

***In vitro* and *In vivo* drug-drug interaction between Sitagliptin phosphate and Atenolol.**

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ABSTRACT

The aim of the present study to evaluate the *in vitro* and *in vivo* complexation nature and strength of complex which may be formed due to interaction between sitagliptin phosphate and atenolol. The interaction of sitagliptin phosphate and atenolol has been studied in aqueous system at a fixed temperature (37°C) at both gastric pH (pH 1.2 and pH 3.2) and intestinal pH (pH 6.8) by using Job's method of continuous variation and Ardon's method. Oral glucose tolerance test (OGTT) was used to identify *in vivo* DDI in mice. From spectrophotometric study, sitagliptin phosphate and atenolol give different spectra when sitagliptin phosphate mixed with atenolol in 1:1 ratio. The intensity of the spectra of sitagliptin phosphate slightly changes due to interaction. The jobs plot was obtained by plotting absorbance difference against the mole fraction of the each drug at pH 1.2, pH 3.2 and pH 6.8. When the spectra of pure sitagliptin phosphate and atenolol were compared with 1:1, 1:2 and 2:1 mixture, there was a change observed which indicate the formation of 1:1, 1:2 and 2:1 complexes of sitagliptin phosphate with atenolol. The values of stability constant is more than one at all pH (1.2, 3.2 and 6.8), which is the indication of strong complex. The stability constant values are 1.2073, 1.1839 and 1.2075 respectively. From the *in vivo* observation of Oral glucose tolerance test, at 1:1 complex, blood glucose level decrease (13.88%) less compared to Sitagliptin phosphate (22.22%) significantly. Complex (1:1) showed different activity rather than pure sitagliptin phosphate treatment due to complexation. Therefore, it can be concluded that a careful consideration is needed during concurrent administration of Sitagliptin phosphate with Atenolol.

Keywords: Drug-drug interaction, Sitagliptin phosphate, Atenolol, Ardon's, Jobs method.

INTRODUCTION

Drug-drug interactions are the important cause of therapeutic problems and increased number of hospital admissions within Asia, where Bangladesh has a leading position. It is because of lack of knowledge. Both physicians and medicine taker (patient) are not concern. As a result, this problem rising dangerously. The United States (US) withdraw half of drugs from the market, because of safety reasons, during the period 1999-2003, was associated with important drug interactions¹. The harmful effects of drugs are sometimes result of drug-drug interactions, this can be prevented by taking appropriate action. It was estimated that more than three-quarters of clinically significant drug interactions can be avoided². For adequate management of drug-drug interactions, the access to appropriate information sources is very important for health professionals³. But problem seriously arise when new drugs come in the market, where lack of information included in its descriptions. So, drug-drug interaction (DDI) or drug-food interaction (DFI) is challenging sector, where huge amount of medicine are present in the market. But multiple drug therapy is a common and useful practice for the treatment of diseases where two or more drugs are given at the same time or concurrently. For this reason DDI should measure selective group of drugs with new medicine.

Sitagliptin phosphate is a dipeptidyl peptidase-4 inhibitor that is thought to act in type 2 diabetes by slowing the inactivation of incretion hormones. It is usually administered orally. This enzyme-inhibiting drug is used either alone or in combination with other oral antihyperglycemic agents (such as metformin or a thiazolidinedione) for treatment of diabetes mellitus type 2⁴. The benefit of this medicine is its fewer side effects (e.g., less hypoglycemia, less weight gain) in the control of blood glucose values. While safety is its advantage, efficacy is often challenged as it is often recommended to be combined with other agents such as metformin. Sitagliptin works to competitively inhibit the enzyme dipeptidyl peptidase 4 (DPP-4). This enzyme breaks down the incretins GLP-1 and GIP, gastrointestinal hormones released in response to a meal⁵. By preventing GLP-1 and GIP inactivation, they are able to increase the secretion of insulin and suppress the release of glucagon by the alpha cells of the pancreas. This drives blood glucose levels towards normal.

