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Pharmacodynamic Interactions of Amlodipine and Enalapril with Glyburide in Normal and Diseased Rats

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ABSTRACT

Drug Interactions have become very common now-a-days. In drug interactions there are mainly three types in that the most important interactions are drug-drug interactions. By keeping in mind about these interactions we have done research by taking Diabetes as the main disease and if same person is suffering any other disease like we have chosen Hypertension. Our main aim is to find out the Interactions between Glyburide and Amlodipine and another scenario is little different with another class of drug that is Enalapril and with Sulphonyl urea derivative drug that is Glyburide. We found that the Glyburide have shown the 49.9% of inhibition. The Amlodipine and Enalapril groups showed 28.9% and 33.7% inhibition rates against the Glyburide group. The combined groups Glyburide with Amlodipine and Glyburide with Enalapril have shown 39% and 43.3% inhibition rates. More results can be obtained if we have done Pharmacokinetic study.

Keywords: Drug Interactions, Glyburide, Amlodipine, Enalapril, Diabetes.

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INTRODUCTION

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 into two phases. Pharmacokinetic phase, which is related to the body's effect on the drug and. Pharmacodynamic phase, which is related to the drug effect on the body³. 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 are 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 another⁴.

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 drug⁵. 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 their extent cannot be easily predicted⁹. 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 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 sulfonylureas 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 concurrently¹². 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 mechanisms¹³. 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}.

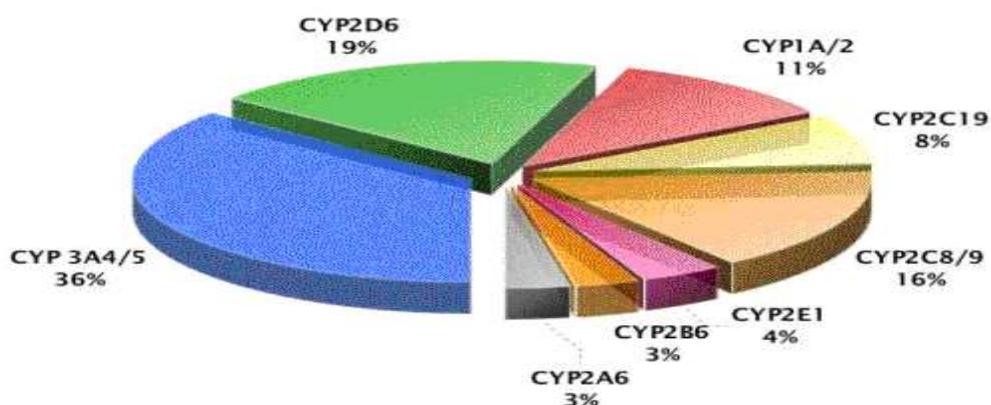


Figure: 1 Proportion of drugs metabolized by CYP450 enzymes.

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 increased¹⁸. 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 mechanism²¹. 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 substrate²².

MATERIALS AND METHOD

Experiments were performed with Wistar rats procured from Sainath agencies (Hyderabad, A.P, India), weighing between 180 to 210 gm. The animals were housed in colony cages 4 per cage under conditions of standard lighting, temperature ($22\pm 1^\circ\text{C}$) and humidity for at least one week before the beginning of the experiment, to adjust to the environment and to overcome the stress possibly occurred during transit. During this period they had free access to food and water.

All wistar rats were feed a normal laboratory chew diet (Nutrilab Rodent Fed, PROVIMI) containing (W/W) of 21.88% crude proteins, 52.15% carbohydrates and 5.97% crude fat. The experiments were planned after the approval of Institutional Animal Ethical Committee (IEAC).

The average body weights of the rats 250-300gm were selected. Before injecting the single dose of Alloxan Monohydrate to animals they were kept overnight fasting and immediately in next day the Alloxan monohydrate mixed in normal saline, is administered to the animals with in 30min in cold condition, should be maintained while using the Alloxan Monohydrate.

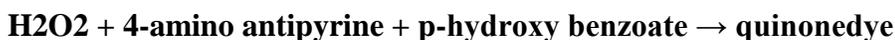
About 750gm of Alloxan monohydrate is dissolved in 10ml of normal saline, this dose is prepared as 75mg/kg body weight of the animals. Each animal receives about 0.3 - 0.4 ml inter-peritoneal respectively. After 72hr, sample was collected from rats by orbital puncture of all surviving animals and the blood was analyzed for glucose levels. Rats with blood glucose levels of 200mg/dl and above were considered as diabetic and selected for the study.

Estimation of Blood Glucose Levels By GOD-POD Method:

The enzymatic Glucose-oxidase-Peroxidase (GOD-POD) method was used for the estimation of blood glucose levels in rats.

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 in the presence of peroxidase (POD) to yield a red quinoneimine dye that is measured at 505nm. The absorbance at 505 nm is proportional to concentration of glucose in the sample.



Glucose kit constitutes following reagents.

1. Glucose reagent \rightarrow glucose oxidase peroxidase
2. Glucose diluent \rightarrow phosphate buffer, PH 7.4 phenol
3. Glucose standard \rightarrow dextrose (100mg/)

Preparation of working reagent:

Dissolve the contents of glucose reagent with glucose diluent, swirl the mixture gently to dissolve the contents, do not shake vigorously.

Specimen:

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 1: Preparations of Specimen Dilution

Sample	Blank	Standard	Sample
Working reagent	1ml	1ml	1ml
Distilled water	0.01	-----	-----
Standard	----	0.01	-----
Sample	-----	-----	0.01

Table 2: Pharmacodynamic interaction study in normal rats:

Group I (Control)	0.2 ml of 0.5 % sodium CMC solution: p.o.
Group II	Glyburide (5mg/kg; p.o) administered for 21days
Group III	Amlodipine (5mg.kg; p.o) administered for 21days
Group IV	Enalapril (10mg/kg; p.o) administered for 21days
Group V	Glyburide (5mg/kg) + Amlodipine (5mg/kg) administered for 21days
Group VI	Glyburide (5mg/kg) + Enalapril (10mg/kg) administered for 21days

Table 3: Pharmacodynamic interaction study in diseased rats:

Group I (Control)	0.2 ml of 0.5 % sodium CMC solution: p.o.
Group II	Glyburide (5mg/kg; p.o) administered for 21days
Group III	Amlodipine (5mg.kg; p.o) administered for 21days
Group IV	Enalapril (10mg/kg; p.o) administered for 21days
Group V	Glyburide (5mg/kg) + Amlodipine (5mg/kg) administered for 21days
Group VI	Glyburide (5mg/kg) + Enalapril (10mg/kg) administered for 21days

In the 21days study blood samples were collected from orbital sinuses at time intervals between 0, 0.5, 1, 2, 3, 4hrs in normal and treated groups and blood glucose levels were determined using GOD-POD method.

Statistical Analysis:

Data obtained from our study were statistically analyzed by Using Graph pad PRISEM and Graph Instant, software version 5 and 6, the data wear analyzed. The data were expressed as mean \pm standard error of mean (SEM) and results were analyzed by Unpaired T test with 2 tail method and One-way ANOVA followed by Dennett's t test. The P- value of <0.05 were considered significant.

RESULTS AND DISCUSSION

Interactions of Glyburide with Amlodipine and Enalapril in healthy rats:

After grouping of all the animals the study was carried out for 21days to study the interactions between the Glyburide with Amlodipine and Enalapril. So, on the first day of the treatment the glucose levels were estimated by collecting the blood from grouped animals at 0min (Fasting Glucose), 30min, 1hr, 2hr, 4hr respectively.

The glucose levels were estimated by using GOD-POD method. For this the overnight fastened animals were fed with oral glucose at a standard dose of 2gm/kg. After this administration, the blood samples were collected at regular intervals. At basal hour the animals were administered with following drugs, Glyburide 5mg/kg, Amlodipine 5mg/kg and Enalapril with 10mg/kg. Similarly for the combination groups administration of Glyburide, followed by Amlodipine and Enalapril after 30min. And the readings are as follows:

Table 4: Showing the Glucose (mg/dL) levels of the normal healthy animals on 1st day of the Treatment.

Groups	0 min (fasting)	30min	1hr	2hr	4hr
Normal	72	98	84	76	70
Glyburide (5mg/kg)	80	102	90	84	78
Amlodipine (5mg/kg)	75	114	106	100	89
Enalapril (10mg/kg)	78	101	89	80	74
Glyburide+Amlodipine	76	110	84	72	68
Glyburide+Enalapril	82	97	92	81	76

Glucose levels increased from 30min in case of Amlodipine and also the glucose levels are also increased up to 30min and then decreased in case of all drugs. In combinations there is a decrease in glucose levels because of Glyburide effect on both the drugs after 30min.

