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Research Article

IN VITRO STUDIES ON THE ANTIDIABETIC ACTIVITY OF 2-THIOHYDANTOIN USING α-amylase AND α-GLUCOSIDASE

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ABSTRACT

Objective: The aim of the present study was to investigate the inhibitory effect of 2-thiohydantoin on the enzymes α-amylase and α-glucosidase *in vitro* as an antidiabetic therapeutic approach to reduce gastrointestinal glucose production.

Methods: Antidiabetic activity of the compound, 2-thiohydantoin was measured using the enzymes α-amylase and α-glucosidase.

Results: Half-maximal inhibitory concentration of the antidiabetic α-amylase was found to be 410.35 µg/ml and α-glucosidase was found to be 356.33 µg/ml, respectively.

Conclusion: The result obtained in the *in vitro* enzyme assay suggests the antidiabetic activity of 2-thiohydantoin.

Keywords: 2-thiohydantoin, α-amylase, α-glucosidase, Antidiabetic activity.

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INTRODUCTION

Diabetes mellitus is a metabolic disorder which affects the blood glucose level in our body. This disease can be considered as the epidemic of the century. At present, an estimated 150 million people worldwide have diabetes and that this will increase 220 million by 2010 and 300 million by 2025 [1]. Globally, type II diabetes (non-insulin-dependent diabetes mellitus) accounts for >90% of the cases [2]. Postprandial hyperglycemia is an important contributing factor for the development of diabetic complications (Monami *et al*., 2013) [3]. It plays an important role in the development of type II diabetes mellitus and its associated chronic complications such as micro- and macro-vascular disorders (neuropathy, cardiovascular, and cerebrovascular diseases) (Boutati and Raptis, 2004) [4]. Postprandial hyperglycemia depends on the absorbed monosaccharides such as glucose. It can be regulated by carbohydrate hydrolyzing enzymes such as alpha-amylase and alpha-glucosidase. Inhibition of these enzymes prolongs overall carbohydrate digestion time causing reduction in the rate of glucose absorption and prevents hyperglycemia (Boutati and Raptis 2004) [5]. Antihyperglycemic agents are used to control blood glucose levels within the normal range. However, these agents are associated with various side effects and limited efficacy [1]. Many compounds were found to have the inhibitory effect on alpha-amylase and alpha-glucosidase. 2-thiohydantoin is a heterocyclic organic compound which possesses a wide range of other pharmaceutical properties including antitumor, anti-inflammatory, anti-HIV, hypolipidemic, antiarrhythmic, and antihypertensive effects [6]. The aim of the present study is to investigate the antidiabetic activity of 2-thiohydantoin on the mammalian carbohydrate-digesting enzymes such as pancreatic α-amylase and intestinal α-glucosidase by determining their inhibitory potential.

METHODS

Sample

The synthetic compound, 2-thiohydantoin ($C_3H_4N_2OS$) is available as brown crystalline powder having molecular weight of 116.14. The drug was purchased from SIGMA-ALDRICH, Germany. The parent compound hydantoin was first isolated in 1861 by Adolf von Baeyer in the course of his study of uric acid. The derivatives of 2-thiohydantoins are produced by heating a mixture of thiourea and alpha-amino acid. The compound was soluble in dimethyl sulfoxide.

Chemicals and reagents

Sodium phosphate buffer, 3, 5-dinitrosalicylic acid, PNPG, starch solution, sodium carbonate, alpha-amylase, alpha-glucosidase, acarbose, and other chemicals needed for this assay were received from Royal Bioresearch Institute, Tamil Nadu, India.

α-amylase inhibition assay

The inhibitory activity of the enzyme, α-amylase was performed using standard methods with slight modifications [7]. Acarbose was used as a standard drug. About 100–500 µg/ml of the test sample, 2-thiohydantoin was taken in test tubes. About 250 µl of α-amylase (1 mg/ml) in 0.2 M sodium phosphate buffer (pH 6.9) was added to each of the test tube and was incubated at 37°C for 20 min. Then, 250 µl of 0.5% starch solution in 0.2 M sodium phosphate buffer (pH 6.9) was added to each of the test tube. The reaction mixtures were then incubated at 37°C for 15 min. The reaction was stopped with the addition of 1 ml of 3, 5-dinitrosalicylic acid. The test tubes were then incubated in a boiling water bath at 100°C for 10 min and then cooled to room temperature. The reaction mixture was then diluted to 10 ml using distilled water and absorbance was measured at 595 nm using ultraviolet (UV)–visible spectrophotometer. The inhibitory activity of α-amylase was calculated using the following formula:

$$\% \text{ inhibition of } \alpha\text{-amylase} = (A \text{ of test} - A \text{ of control}) / A \text{ of control} \times 100,$$

Where, A indicates the absorbance value.