Atenolol is a selective β_1 receptor antagonist, a drug belonging to the group of beta blockers (sometimes written β -blockers), a class of drugs used primarily in cardiovascular diseases. Introduced in 1976, atenolol was developed as a replacement for propranolol in

the treatment of hypertension. It works by slowing down the heart and reducing its workload. Unlike propranolol, atenolol does not pass through the blood-brain barrier to a large extent thus decreasing the incidence of various central nervous system side effects⁶. Atenolol is one of the most widely used β -blockers in the United Kingdom and was once the first-line treatment for hypertension. The role for β -blockers in hypertension was downgraded in June 2006 in the United Kingdom to fourth-line, as they perform less appropriately or effectively than newer drugs, particularly in the elderly⁷.

The present study was to find out the drug-drug interactions (DDIs) as well as to determine the stability of the complexes, which could be formed after interaction between Sitagliptin phosphate and Atenolol at various pH. The values of stability constants were determined by using Job's continuous-variation analysis and Ardon's spectrophotometric measurement methods. DDI also measured with *in vivo* Oral glucose tolerant test (OGTT).

MATERIALS AND METHODS

Drugs and chemicals

Sitagliptin phosphate and Atenolol were collected from Albion Laboratories Limited. Sodium dihydrogen orthophosphate and di-sodium hydrogen orthophosphate, used for the preparation of buffer solutions were purchased from Merck (Germany). Sodium hydroxide and Sodium chloride were purchased from Riedel-De Haen Ag, Seelze-Hannover, Germany. All chemicals and reagents were of analytical grade.

Equipment

UV-Visible spectrometer (Model No. UV-1600, Shimadzu, Japan) was used for the test. Rapid ViewTM (Blood glucose monitoring system, Model: BIO-M1, BIOUSA Inc, California, USA) with strips were purchased from Andorkilla, Chittagong.

Animals and experimental set-up

Swiss albino mice, weighing about 28–35 g, were collected from Jahangir Nagar University, Savar, Bangladesh. The animals were furnished with standard lab nourishment and refined water ad libitum and maintained at natural regular day-night cycle having legitimate ventilation in the room. All the experiments were conducted in an isolated and noiseless condition. The study protocol was approved by the P&D Committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh. The mice were acclimatized to laboratory condition for 7 days prior to

experimentation. All animal experiments were carried out according to the guidelines of Institutional Animals Ethics Committee and study protocols were approved by the Department of Pharmacy, International Islamic University Chittagong Medical Ethics, Biosafety. Ethical committee approval no Pharm- 71/09-15/46.

Preparation of standard solutions

100 ml of 10^{-3} M solution of Sitagliptin phosphate was prepared as the stock solution by dissolving 0.0523 gm of Sitagliptin phosphate in 100ml of DW in a 100 ml volumetric flask. 100 ml of 10^{-3} M solution of Atenolol was prepared as the stock solution by dissolving 0.0266 gm of Atenolol in 100ml of DW in a 100 ml volumetric flask.

Absorption spectrum analysis

In observation of the spectra, the absorption characteristics of Sitagliptin phosphate and Atenolol and their 1:1 mixtures in the solutions of buffers ⁸ pH 1.2, 3.2 and 6.8 were compared with those of each interacting species. The concentrations of the sample were kept at very dilute levels in each case and the measurements made using UV-VIS spectrophotometer. The spectra of the working standard solutions (1×10^{-3} M) were recorded between 400 - 200 nm. The spectra were compared with those of the pure samples in each case.

Job's Spectrophotometric method

According to Job's method ^{9,10} the absorbance of series of Sitagliptin phosphate with Atenolol in different molar ratios 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 were measured by keeping the total mole constant. The observed absorbance of the mixtures at various mole fractions was subtracted from sum of the values for free drugs (Sitagliptin phosphate and Atenolol). The absorbance difference (D) was then plotted against the mole fractions of the drug in the mixtures. If the formation constant is reasonably favorable, two straight lines of different slopes that intersect at a mole ratio that corresponds to the combining ratio in the complex are obtained ^[20].