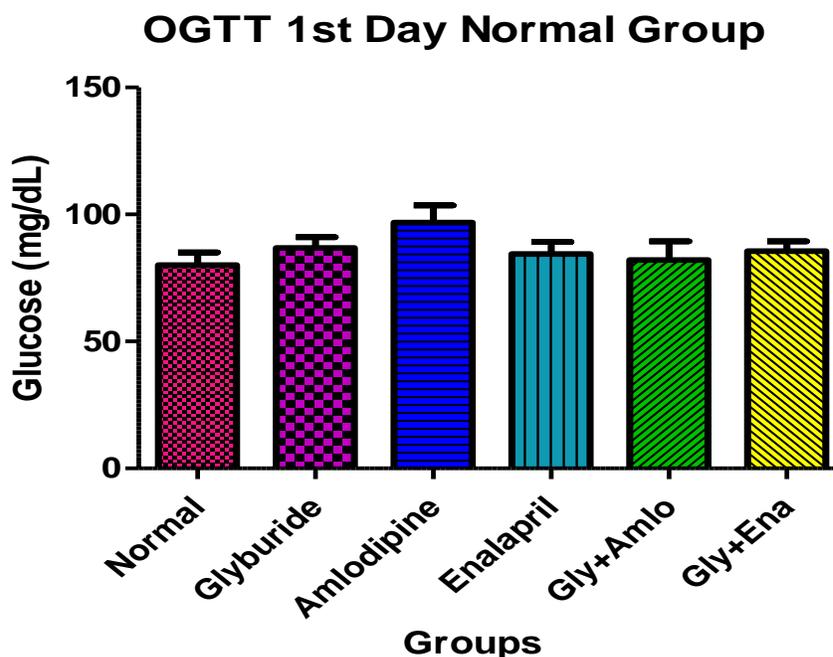


Figure 2: Graph showing the differences between groups

Values are expressed as mean \pm S.E.M; n=6 One way ANOVA followed by Dunnet Multiple comparison test using Graph pad INSTANT version 5.

After plotting the graph and the result obtained from ANOVA shows that the Amlodipine doesn't cause the significant reduction in glucose levels when compared with only glyburide group, but whereas Only Enalapril showed minimum reduction of glucose levels compared to Amlodipine. And in case of the combination is also the same. Glyburide with Enalapril showed better reduction in the glucose levels compared with that of Glyburide with Amlodipine group.

From the above obtained data we found that the glucose levels of Amlodipine group reduced to 85mg/dl, whereas Enalapril showed much better effect as compared to Amlodipine 74mg/dl. And when we are comparing with our standard drug i.e Glyburide caused the significant reduction of glucose levels from 80mg/dl after fasting to 78mg/dl after 4hrs of oral glucose load administration. The drug showing the maximum peak plasma concentrations after one hour of the administration.

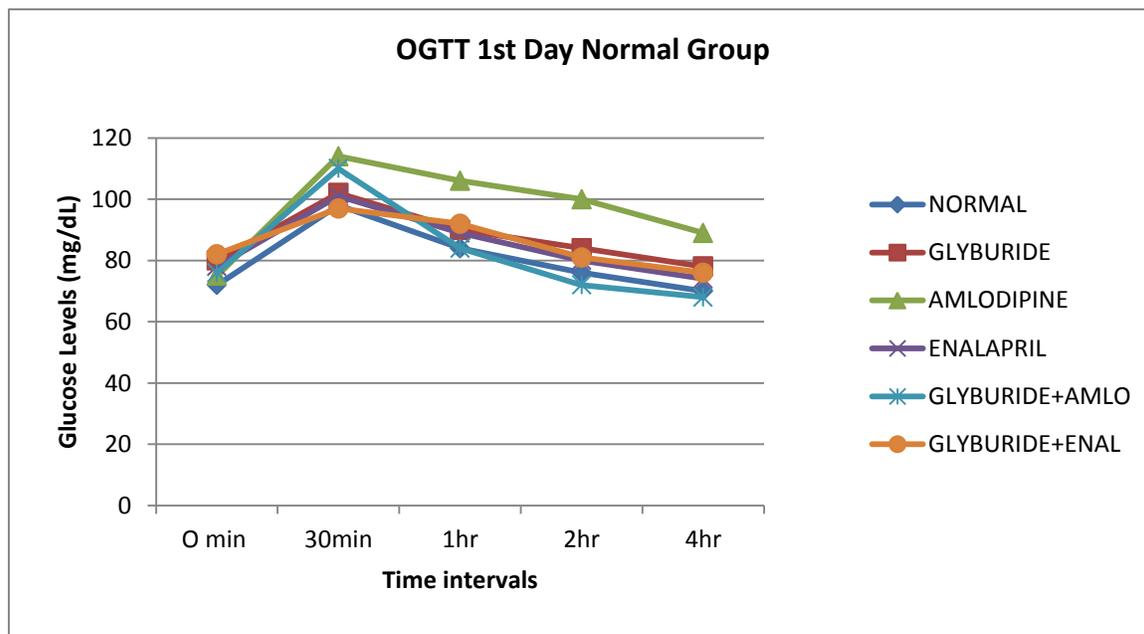


Figure 3: Graph Significant reductions of Glucose levels (mg/dl)

OGTT 7th DAY (NORMAL GROUP) INTERACTIONS:

During the continuous administration of drugs from day one, on 6th day animals were kept overnight fasten and on the 7th day the blood was withdrawn from the respective groups and glucose levels were estimated.

After the initial collection of blood the groups were administered with a glucose load of 2gm/kg, followed by the respective drug administrations.

The data obtained on 7th day of treatment in Normal Groups are as follows:-

Table 5: Showing the Glucose levels (mg/dL) of the Normal Group on 7th Day.

Groups	0 min (fasting)	30min	1hr	2hr	4hr
Normal	75	101	87	79	73
Glyburide (5mg/kg)	83	105	93	87	81
Amlodipine (5mg/kg)	78	117	109	103	92
Enalapril (10mg/kg)	81	104	94	84	77
Glyburide+Amlodipine	79	114	88	76	71
Glyburide+Enalapril	85	100	96	87	78

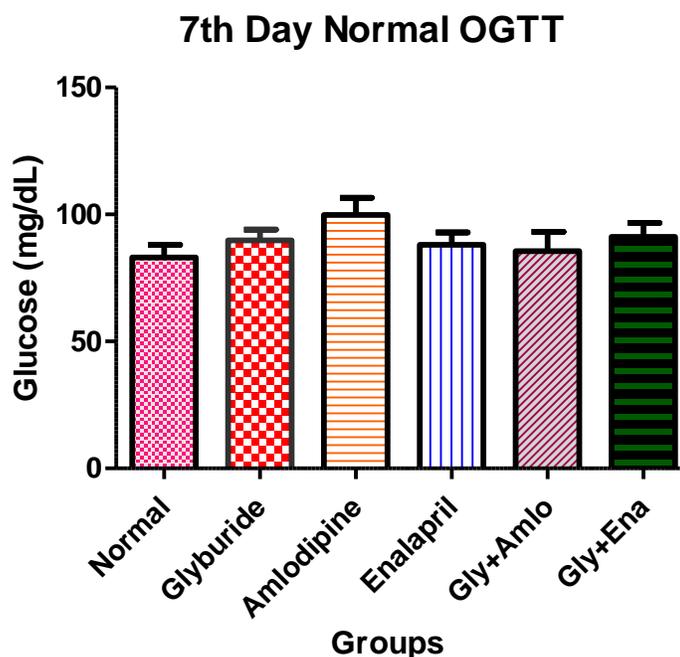


Figure 4: Graph showing the differences between groups

Values are expressed as mean \pm S.E.M; n=6 One way ANOVA followed by Dunnet Multiple comparison test using Graph pad INSTANT version 5.

After plotting the graph and the result obtained from ANOVA shows that the Amlodipine doesn't cause the significant reduction in glucose levels when compared with only glyburide group, but whereas Only Enalapril showed minimum reduction of glucose levels compared to Amlodipine. And in case of the combination is also the same. Glyburide with Enalapril showed better reduction in the glucose levels compared with that of Glyburide with Amlodipine group.

From the above obtained data we found that the glucose levels of Amlodipine group reduced to only 92mg/dl, whereas Enalapril showed much better effect as compared to Amlodipine 81mg/dl to 77mg/dl. And when we are comparing with our standard drug i.e Glyburide caused the significant reduction of glucose levels from 83mg/dl after fasting to 81mg/dl after 4hrs of oral glucose load administration. The drug showing the maximum peak plasma concentrations after one hour of the administration. And in the combined groups the Glyburide with Amlodipine showing glucose levels 79mg/dl at fasting stage i.e. 0min and followed by rise in levels up to one hour and by reduction of glucose levels to 71mg/dl. Similarly for Glyburide with Enalapril also from 85mg/dl to 78mg/dl.