α-glucosidase inhibition assay

The inhibitory activity of the enzyme, α-glucosidase was performed using standard methods [8,9]. Acarbose was used as a standard drug. About 50 µl of the enzyme, α-glucosidase was mixed with the test sample, 2-thiohydantoin (100–500 µg/ml) taken in different test tubes. The tubes were vortexed and incubated at 37°C for 30 min. About 800 µl of 0.1 M sodium carbonate was added. Absorbance was measured at 405 nm using UV–visible spectrophotometer [10].

The inhibitory activity of α-glucosidase was calculated using the following formula:

% inhibition of α-glucosidase=(A of test−A of control)/A of control×100,

Where, A indicates the absorbance value.

Table 1: IC_{50} value for acarbose in α-amylase inhibitory assay of *in vitro* antidiabetic activity

S. No.	Concentration of the standard (acarbose) (µg/ml)	% of α-amylase inhibition	IC_{50} (µg/mL)
1.	100	4.1	427.51
2.	200	17.8	
3.	300	32.3	
4.	400	45.2	
5.	500	61.0	

IC_{50}: Half-maximal inhibitory concentration

Table 2: IC_{50} value for 2-thiohydantoin in α-amylase inhibitory assay of *in vitro* antidiabetic activity

S. No.	Concentration of the test sample (2-thiohydantoin) (µg/ml)	% of α-amylase inhibition	IC_{50} (µg/mL)
1.	100	5.2	410.35
2.	200	21.7	
3.	300	33.3	
4.	400	43.7	
5.	500	51.3	

IC_{50}: Half-maximal inhibitory concentration

The concentration of the test sample, 2-thiohydantoin to inhibit half (50%) of α-glucosidase activity is known as the half-maximal inhibitory concentration (IC_{50}) value.

Statistical analysis

The results were expressed as mean values and standard deviation. Linear regression analysis was used to calculate IC_{50} value.

RESULTS

α-amylase inhibitory activity for standard drug, acarbose and the test sample, 2-thiohydantoin were presented in Tables 1 and 2 respectively. The inhibition of the enzyme, α-amylase shows optimum activity of 61% at a maximum concentration of the standard drug, acarbose (500 µg/ml) was shown in Fig. 1a. The inhibition of the enzyme, α – amylase shows optimum activity of 51.3% at a maximum concentration of the test drug, 2-thiohydantoin (500 µg/ml) was shown in Fig. 1b. The IC_{50} values for the standard drug, Acarbose and the test sample, 2-thiohydantoin and was found to be 427.51 and 410.35 respectively.

α-glucosidase inhibitory activity for standard drug, acarbose and the test sample, 2-thiohydantoin were presented in Tables 1 and 2 respectively. The inhibition of the enzyme, α-glucosidase shows optimum activity of 80.01% at a maximum concentration of the standard drug, acarbose (500 µg/ml) was shown in Fig. 1a. The inhibition of the enzyme, α-glucosidase shows optimum activity of 56% at a maximum concentration of the test drug, 2-thiohydantoin (500 µg/ml) was shown in Fig. 1b. The IC_{50} values for the standard drug, Acarbose and the test sample, 2-thiohydantoin and was found to be 263.33 and 356.33 respectively.

DISCUSSION

The treatment strategy of diabetes is controlling postprandial hyperglycemia after the consumption of the meals. As the digestion