Ardon's spectrometric method

In the Ardon's Spectrophotometric method, ^{11,12} concentrations of Sitagliptin phosphate was varied while keeping the concentrations of Atenolol fixed at 1×10^{-3} M. All the experiments were performed in buffer at pH 1.2, 3.2 and 6.8. The absorbances of solutions were measured at 267 nm using UV-VIS spectrophotometer. The Ardon's equation was used for calculation. This equation is given below-

$$\frac{1}{(D - \epsilon_A C)} = \frac{1}{KC (\epsilon_{com} - \epsilon_A) [B]^n} + \frac{1}{C(\epsilon_{com} - \epsilon_A)}$$

Where, D = Absorbance of the mixture, B = Molar concentration of the Sitagliptin phosphate. C = Molar concentration of the Atenolol, n=Number of Mole, ϵ_{com} = Molar extinction co-efficient of the complex, ϵ_A = Molar extinction co-efficient of the Sitagliptin phosphate.

The value of n was chosen as 1, which is an essential condition for validation of the method. The value for $1 / (D - \epsilon_A C)$ was plotted versus $1 / D$ to get the straight lines.

The stability constant of the complex was given by the relation,

$K = \text{intercept} / \text{slope}$

It is to be mentioned that this method is only valid for the systems where 1:1 complexes are found.

Hypoglycemic effect in glucose induced hyperglycemic mice (OGTT)

Oral glucose tolerance test (OGTT) was performed according to the standard method ¹³ with minor modification. Group I was treated as normal control group, Group II treated with Sitagliptin phosphate (50mg/kg, p.o.), Group III were treated with Atenolol (50mg/kg, p.o.) and Group VIII were treated with Sitagliptin phosphate (50mg/kg, p.o.)+ Atenolol (50mg/kg, p.o.) of 1:1 complex respectively. Glucose solution (1 g/kg body weight) was administered at first. Then drugs and complex solutions were administered to the glucose fed mice. Serum glucose level of blood sample from tail vein was estimated by using glucometer (Rapid ViewTM) at 0, 30min, 60min, 90 min and 120 min. Percent decrease of blood glucose level after 120 min measured by following equation,

$$\% \text{ decrease} = \frac{GL_{0 \text{ min}} - GL_{120 \text{ min}}}{GL_{0 \text{ min}}} \times 100$$

$GL_{0 \text{ min}}$ = Blood Glucose level at 0 min,

$GL_{120 \text{ min}}$ = Blood Glucose level at 120 min.

Statistical analysis

The results were expressed as the mean. Regression analysis was performed to calculate slope and intercept. Student's t test was performed between Stability Constants. Statistical programs used were GRAPHPAD PRISM® (version 6.00; GraphPad Software Inc., San Diego, CA, USA), which also used for graph preparation. The results were

expressed for OGTT as mean±SEM. The results were statistically analyzed using repeated measures analysis of variance with Dunnett's test by using SPSS (version 22.0), when compared against negative control in OGTT. P<0.05 and P<0.01 were considered as statistically significant.

RESULTS

Spectral study

The drugs studied showed absorption in UV-VIS region. The molecular species of Sitagliptin phosphate when mixed with Atenolol showed some changes in absorption characteristics of this molecule (Atenolol) including some shifts in the absorption maxima. Thus alteration in spectral pattern may be regarded as an indicator for the primary interaction among these drugs. The UV absorption values of the drug and drug mixtures were measured at 200-400 nm. 1 ml of 10⁻³ M Sitagliptin phosphate and 1 ml of 10⁻³ M Atenolol were mixed and absorbances were measured within the range of 200-400 nm. Before that individual absorbance of 10⁻³ M Sitagliptin phosphate and Atenolol were measured (Figure 1).

Study of Job's method

The molar ratios of the complexes of Sitagliptin phosphate with Atenolol were estimated by Job's method of continuous variation. The observed absorbance values were measured in pH 1.2, 3.2 and 6.8 at various concentrations (0.1 x 10⁻³ to 0.9 x 10⁻³ M) Sitagliptin phosphate with Atenolol of at 267 nm. The Job's plots at pH 1.2, 3.2 and 6.8 were obtained by plotting absorbance differences against the mole fraction of the drug (Sitagliptin phosphate) which are presented in Table 1 and Figure 2.