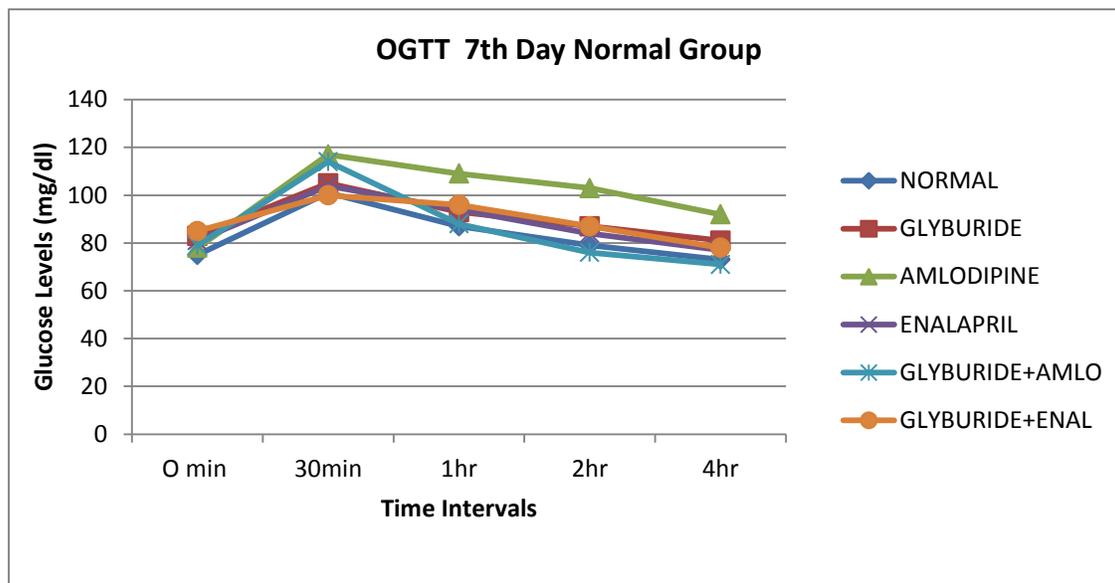


Figure 5: Graph Significant reductions of Glucose levels (mg/dl)

14th DAY OGTT (NORMAL GROUP) INTERACTION:

During the continuous administration of drugs from day one, on 13th day animals were kept overnight fasten and on the 14th day the blood was withdrawn from the respective groups and glucose levels were estimated.

After the initial collection of blood the groups were administered with a glucose load of 2gm/kg, followed by the respective drug administrations.

The data obtained on 14th day of treatment in Normal Groups are as follows:-

Table 6: Showing the Glucose levels (mg/dL) of the Normal Group on 14th Day.

Groups	0 min (fasting)	30min	1hr	2hr	4hr
Normal	77	103	89	81	75
Glyburide (5mg/kg)	85	107	95	89	73
Amlodipine (5mg/kg)	80	119	111	105	95
Enalapril (10mg/kg)	83	105	96	87	79
Glyburide+Amlodipine	81	113	91	78	72
Glyburide+Enalapril	84	102	98	89	76

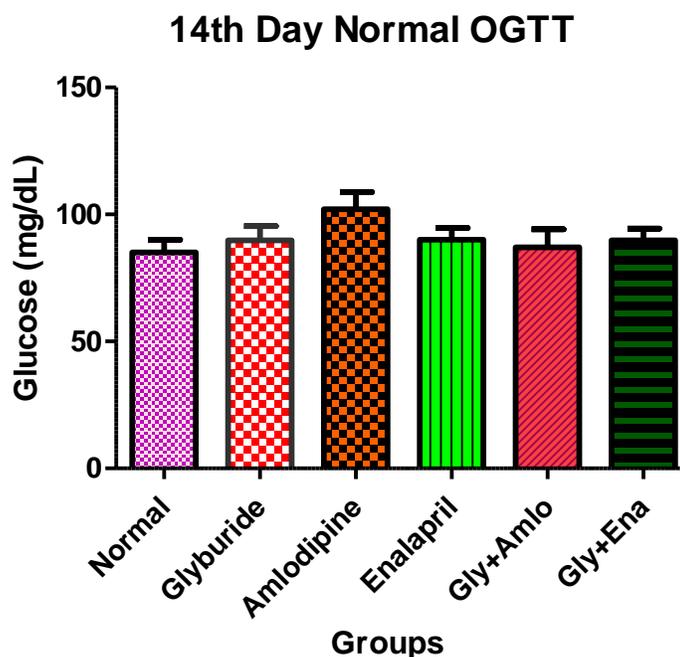


Figure 6: Graph showing the differences between groups

Values are expressed as mean \pm S.E.M; n=6 One way ANOVA followed by Dunnet Multiple comparison test using Graph pad INSTANT version 5.

After plotting the graph and the result obtained from ANOVA shows that the Amlodipine doesn't cause the significant reduction in glucose levels when compared with only Glyburide group, but whereas Only Enalapril showed minimum reduction of glucose levels compared to Amlodipine. And in case of the combination is also the same. Glyburide with Enalapril showed better reduction in the glucose levels compared with that of Glyburide with Amlodipine group.

From the above obtained data we found that the glucose levels of Amlodipine group reduced to only 95mg/dl, whereas Enalapril showed much better effect as compared to Amlodipine 83mg/dl to 79mg/dl. And when we are comparing with our standard drug i.e Glyburide caused the significant reduction of glucose levels from 85mg/dl after fasting to 73mg/dl after 4hrs of oral glucose load administration. The drug showing the maximum peak plasma concentrations after one hour of the administration. And in the combined groups the Glyburide with Amlodipine showing glucose levels 81mg/dl at fasting stage i.e. 0min and followed by rise in levels up to one hour and by reduction of glucose levels to 72mg/dl. Similarly for Glyburide with Enalapril also from 84mg/dl to 76mg/dl.

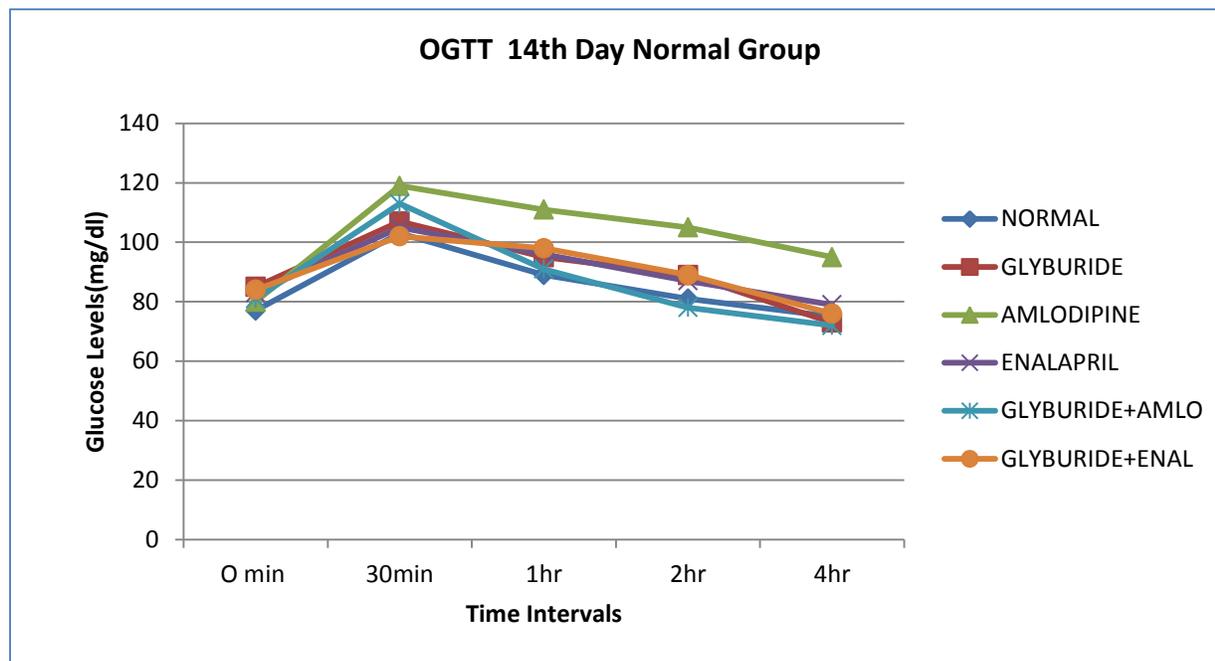


Figure 7: Graph Significant reductions of Glucose levels (mg/dl)

21st DAY OGTT (NORMAL GROUP) INTERACTION:

During the continuous administration of drugs from day one, on 20th day animals were kept overnight fasten and on the 21st day the blood was withdrawn from the respective groups and glucose levels were estimated.

