.001; * represent that comparison of Pioglitazone with Pioglitazone + Bosentan interaction. *** Very highly significant at p<0.0001

Effect of Bosentan pre-treatment on antidiabetic activity of pioglitazone and Nateglinide in diabetic rats:

In this study Bosentan pre-treatment, i.e. 10 mg/kg has not significantly altered the onset of hypoglycemia (27.69 2.39% reduction before treatment to 19.31 0.52% reduction after treatment) at 2nd hr but peak hypoglycemia was reduced significantly (44.50 2.56% reduction before treatment to 34.59 0.25% reduction after treatment, P< 0.001) at 6th hr of pioglitazone. However duration of hypoglycemia was only 16 hours before treatment and reduced to 12 hours after treatment (25.46 2.40% reduction before treatment to 30.44 1.13% reduction after treatment, p< 0.05).

The results are shown in table No. 3 and 4 and graphically shown in figure No. 1. In this study Bosentan pre-treatment, i.e. 10 mg/kg has not significantly altered the onset of hypoglycemia (25.70 0.58% reduction before treatment to 18.17 1.18% reduction after treatment) at 1st hr but peak hypoglycemia was reduced significantly (46.16 1.25% reduction before treatment to 37.57 1.61% reduction after treatment, P<0.0001) at 5th hr of Nateglinide. However duration of hypoglycemia was only 11 hours before treatment and reduced to 7 hours after treatment (23.14 0.94 % reduction before treatment to 26.03 1.23% reduction after treatment, P< 0.0001). The results are shown in table No. 7 and 8 and graphically shown in figure No. 2.

Table No 3: Blood Glucose Levels with Pioglitazone (0.3 mg/kg) in Diabetic Albino Rats before and after Bosentan treatment

Sr. No.	Time in Hrs	Blood Glucose Levels (mg%) with Pioglitazone (MEAN± SEM)	Blood Glucose Levels (mg%) with Pioglitazone + Bosentan (MEAN± SEM)
1	0	248.24±7.20	255.96±6.76
2	30	229.66±7.04	239.00±6.96
3	1	208.53±7.38	218.24±6.62
4	2	179.22±6.36	188.47±4.30
5	4	165.00±7.51	161.45±4.33
6	6	149.15±7.11	152.58±4.03
7	8	137.44±6.08	141.85±4.04
8	12	157.46±2.41	159.88±2.91
9	18	184.35±3.71	192.68±4.01
10	24	218.55±6.31	224.40±6.33

Table No 4: Percentage Blood Glucose Levels with Pioglitazone (0.3 mg/kg) in Diabetic Albino Rats before and after Bosentan treatment

Sr. No.	Time in Hrs	Blood Glucose Levels (mg%) with Pioglitazone (MEAN± SEM)	Blood Glucose Levels (mg%) with Pioglitazone + Bosentan (MEAN± SEM)
1	0	7.49±0.57	4.63±0.55
2	30	13.02±1.12	10.73±0.88***
3	1	27.69±2.39	19.31±0.52***
4	2	33.42±2.75	26.89±0.86*
5	4	39.84±2.60	30.39±0.50**
6	6	44.50±2.56	34.59.±0.25**
7	8	36.24±2.41	30.44±1.13*
8	12	25.46±2.40	14.49±2.39***
9	18	11.91±1.27	6.50±1.22**
10	24	7.49±0.57	4.63±0.55

* Significant at p< 0.05; ** Highly significant at p<0.01; *** Very highly significant at p<0.001

*represent that comparison of Pioglitazone with Pioglitazone + Bosentan interaction