Effect of Sitagliptin phosphate on Atenolol using Ardon's method

Ardon's plot confirmed the formation of 1:1 complex of Sitagliptin phosphate and Atenolol at pH 1.2, 3.2 and 6.8, since the method is valid only for 1:1 complexes. The values of 1/[drug] by using the Ardon's equation:

$$\frac{1}{(D - \epsilon_A C)} = \frac{1}{KC (\epsilon_{com} - \epsilon_A) [B]^n} + \frac{1}{C (\epsilon_{com} - \epsilon_A)}$$

This experiment was performed in buffer systems at pH 1.2, 3.2 and 6.8. The data for Ardon's gave straight lines with intercept which are presented in Table 2 and Figure 3 indicates the formation of 1:1 complexes for the system at both pH.

Estimation of Stability Constant

The value of stability constant for the complexation of Sitagliptin phosphate with Atenolol at pH 1.2, 3.2 and 6.8 were obtained from the spectral data using Ardon's plot. The values for stability constant were calculated from the slopes and intercepts of the straight lines from these plots. It was seen from the Ardon's equation that the values of stability constant was given as [(intercept) / (slope)] of straight line so obtained. i. e. k = (intercept) / (slope). The value of intercept and slope were calculated by Least Squares Method using the following equation:

$$y = mx + C$$

The values of stability constants for the drug-metal system at pH 1.2, 3.2 and 6.8 presented in the Table 3.

In vivo drug interactions study of Sitagliptin phosphate with Atenolol was evaluated by Oral Glucose Tolerance Test

Investigational induction of hyperglycemia resulted in increased glucose level in blood (comparing the bar diagram of control of 0 h and 1 hour, Figure 4). All the treatments significantly reduce blood glucose level in 30 min after administration. Most significant reduction (P<0.01) was observed for Sitagliptin and the complex (1:1) at 120 min. But, Atenolol didn't reduce blood glucose level after 120 min of the treatment. At 1:1 complex, blood glucose level decrease (13.88%) less compared to Sitagliptin phosphate (22.22%) significantly (P<0.01). Time interaction with each specific hour in this experiment was also found significant (P<0.05-0.01). All results are presented in Table 4 and Figure 5. Percentage of decrease of blood glucose level in glucose induced mice after 120 min with different treatment are showed in Figure 5.

DISCUSSION

Initial evidence for complexation of Sitagliptin phosphate with Atenolol came from differences between the spectra of the drugs and those of their mixtures in buffer solutions. Each compound has its unique molecular structure or electronic configuration which is responsible for absorption of light. It is obvious that each compound has its unique molecular structure or electronic configuration which is responsible for absorption of light in the form of ultraviolet or visible form^{14,15}. When Atenolol mixed with Sitagliptin phosphate in 1:1 ratio the intensity of the peak of Sitagliptin phosphate change slightly (absorbance decreases) i.e. absorption characteristics are altered due to interaction but the position of the compound do not shift. The Ardon's plots have been used to evaluate the stability constants and it has been

observed that when values of $1 / (D - C\epsilon A)$ are plotted against $1 / \text{Drug}$ (Figure 5), these lines are obtained obeying the Ardon's equation. The values of stability constants at different pH are shown in table 3. Stability constants data showed that Sitagliptin phosphate-Atenolol system formed relatively stronger complexes at all pH conditions.

From the *in vivo* observation of OGTT test, at 1:1 complex, blood glucose level decrease (13.88%) less compared to Sitagliptin phosphate (22.22%) significantly. Because when drug-drug interaction happened, then drug cannot exhibit its main activity. That's why in this test complex showed different activity. But, Sitagliptin showed its activity low in the form of complex, but showed its activity.

CONCLUSION

Interaction of Sitagliptin phosphate with Atenolol decreased the free drug concentration of both drugs

which can result in decreased availability of the drugs at receptors. Ultimately, one or both drugs may show diminished pharmacologic activity. In addition, Sitagliptin phosphate and Atenolol lowered protein binding of Atenolol, could increase the volume of distribution of Atenolol. The results from all these allowed to conclude that Sitagliptin phosphate formed stable complex with Atenolol. *In vivo* result also supports this. Therefore, precaution and monitoring must be exercised during concurrent therapy of both drugs.