After the initial collection of blood the groups were administered with a glucose load of 2gm/kg, followed by the respective drug administrations.

The data obtained on 21st day of treatment in Normal Groups are as follows:-

Table 7: Showing the Glucose levels (mg/dL) of the Normal Group on 21st Day.

Groups	0 min (fasting)	30min	1hr	2hr	4hr
Normal	74	102	85	78	72
Glyburide (5mg/kg)	82	106	94	88	79
Amlodipine (5mg/kg)	76	115	108	101	93
Enalapril (10mg/kg)	79	103	93	85	77
Glyburide+Amlodipine	80	112	89	76	69
Glyburide+Enalapril	77	98	92	88	73

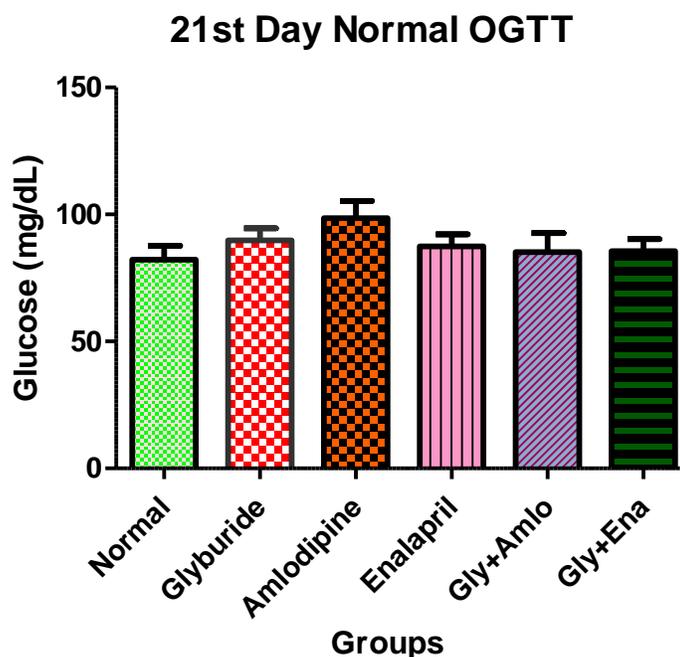


Figure 8: Graph showing the differences between groups

Values are expressed as mean \pm S.E.M; n=6 One way ANOVA followed by Dunnet Multiple comparison test using Graph pad INSTANT version 5.

After plotting the graph and the result obtained from ANOVA shows that the Amlodipine doesn't cause the significant reduction in glucose levels when compared with only glyburide group, but whereas Only Enalapril showed minimum reduction of glucose levels compared to Amlodipine. And in case of the combination is also the same. Glyburide with Enalapril showed better reduction in the glucose levels compared with that of Glyburide with Amlodipine group.

From the above obtained data we found that the glucose levels of Amlodipine group reduced to only 93mg/dl, whereas Enalapril showed much better effect as compared to Amlodipine 79mg/dl to 77mg/dl. And when we are comparing with our standard drug i.e Glyburide caused the significant reduction of glucose levels from 82mg/dl after fasting to 79mg/dl after 4hrs of oral glucose load administration. The drug showing the maximum peak plasma concentrations after one hour of the administration. And in the combined groups the Glyburide with Amlodipine showing glucose levels 80mg/dl at fasting stage i.e. 0min and followed by rise in levels up to one hour and by reduction of glucose levels to 69mg/dl. Similarly for Glyburide with Enalapril also from 77mg/dl to 73mg/dl.

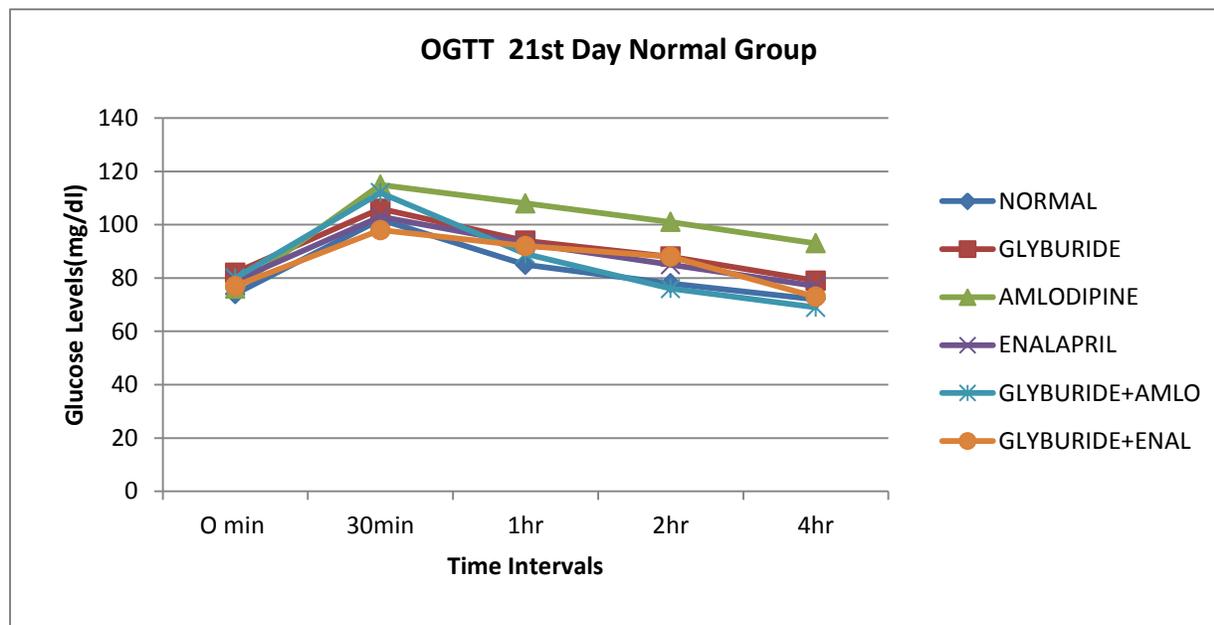


Figure 9: Graph Significant reductions of Glucose levels (mg/dl)

Interactions of glyburide with amlodipine and enalapril in diseased rats:

For carrying out the interactions of Glyburide with Amlodipine and Enalapril in diseased rats, the animals were administered with Alloxan (130mg/kg). After administration of alloxan within seven days the glucose levels have reached above 250mg/dl. Then according to the glucose levels the animals were grouped and the interactions were carried out for 21days. From the first day of treatment the drugs were administered like Glyburide 5mg/kg, Enalapril 10mg/kg, and Amlodipine 5mg/kg. And at an interval of every seven days the glucose levels were estimated by GOD-POD method i.e., on 1st, 7th, 14th and finally on 21st day.

After grouping of all the animals the study was carried out for 21days to study the interactions between the Glyburide with Amlodipine and Enalapril. So, on the first day of the treatment the glucose levels were estimated by collecting the blood from grouped animals at 0min (Fasting Glucose), 30min, 1hr, 2hr, 4hr respectively.

The glucose levels were estimated by using GOD-POD method. For this the overnight fastened animals were fed with oral glucose at a standard dose of 2gm/kg. After this administration, the blood samples were collected at regular intervals. At basal hour the animals were administered with following drugs, Glyburide 5mg/kg, Amlodipine 5mg/kg and Enalapril with 10mg/kg. Similarly for the combination groups administration of Glyburide, followed by Amlodipine and Enalapril after 30min. And the readings are as follows

1st DAY OGTT (DISEASED GROUP) INTERACTION:

Table 8: Showing the Glucose levels (mg/dL) of the Diseased Group on 1st Day

Groups	0 min (Fasting)	30min	1hr	2hr	4hr
Diseased Control	268	290	302	320	322
Glyburide (5mg/kg)	260	280	295	270	254
Amlodipine (5mg/kg)	258	290	301	294	280
Enalapril (10mg/kg)	272	304	310	290	266
Glyburide+Amlodipine	290	316	320	301	272
Glyburide+Enalapril	256	296	307	298	287

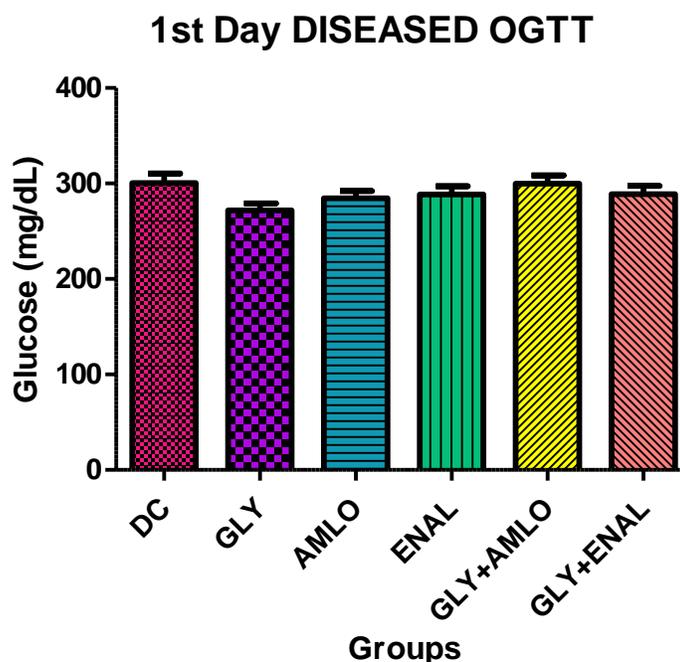


Figure 10: Graph showing the differences between groups.