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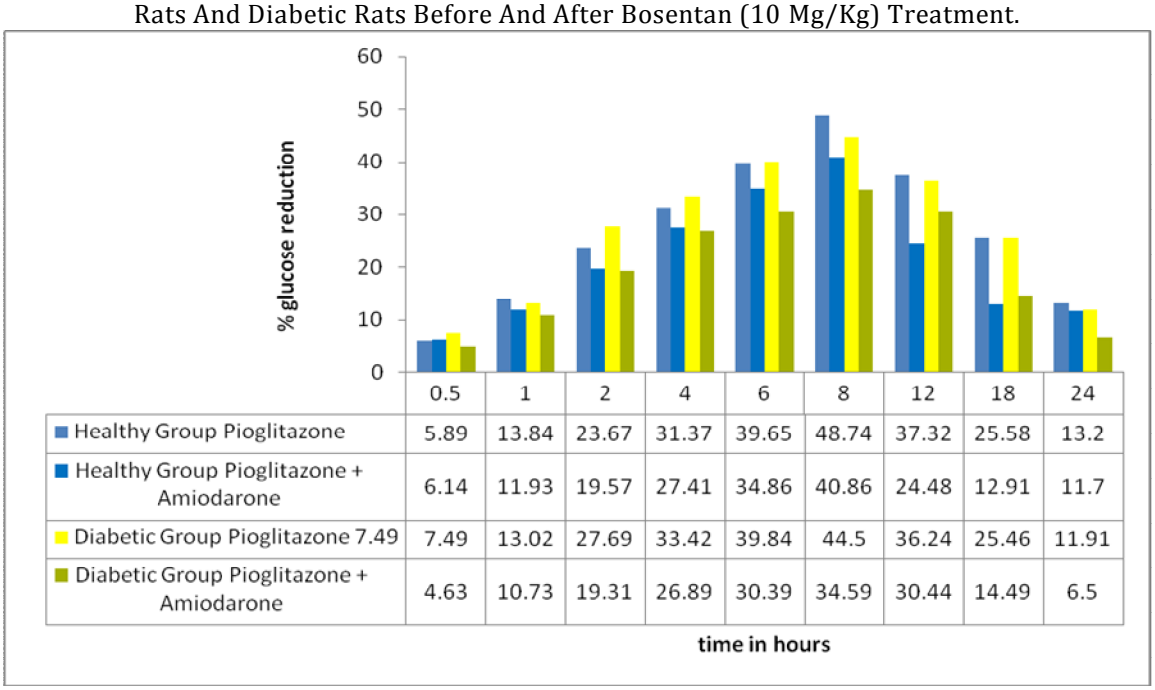
Figure No 1: Percentage Blood Glucose Reduction With Pioglitazone (0.3 Mg/Kg) In Healthy Albino Rats And Diabetic Rats Before And After Bosentan (10 Mg/Kg) Treatment.



	0.5	1	2	4	6	8	12	18	24
Healthy Group Pioglitazone	5.89	13.84	23.67	31.37	39.65	48.74	37.32	25.58	13.2
Healthy Group Pioglitazone + Amiodarone	6.14	11.93	19.57	27.41	34.86	40.86	24.48	12.91	11.7
Diabetic Group Pioglitazone 7.49	7.49	13.02	27.69	33.42	39.84	44.5	36.24	25.46	11.91
Diabetic Group Pioglitazone + Amiodarone	4.63	10.73	19.31	26.89	30.39	34.59	30.44	14.49	6.5

Table No 5: Blood Glucose Levels with Nateglinide (50 mg/kg) in healthy Albino Rats before and after Bosentan (10 mg/kg) treatment

Sr. No.	Time in Hrs	Blood Glucose Levels (mg%) with Nateglinide (MEAN± SEM)	Blood Glucose Levels (mg%) with Nateglinide + Bosentan (MEAN± SEM)
1	0	94.57±3.61	98.73±2.61
2	30	88.08±3.41	87.44±2.80
3	1	75.99±3.72	69.68±1.18
4	2	68.30±3.08	50.20±0.99
5	4	59.80±2.49	38.88±1.04
6	6	55.65±1.65	64.58±2.11
7	8	73.73±1.53	75.00±2.22
8	12	83.99±2.23	82.97±1.92
9	18	90.92±2.47	92.07±1.47
10	24	93.30±2.42	98.70±2.34

Table No 6: Percentage Blood Glucose Levels with Nateglinide (50 mg/kg) in healthy Albino Rats before and after Bosentan (10 mg/kg) treatment

Sr. No.	Time in Hrs	Blood Glucose Levels (mg%) with Nateglinide (MEAN± SEM)	Blood Glucose Levels (mg%) with Nateglinide + Bosentan (MEAN± SEM)
1	0	-	-
2	30	12.84±1.02	06.48±0.76
3	1	24.70±1.93	21.30±1.21*
4	2	30.76±1.74	26.88±1.21**
5	4	42.45±2.45	32.38±0.97***
6	6	49.80±2.54	41.27±1.30***
7	8	30.49±3.32	23.66±6.86**
8	12	-23.64±3.72	13.65±1.21*
9	18	-14.51±2.70	6.54±1.29***
10	24	-0.95±2.35	-0.010±2.18***

*Significant at p< 0.01; ** Highly significant at p<0.001; *** Very highly significant at p<0.0001* represent that comparison of Nateglinide with Nateglinide + Bosentan interaction