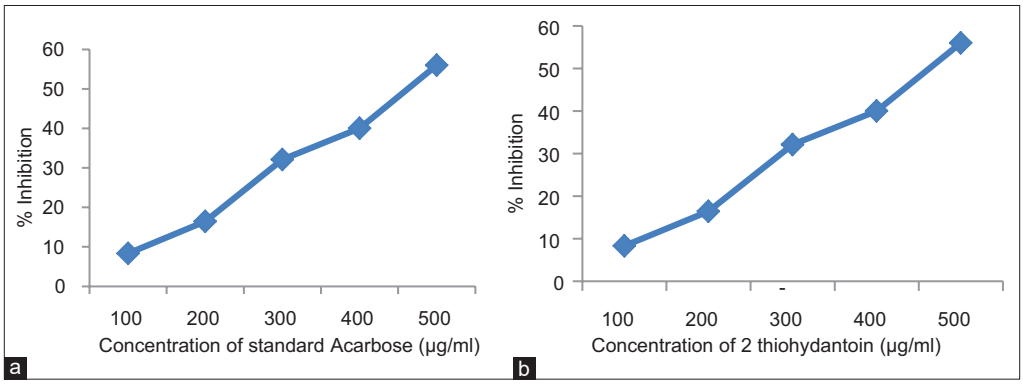

Fig. 1: (a and b) Half-maximal inhibitory concentration value for 2-thiohydantoin in α-amylase inhibitory assay of *in vitro* antidiabetic activity

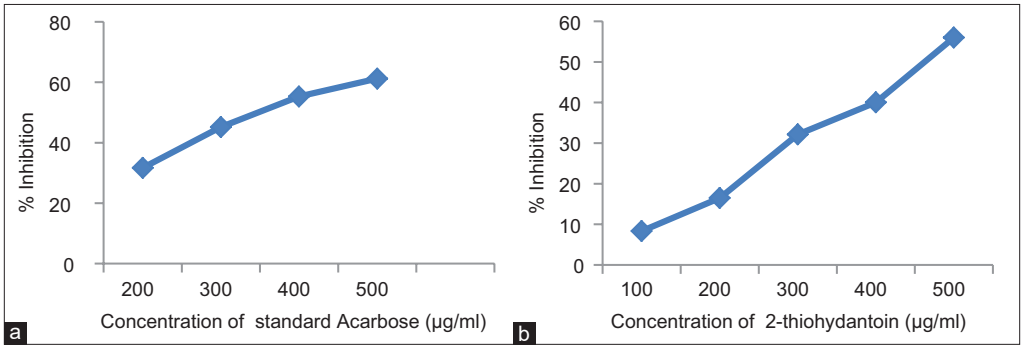

Fig. 2: (a and b) Half-maximal inhibitory concentration value for 2-thiohydantoin in α-glucosidase inhibitory assay of *in vitro* antidiabetic activity

Table 3: IC_{50} value for acarbose in α-glucosidase inhibitory assay of *in vitro* antidiabetic activity

S. No.	Concentration of the standard (acarbose) (µg/ml)	% of α-glucosidase inhibition	IC_{50} (µg/mL)
1.	100	28.2	263.33
2.	200	44.6	
3.	300	56.1	
4.	400	64.3	
5.	500	80.1	

IC_{50}: Half-maximal inhibitory concentration

Table 4: IC_{50} value for 2-thiohydantoin in α-glucosidase inhibitory assay of *in vitro* antidiabetic activity

S. No.	Concentration of 2-thiohydantoin (µg/ml)	% of α-glucosidase inhibition	IC_{50} (µg/mL)
1.	100	16.46	356.33
2.	200	32.12	
3.	300	40.05	
4.	400	56.01	
5.	500	69.01	

IC_{50}: Half-maximal inhibitory concentration

of carbohydrates starts from the salivary amylase, polysaccharides such as starch and glycogen are broken down to disaccharides. Then, the pancreatic amylase and alpha-glucosidase break down the disaccharides into monosaccharide such as glucose increasing the blood glucose level, leading to postprandial hyperglycemia. The results of the present study indicate that the synthetic drug, 2-thiohydantoin shows alpha-amylase and alpha-glucosidase inhibitory activity. Amylase is present in saliva and pancreatic juices. Amylase and glucosidase break down the glycogen and starch present in the meals to dextrins which is further broken down into glucose units, leading to postprandial hyperglycemia. Alpha-amylase and glucosidase inhibition provides a therapeutic approach to control the glucose level in the blood preventing hyperglycemia. IC_{50} value of 2-thiohydantoin shows significant glycoside enzyme inhibitory activity.

CONCLUSION

This study shows that 2-thiohydantoin has antidiabetic potential by inhibiting the glucose hydrolysing enzymes such α-amylase and α-glucosidase and can be used as a therapeutic agent for treating diabetes.

AUTHORS' CONTRIBUTIONS

Principal author: Performed collection of sample, extraction, analysis, interpreted data, wrote the manuscript, and acted as corresponding author.

Coauthor contribution: Supervised the development of work and helped in the evaluation of the manuscript.

CONFLICTS OF INTEREST

There are no conflicts of interest. This article is the independent work of the authors. This article does not contain any studies with human or animal subjects performed by the any of the authors.

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