One-way analysis of variance

P value 0.0002

P value summary ***

Are means signif. different? (P < 0.05) Yes

Number of groups 6

F 7.425

R square 0.6074

Values are expressed as mean \pm S.E.M; n=6 One way ANOVA followed by Dunnet Multiple comparison test using Graph pad INSTANT version 5.

After plotting the graph and the result obtained from ANOVA shows that the Amlodipine doesn't cause the significant reduction in glucose levels when compared with only glyburide group, but whereas Only Enalapril showed minimum reduction of glucose levels compared to Amlodipine. And in case of the combination is also the same. Glyburide with Enalapril showed better reduction in the glucose levels compared with that of Glyburide with Amlodipine group.

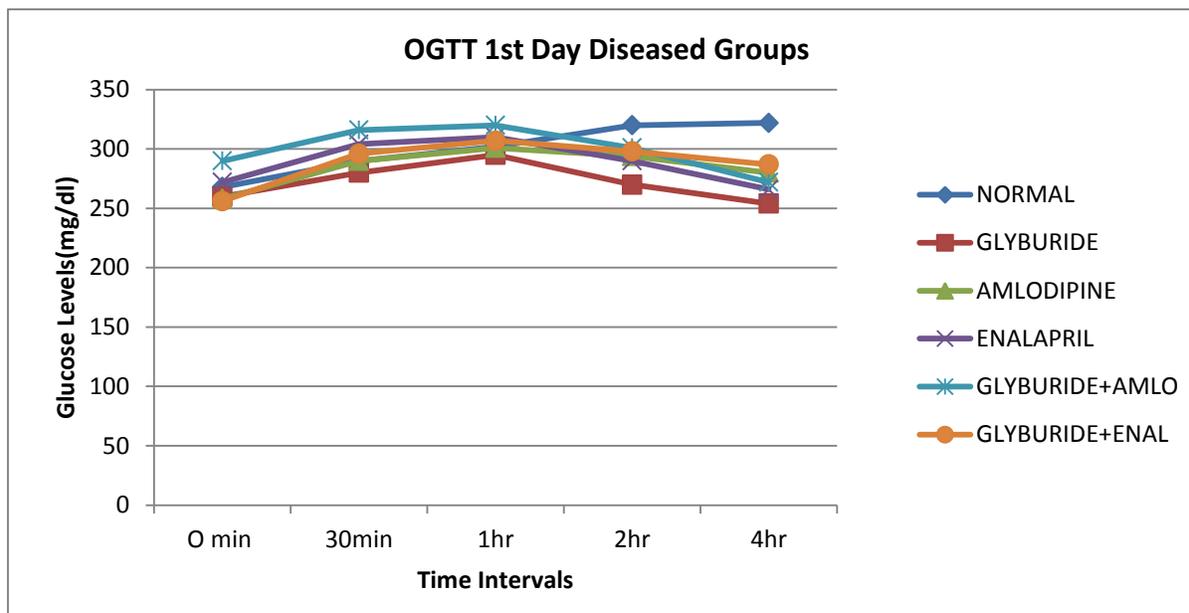


Figure 11: Graph Significant reductions of Glucose levels (mg/dl)

From the above obtained data we found that the glucose levels of Amlodipine group reduced to only 280mg/dl, whereas Enalapril showed much better effect as compared to Amlodipine 272mg/dl to 266mg/dl. And when we are comparing with our standard drug i.e Glyburide caused the significant reduction of glucose levels from 260mg/dl after fasting to 254mg/dl after 4hrs of oral glucose load administration. The drug showing the maximum peak plasma concentrations after one hour of the administration. And in the combined groups the Glyburide with Amlodipine showing glucose levels 290mg/dl at fasting stage i.e. 0min and followed by rise in levels up to one hour and by reduction of glucose levels to 272mg/dl. Similarly for Glyburide with Enalapril also from 256mg/dl to 287mg/dl.

7th DAY OGTT (DISEASED GROUP) INTERACTION:

Table 9: Showing the Glucose levels (mg/dL) of the Diseased Group on 7th Day.

Groups	0 min (Fasting)	30min	1hr	2hr	4hr
Diseased Control	280	299	306	321	338
Glyburide (5mg/kg)	216	230	261	250	202
Amlodipine (5mg/kg)	237	262	298	282	273
Enalapril (10mg/kg)	240	274	285	276	247
Glyburide+Amlodipine	220	258	274	269	229
Glyburide+Enalapril	210	238	267	256	233

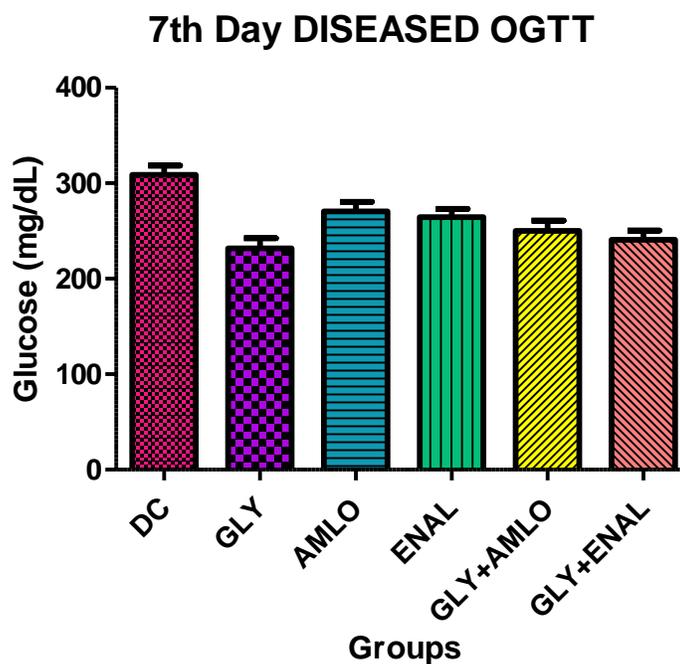


Figure 12: Graph showing the differences between groups.

One-way analysis of variance

P value 0.0002

P value summary ***

Are means signif. different? (P < 0.05) Yes

Number of groups 6

F 7.425

R square 0.6074

Values are expressed as mean \pm S.E.M; n=6 One way ANOVA followed by Dunnet Multiple comparison test using Graph pad INSTANT version 5.

After plotting the graph and the result obtained from ANOVA shows that the Amlodipine doesn't cause the significant reduction in glucose levels when compared with only glyburide group, but whereas Only Enalapril showed minimum reduction of glucose levels compared to Amlodipine. And in case of the combination is also the same. Glyburide with Enalapril showed better reduction in the glucose levels compared with that of Glyburide with Amlodipine group.

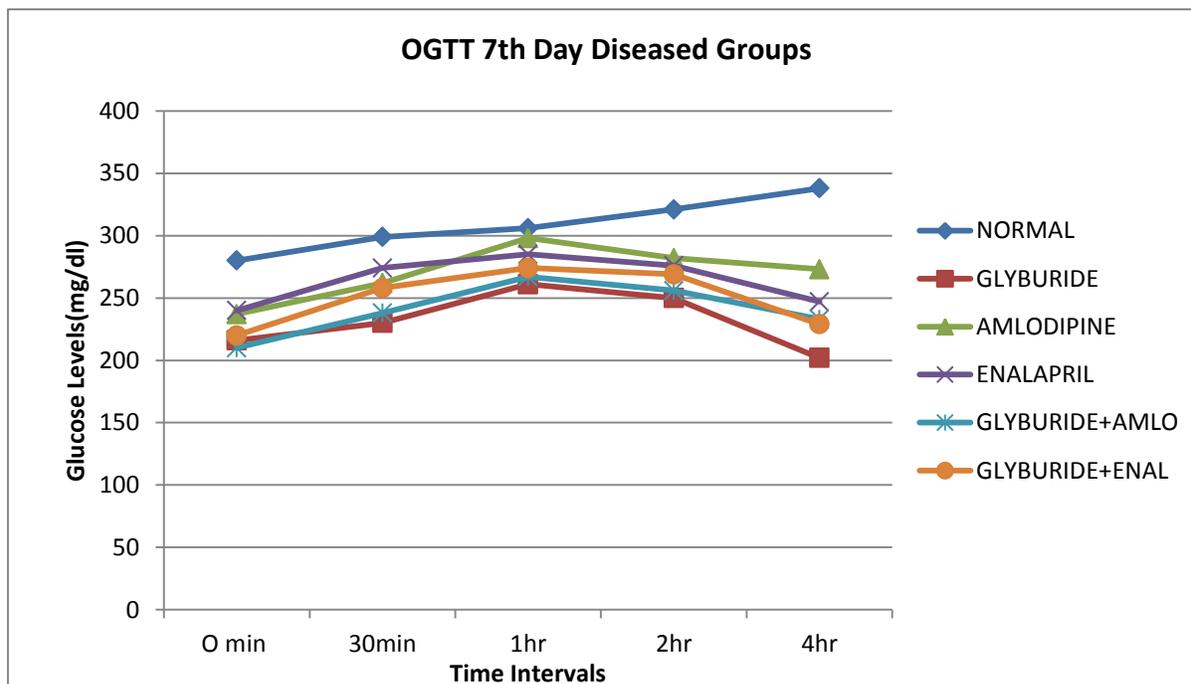


Figure 13: Graph Significant reductions of Glucose levels (mg/dl)