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Table No 7: Blood Glucose Levels with Nateglinide (50 mg/kg) in Diabetic Albino Rats before and after Bosentan (10 mg/kg) treatment

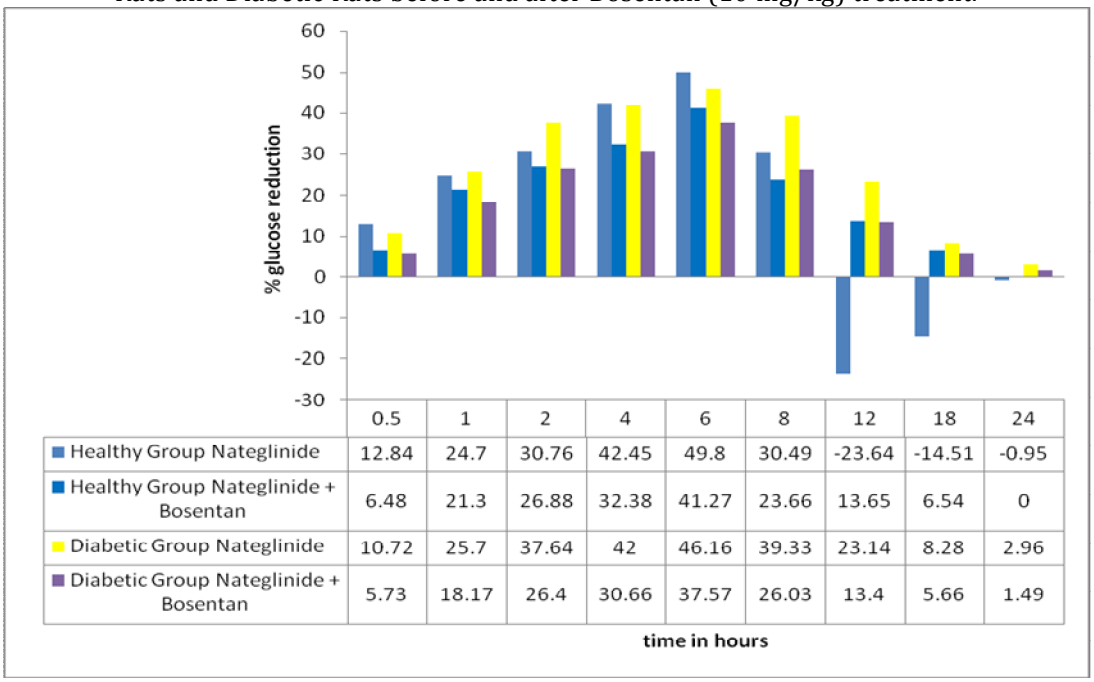
Sr. No.	Time in Hrs	Blood Glucose Levels (mg%) with Nateglinide (MEAN± SEM)	Blood Glucose Levels (mg%) with Nateglinide + Bosentan (MEAN± SEM)
1	0	261.63±5.63	264.24±9.83
2	30	233.56±4.00	238.42±4.66
3	1	194.35±3.44	203.00±5.38
4	2	163.11±3.64	171.34±3.84
5	4	151.72±3.91	141.66±2.16
6	6	140.97±3.95	175.34±3.40
7	8	158.66±5.02	195.37±3.08
8	12	200.94±2.51	228.56±3.67
9	18	239.79±3.00	249.24±4.32
10	24	256.55±5.57	260.37±4.81

Table No 8: Percentage Blood Glucose Levels with Nateglinide (50 mg/kg) in Diabetic Albino Rats before and after Bosentan (10 mg/kg) treatment

Sr. No.	Time in Hrs	Blood Glucose Levels (mg%) with Nateglinide (MEAN± SEM)	Blood Glucose Levels (mg%) with Nateglinide + Bosentan (MEAN± SEM)
1	0	-	-
2	30	10.72±0.42	5.73±0.92
3	1	25.70±0.58	18.17±1.18***
4	2	37.64±0.82	26.40±1.35***
5	4	42.00±1.07	30.66±0.59**
6	6	46.16±1.25	37.57±1.61***
7	8	39.33±0.89	26.03±1.23***
8	12	23.14±0.94	13.40±1.69
9	18	8.28±1.17	5.66±0.69**
10	24	2.96±0.66	1.49±0.30***

** Highly significant at p<0.01; *** Very highly significant at p<0.001 *represent that comparison of Nateglinide with Nateglinide + Bosentan interaction

Figure No 2: Percentage Blood Glucose reduction with Nateglinide (50 mg/kg) in healthy Albino Rats and Diabetic Rats before and after Bosentan (10 mg/kg) treatment.