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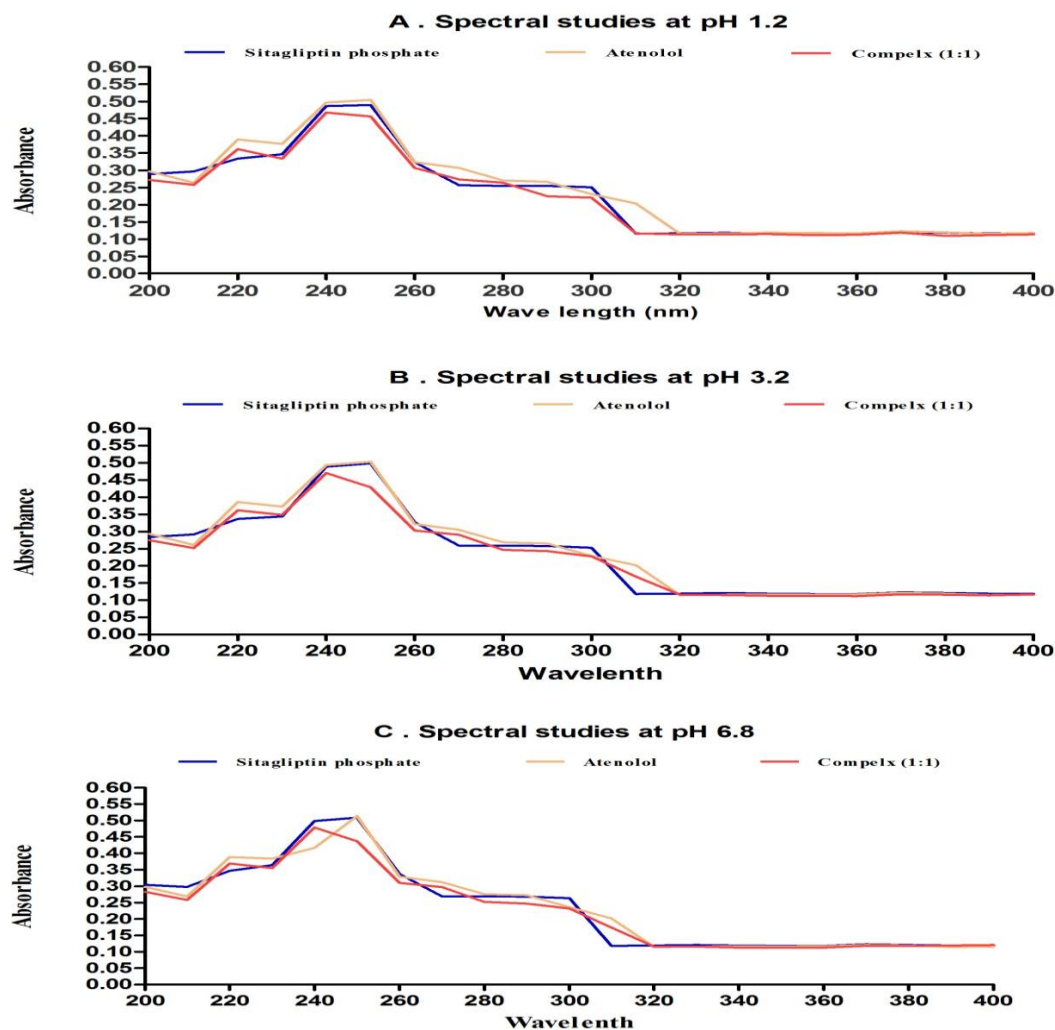
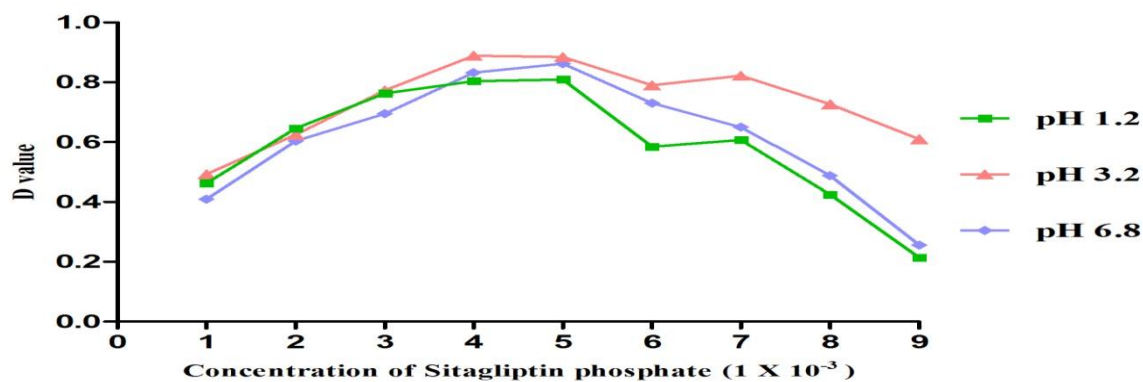


Figure 1: Spectral studies of Sitagliptin phosphate, Atenolol and their (1:1) complex for A. pH 1.2, B. pH 3.2 and C. pH 6.8.