From the above obtained data we found that the glucose levels of Amlodipine group reduced to only 273mg/dl, whereas Enalapril showed much better effect as compared to Amlodipine 240mg/dl to 247mg/dl. And when we are comparing with our standard drug i.e Glyburide caused the significant reduction of glucose levels from 216mg/dl after fasting to 202mg/dl after 4hrs of oral glucose load administration. The drug showing the maximum peak plasma concentrations after one hour of the administration. And in the combined groups the Glyburide with Amlodipine showing glucose levels 220mg/dl at fasting stage i.e. 0min and followed by rise in glucose levels to 229mg/dl. Similarly for Glyburide with Enalapril also from 210mg/dl to 233mg/dl.

14th DAY OGTT (DISEASED GROUP) INTERACTION:

Table 10: Showing the Glucose levels (mg/dL) of the Diseased Group on 14th Day.

Groups	0 min (Fasting)	30min	1hr	2hr	4hr
Diseased Control	283	297	309	315	321
Glyburide (5mg/kg)	173	197	248	203	164
Amlodipine (5mg/kg)	206	225	252	247	221
Enalapril (10mg/kg)	197	236	259	241	206
Glyburide+Amlodipine	176	212	259	226	187
Glyburide+Enalapril	185	209	234	217	199

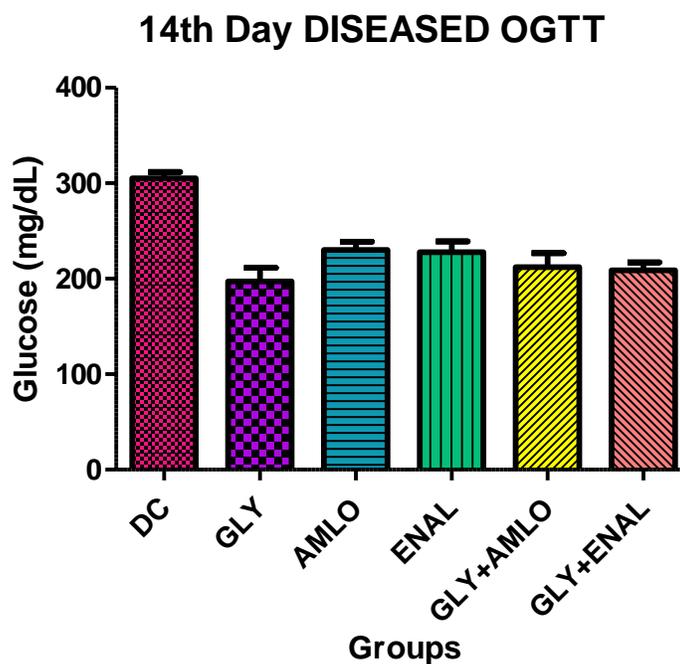


Figure 14: Graph showing the differences between groups.

One-way analysis of variance

P value < 0.0001

P value summary ***

Are means signif. different? (P < 0.05) Yes

Number of groups 6

F 11.98

R square 0.7140

Values are expressed as mean \pm S.E.M; n=6 One way ANOVA followed by Dunnet Multiple comparison test using Graph pad INSTANT version 5.

After plotting the graph and the result obtained from ANOVA shows that the Amlodipine doesn't cause the significant reduction in glucose levels when compared with only glyburide group, but whereas Only Enalapril showed minimum reduction of glucose levels compared to Amlodipine. And in case of the combination is also the same. Glyburide with Enalapril showed better reduction in the glucose levels compared with that of Glyburide with Amlodipine group.

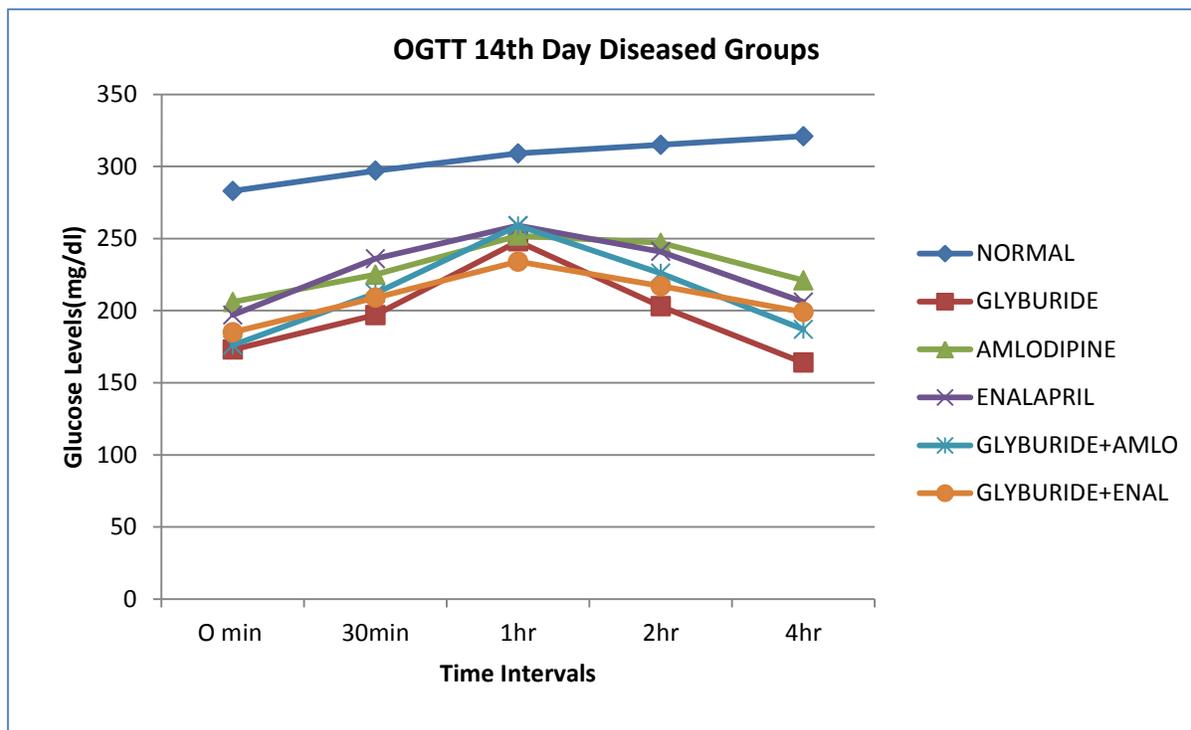


Figure 15: Graph Significant reductions of Glucose levels (mg/dl)

From the above obtained data we found that the glucose levels of Amlodipine group reduced to only 221mg/dl, whereas Enalapril showed much better effect as compared to Amlodipine 197mg/dl to 206mg/dl. And when we are comparing with our standard drug i.e Glyburide caused the significant reduction of glucose levels from 173mg/dl after fasting to 164mg/dl after 4hrs of oral glucose load administration. The drug showing the maximum peak plasma concentrations after one hour of the administration. And in the combined groups the Glyburide with Amlodipine showing glucose levels 176mg/dl at fasting stage i.e. 0min and followed by rise in glucose levels to 187mg/dl. Similarly for Glyburide with Enalapril also from 185mg/dl to 199mg/dl.

21st DAY OGTT (DISEASED GROUP) INTERACTION:

Table 11: Showing the Glucose levels (mg/dL) of the Diseased Group on 14th Day.