	0.5	1	2	4	6	8	12	18	24
Healthy Group Nateglinide	12.84	24.7	30.76	42.45	49.8	30.49	-23.64	-14.51	-0.95
Healthy Group Nateglinide + Bosentan	6.48	21.3	26.88	32.38	41.27	23.66	13.65	6.54	0
Diabetic Group Nateglinide	10.72	25.7	37.64	42	46.16	39.33	23.14	8.28	2.96
Diabetic Group Nateglinide + Bosentan	5.73	18.17	26.4	30.66	37.57	26.03	13.4	5.66	1.49

DISCUSSION

The patient suffering from diabetes mellitus are more prone to develop Hypertension. In such cases anti-diabetic drugs like Nateglinide and Pioglitazone and antihypertensive agents are needed to be

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administered simultaneously. There are reports that Rifampicin induces the metabolism of Pioglitazone [22].

In addition there are reports indicating that Bosentan a relatively newer pulmonary antihypertensive agent inhibits human cytochrome p450 enzyme system. The most important CYP isoenzyme that are inhibited or affected by antihypertensive drug like Bosentan, are CYP3A4 and CYP2C9. There is a possibility that drug-drug interaction may occur between the antihypertensive drug and the drugs metabolized by these enzymes. Pioglitazone and Nateglinide are metabolized by CYP3A4, CYP2C8 and CYP2C9 [23,24].

Our studies in rats and rabbits suggested that drug-drug interaction occurs between Bosentan and oral antidiabetic agents when they are used concomitantly in healthy conditions. However the interaction in the pathophysiological conditions like in diabetes was not clear. Hence, in the fourth phase of our study the diabetic rats (Alloxan induced diabetic rats) were used, Pioglitazone and Nateglinide were given to diabetic animals and the onset of hypoglycemia duration of hypoglycemia and peak antidiabetic effect was determined. To the same animals Bosentan (10 mg/kg) pretreatment for one week as usual and again oral antidiabetic agents (Pioglitazone and Nateglinide) were given, with Pioglitazone the onset is not significantly altered (27.692.39% reduction to 19.310.52% reduction) at 2nd hr but peak effect was reduced (44.502.56% reduction before treatment to 34.59.25% reduction after treatment, $P< 0.001$) at 6th hr and changed the duration from 18 hours to less than 16 hrs (25.462.40 % reduction before treatment to 30.441.13 % reduction after treatment, $P< 0.05$) were determined. The results are compiled in table-16 and table-17 and graphically depicted in figure-11. With Nateglinide has not significantly altered the onset of hypoglycemia (25.700.58% reduction to 18.171.18% reduction) at 1st hr but significantly decreased the peak hypoglycemia (i.e. from 46.16.25% reductions to 37.57% reductions, $P< 0.0001$) at 5th hr and duration of hypoglycemia reduced i.e. from 11 hrs to less than 7 hrs (23.140.94% reduction before treatment to 26.031.23% reduction after treatment, $P< 0.0001$) induced by nateglinide.

CONCLUSION

Bosentan single dose treatment has not influenced the blood glucose levels in healthy albino rats and diabetic rats. These findings are indicating that Bosentan does not possess hypoglycemic activity therefore it may be inferred that drug-drug interaction with Pioglitazone and Nateglinide is pharmacokinetic type.

Bosentan pre-treatment for one week has not significantly influenced the onset with Pioglitazone and Nateglinide but peak hypoglycemia and the duration of hypoglycemia were reduced. However, Bosentan (10mg/kg) has reduced the duration of hypoglycemia, peak hypoglycemia has been reduced, and not significantly altered onset of hypoglycemia induced by Pioglitazone in healthy albino rats. Similarly Bosentan (10mg/kg) has significantly reduced effect on the peak hypoglycemia, duration of hypoglycemia and the onset of hypoglycemia has no any significant change by Nateglinide in albino rats.

The potentiation of hypoglycemic effect of oral antidiabetic agents is may be due to the induction of CYP3A4 and CYP2C8 isoenzyme system by Bosentan.

From the above conclusion it may be suggested that monitoring of blood glucose levels is essential during concomitant use of Pioglitazone or Nateglinide with Bosentan. Therefore it is further suggested that readjustment of dose and frequency of administration of oral antidiabetic agents may be made when they are used simultaneously with Bosentan.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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