Table 1: Absorbance of Sitagliptin phosphate at different pH (using Job's method)

Concentration of Sitagliptin phosphate (1 X 10 ⁻³)	Absorbance(D value)		
	pH 1.2	pH 3.2	pH 6.8
1	0.463	0.493	0.409
2	0.646	0.625	0.604
3	0.763	0.773	0.695
4	0.804	0.889	0.832
5	0.809	0.885	0.862
6	0.585	0.790	0.730
7	0.607	0.822	0.650
8	0.425	0.727	0.488
9	0.214	0.610	0.256

Values are presented as Mean (n=3).

**Figure 2:** Job's plot for complexation of Sitagliptin phosphate with Atenolol at 267 nm.**Table 2: Absorbance of Sitagliptin phosphate at different pH (using Ardon's method, when concentration of Atenolol is constant)**

Concentration of Sitagliptin phosphate (1/D X 10 ⁻³)	Absorbance (D value)		
	pH 1.2	pH 3.2	pH 6.8
1.000	10	10.42	8.40
0.500	2.59	4.10	2.43
0.333	2.33	2.33	1.05
0.250	1.54	1.47	1.24
0.200	1.28	1.16	1.07
0.167	1.02	0.97	0.90
0.143	0.89	0.86	0.79

Values are presented as Mean (n=3).