Groups	0 min (Fasting)	30min	1hr	2hr	4hr
Diseased Control	276	289	301	312	310
Glyburide (5mg/kg)	127	156	172	133	92
Amlodipine (5mg/kg)	158	184	216	204	197
Enalapril (10mg/kg)	169	209	237	214	182
Glyburide+Amlodipine	144	177	223	196	175
Glyburide+Enalapril	136	168	195	172	149

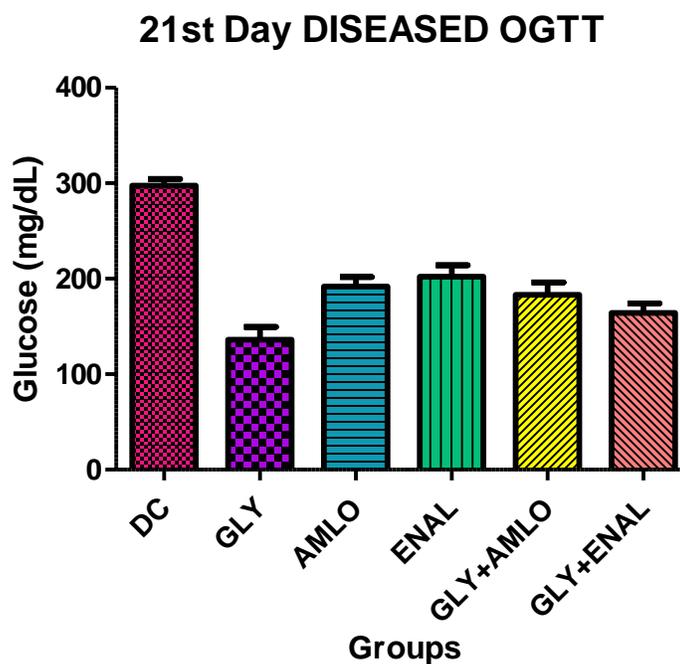


Figure 16: Graph showing the differences between groups

One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	24.36
R square	0.8354

Values are expressed as mean \pm S.E.M; n=6 One way ANOVA followed by Dunnet Multiple comparison test using Graph pad INSTANT version 5.

After plotting the graph and the result obtained from ANOVA shows that the Amlodipine doesn't cause the significant reduction in glucose levels when compared with only glyburide group, but whereas Only Enalapril showed minimum reduction of glucose levels compared to Amlodipine. And in case of the combination is also the same. Glyburide with Enalapril showed better reduction in the glucose levels compared with that of Glyburide with Amlodipine group.

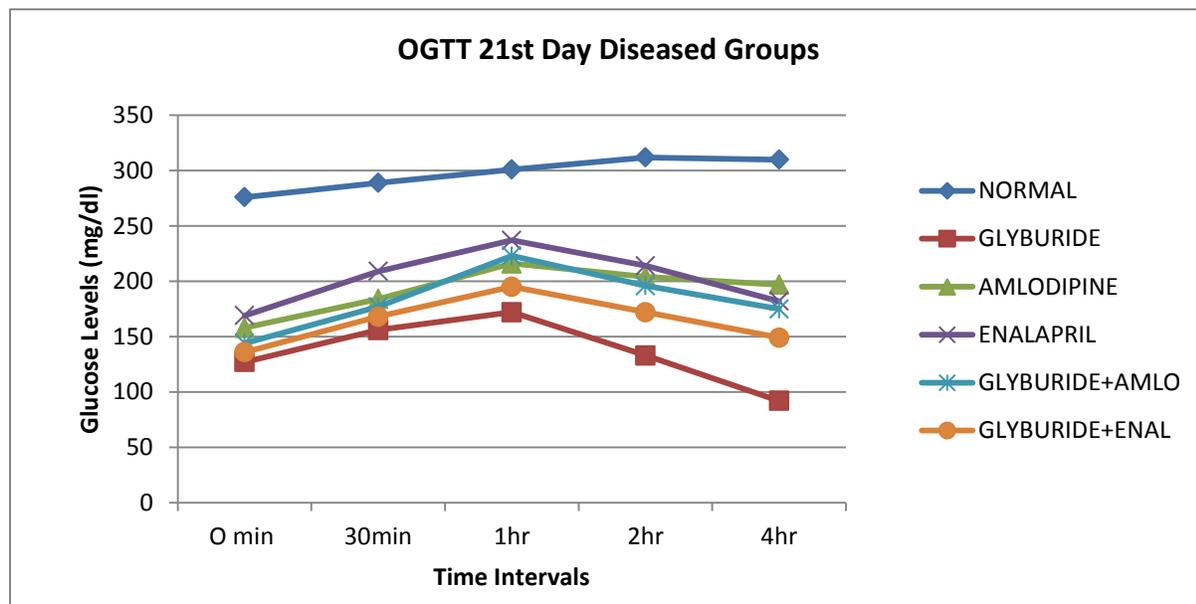


Figure 17: Graph Significant reductions of Glucose levels (mg/dl)

From the above obtained data we found that the glucose levels of Amlodipine group reduced to only 197mg/dl, whereas Enalapril showed much better effect as compared to Amlodipine 169mg/dl to 182mg/dl. And when we are comparing with our standard drug i.e Glyburide caused the significant reduction of glucose levels from 127mg/dl after fasting to 92mg/dl after 4hrs of oral glucose load administration. The drug showing the maximum peak plasma concentrations after one hour of the administration. And in the combined groups the Glyburide with Amlodipine showing glucose levels 144mg/dl at fasting stage i.e. 0min and followed by rise in glucose levels to 175mg/dl. Similarly for Glyburide with Enalapril also from 136mg/dl to 149mg/dl.

Percentage inhibition:

Table 12: Showing Percentage inhibition between Diseased groups from 1st Day to 21st Day.

Groups	1 ST Day	7 th Day	14 th Day	21 st Day
Diseased Control	300	309	305	297
Glyburide	271	231	197	136
Amlodipine	284	270	230	202
Enalapril	288	264	227	191
Gly + Amlo	300	250	212	183
Gly + Enala	289	240	208	164

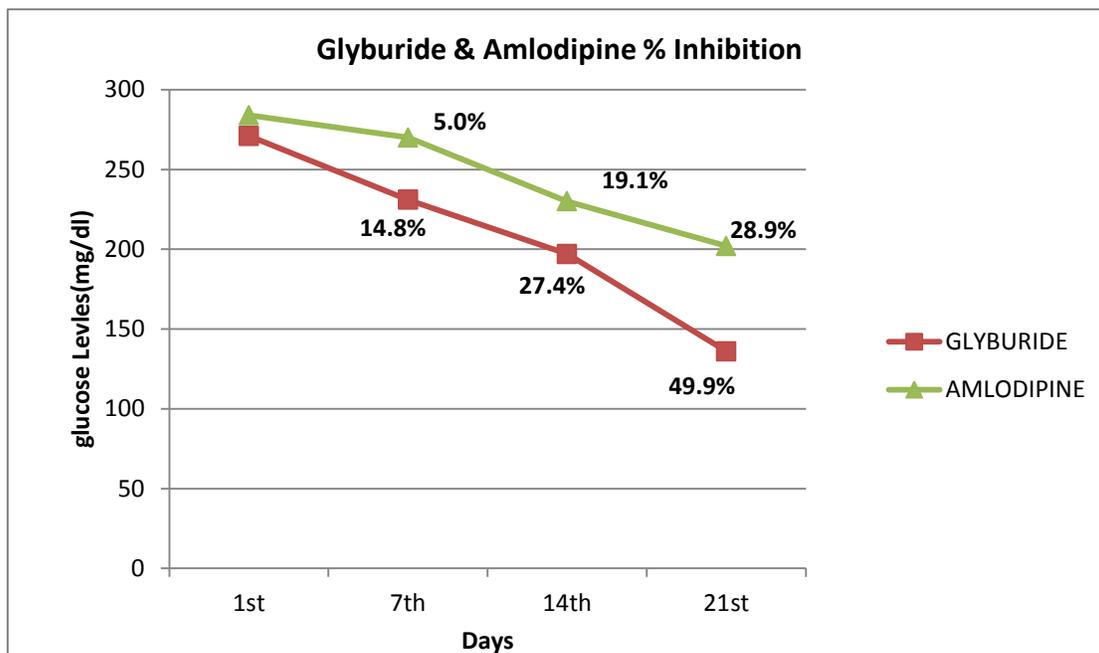


Figure 18: Showing percentage inhibition of Glyburide and Amlodipine

From the above graph we found that the inhibition of Glyburide is from 14.8% at day 7 to 49.9% at 21st Day. But when compare it with only Amlodipine group it is more Significant where as in case of Amlodipine it is showing only reduction from 5% to 28.9%. This shows that the Glyburide is having better Antihyperglycemic activity.