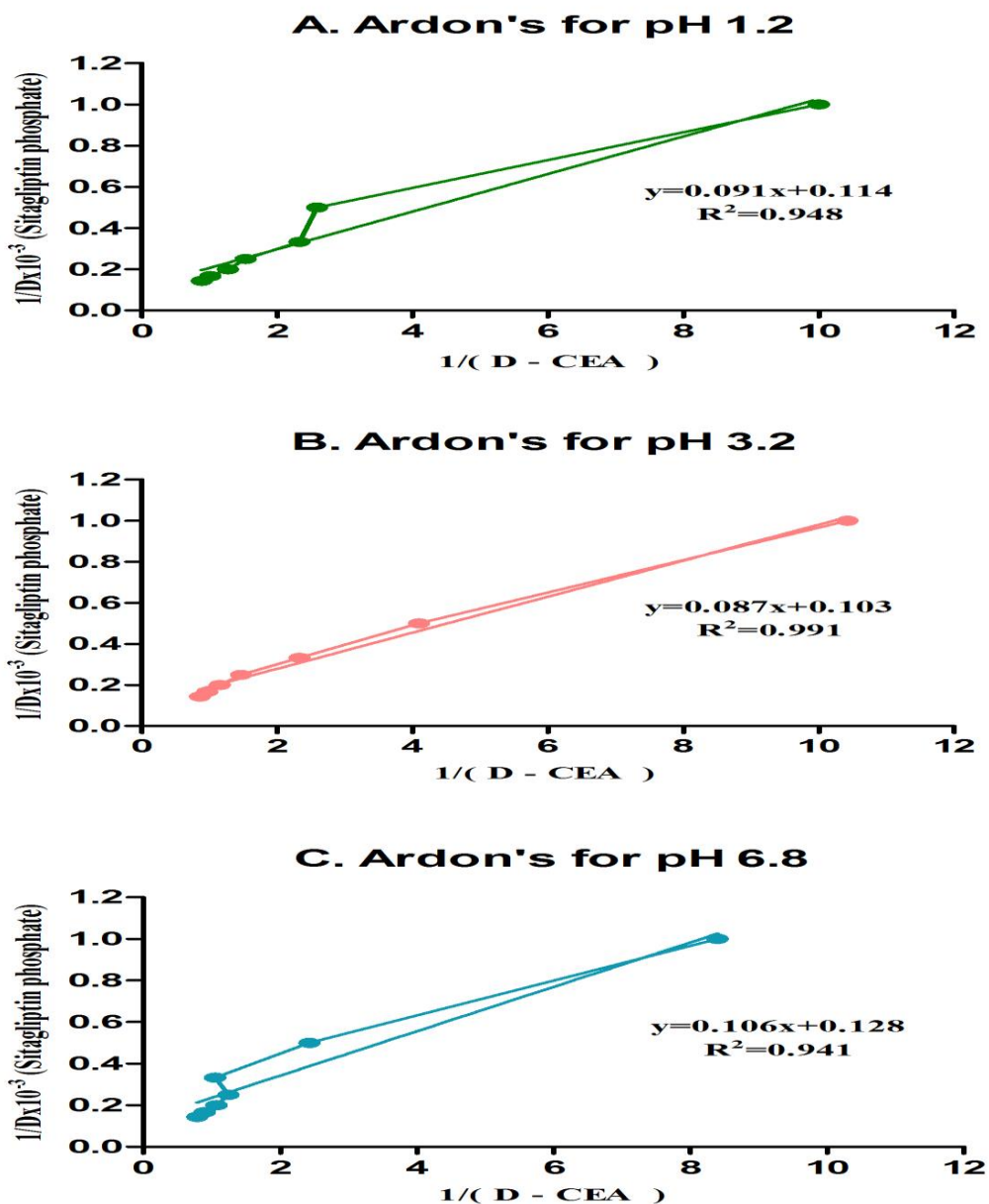


FIGURE 3: Ardon’s plot for complexation of Sitagliptin phosphate with Atenolol at 267 nm for A. pH 1.2, B. pH 3.2 and C. pH 6.8.

TABLE 3: Stability constant of Sitagliptin phosphate with Atenolol at different pH.

System	pH	Stability Constants
Interaction of Sitagliptin phosphate with Atenolol	1.2	1.2073**
	3.2	1.1839*
	6.5	1.2075**

Values are the mean of triplicate experiments and represented as mean. Values in the same column with different superscripts (*) are significantly different *P < 0.05 and **P < 0.01. Student’s t test was performed to analyze this data set.

Table 4: Effect of Sitagliptin phosphate with Atenolol and their mixture of 1:1 on glucose induced hyperglycemia (mmol/L) in normal mice.

Group	0min	30min	60min	90min	120min	% decrease
Control	8.02±0.86	7.32±0.89	5.98±0.86	7.04±0.73	8.294±1.35	-
Sitagliptin phosphate	5.94±0.14	7.98±1.08*	5.5±0.36*	5.5±0.53*	4.62±0.31**	22.22
Atenolol	5.16±0.39	6.86±0.49*	5.06±0.68	5.56±0.24*	5.74±0.46	-
Complex (1:1)	7.06±0.54	8.48±0.65*	6.52±0.7	6.08±0.54*	6.08±0.53**	13.88

Values are presented in mean±SEM (n=5). Values with different superscripts in same column are significantly different from control at each specific hour after the administration of Sitagliptin phosphate with Atenolol and their mixture of different ratio. For * $P>0.05$ and ** $P<0.01$. One-way ANOVA followed by Dunnett's multiple comparison was performed to analyze this comparison. "-" means no decrease.

Glucose level for OGTT test

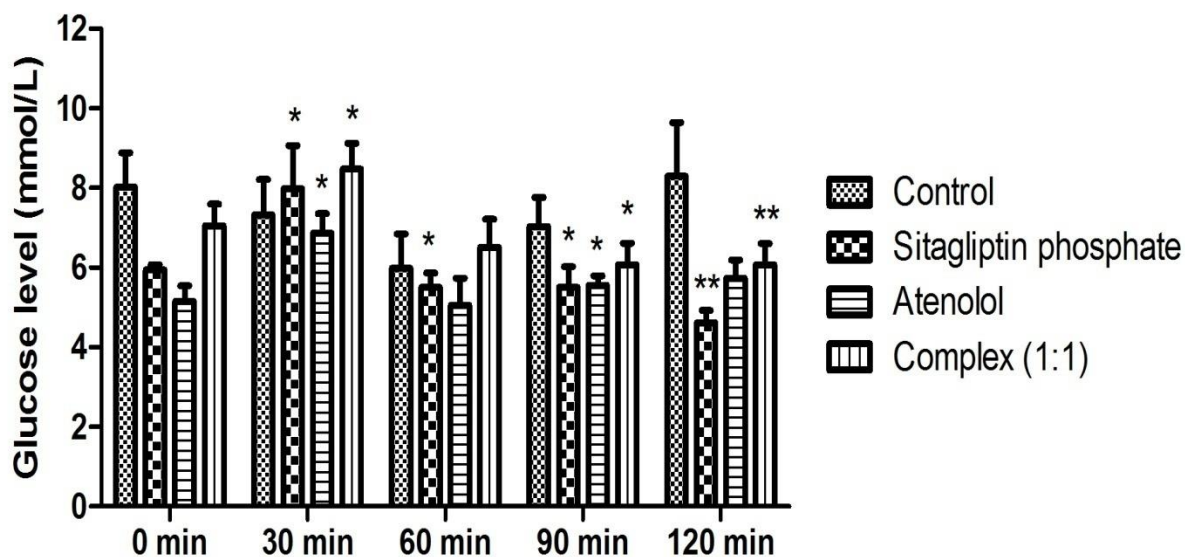


Figure 4: Effect of Sitagliptin phosphate with Atenolol and their mixture of 1:1 ratio on glucose induced hyperglycemia (mmol/L) in normal mice. Values are presented in mean±SEM (n=5). Values with different superscripts in same column are significantly different from control at each specific hour after the administration of Sitagliptin phosphate with Atenolol and their mixture of different ratio. For * $P>0.05$ and ** $P<0.01$. One-way ANOVA followed by Dunnett's multiple comparison was performed to analyze this comparison.

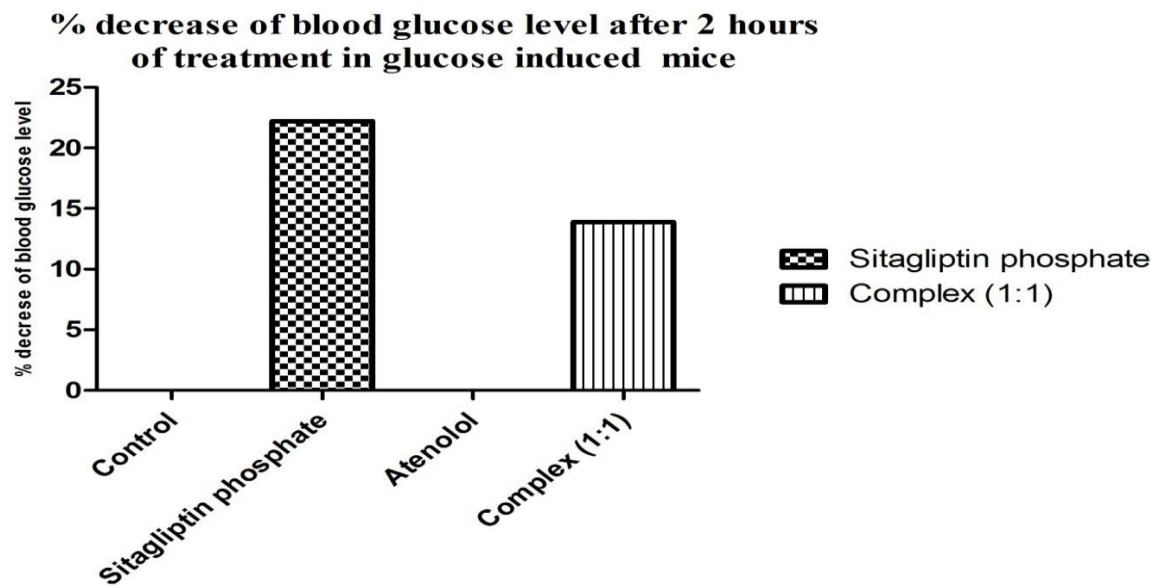


Figure 5: Percentage decrease of blood glucose level after treatment with Sitagliptin phosphate with Atenolol and their mixture of 1:1 ratio in glucose induced mice.

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