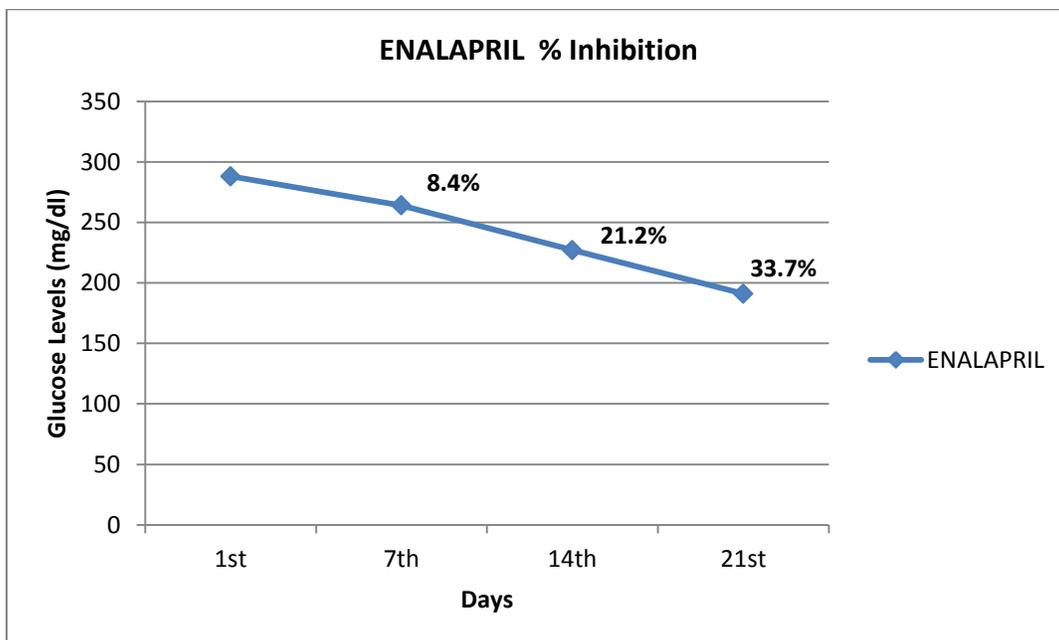


Figure 19: Showing percentage inhibition of only Enalapril

From above Figure: 23 we state that the percentage inhibition of Enalapril at 7th Day is 8.4% and at 14th Day it is 21.2% at final day i.e. on 21st Day it is showing a reduction of 33.7% when we compare it with glyburide it is less significant.

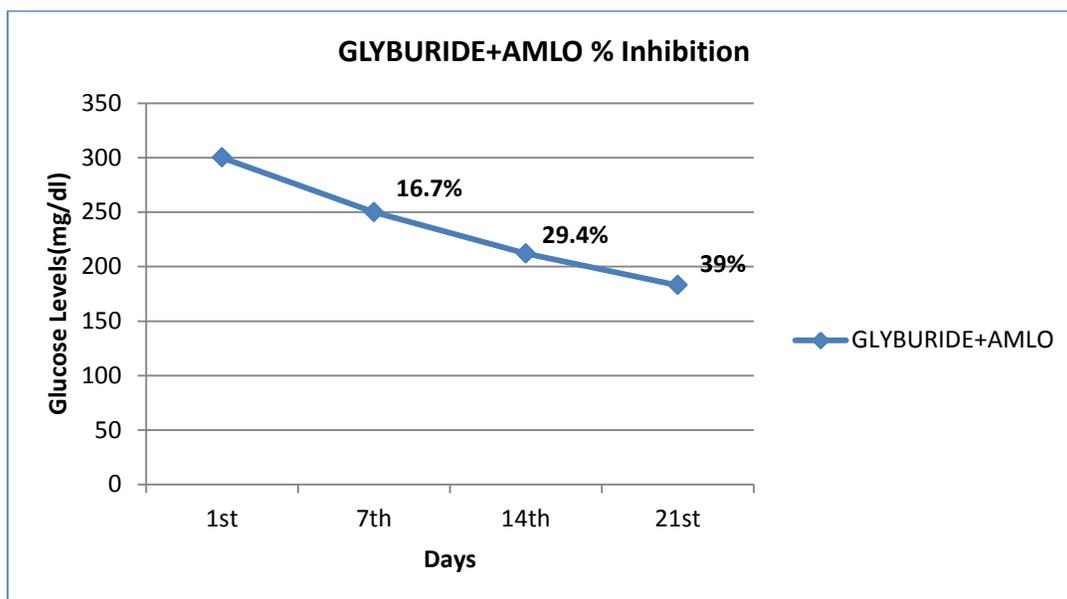


Figure 20: Showing percentage inhibition of Glyburide with Amlodipine

From above Figure: 24 we state that the percentage inhibition of Glyburide with Amlodipine at 7th Day is 16.7% and at 14th Day it is 29.4% at final day i.e. on 21st Day it is showing a reduction of 39% when we compare it with Glyburide it is less significant.

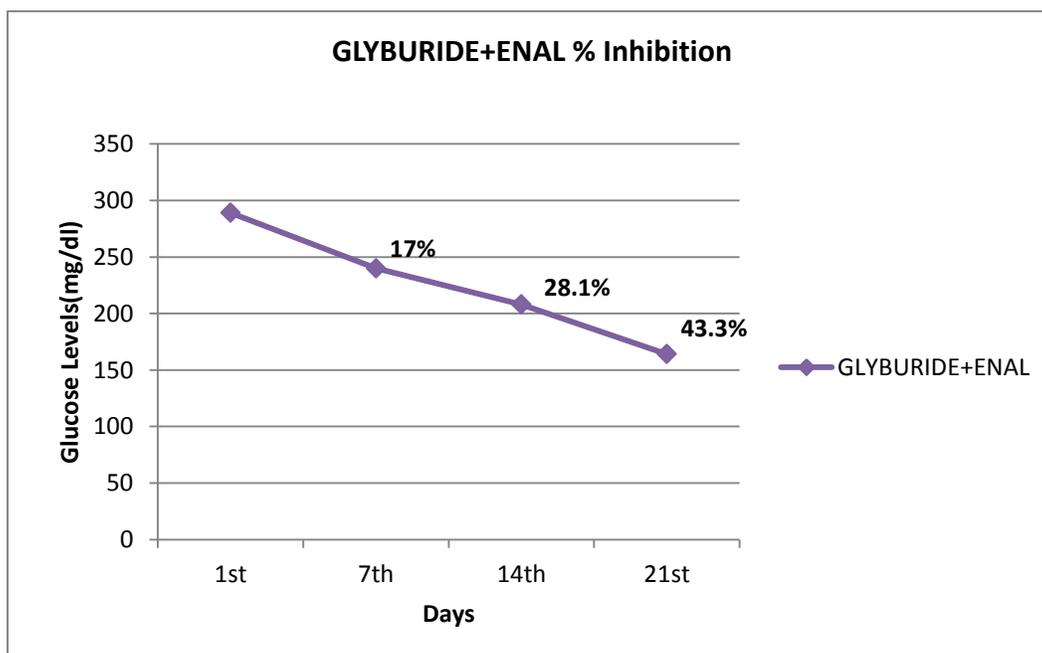


Figure 21: Showing percentage inhibition of Glyburide with Enalapril

From above Figure: 25 we state that the percentage inhibition of Glyburide with Enalapril at 7th Day is 17% and at 14th Day it is 28.1% at final day i.e. on 21st Day it is showing a reduction of 43.3% when we compare it with Glyburide it is less significant.

From all the obtained data we conclude that the drug only Amlodipine is having no effect on glucose levels when compared with Glyburide. But Enalapril is showing good results compared with Amlodipine i.e. Amlodipine is showing only 28.9%inhibition and Enalapril is showing 33.7% inhibition in case of diseased conditions.

In combined groups like Glyburide with Amlodipine is showing a percentage inhibition of 39% and Glyburide with Enalapril is showing 43.3% inhibition.

By this we conclude that Enalapril is having antihyperglycemic effect compared with Amlodipine. And the Combination can be suggested.

CONCLUSION

After the 21days of the study we found that the Glyburide have shown the 49.9% of inhibition. The Amlodipine and Enalapril groups showed 28.9% and 33.7% inhibition rates against the Glyburide group. The combined groups Glyburide with Amlodipine and Glyburide with Enalapril have shown 39% and 43.3% inhibition rates. From this above results we conclude that the combination of Glyburide with Enalapril does affect the blood glucose levels and the combination of Glyburide with Amlodipine also showed good results but comparatively less than the Glyburide with Enalapril group. But where as in the single groups of Enalapril and Amlodipine showed less effects comparing with Amlodipine and Enalapril the Enalapril showed little diabetic activity. When we compared the body weights of the all groups there showed much similar results to one another showing that no effect on their body weights. From all the results obtained we conclude that Enalapril has no effect on the blood glucose levels in using alone or in the combination with Glyburide. The Amlodipine also have similar effect but less when compared with Enalapril. More results can be obtained if we have done Pharmacokinetic study.

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