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Antidiabetic Potential: Effect of Resorcinol on α-Glycosidase and α-Amylase Enzymes

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Abstract. The vast majority of the carbohydrates in the biomolecule class are formed by photosynthesis using sunlight. One of the important enzymes in this class is α-glycosidase. This enzyme converts complex carbohydrates to monosaccharides. On the other hand, another important enzyme species is the α-amylase enzyme. Which directly related to starch. Starch is a type of polymer formed by the oxygen binding of glucose units to each other at the C1 position with glycosidic bonds. α-Amylase is an enzyme that catalyzes the hydrolysis of glycosidic bonds in starch, and this enzyme is purified from a wide variety of animals, plants, and microorganisms. Resorcinol, a phenol derivative, is an important organic chemical substance in the production of textile, medicine, hair dye, plastic and agricultural products of many industrial materials. Resorcinol, a phenol derivative, is present as white crystals, and resorcinol consists of two hydroxyl molecules attached to the benzene ring, and also was defined as α-glycosidase and α-amylase inhibitor to detect antidiabetic properties. Resorcinol was used as *α*-glycosidase and *α*-amylase inhibitor. Accordingly, IC_{50} values of α-amylase and α-glycosidase were calculated as 1.34 μM and 77.00 nM, respectively. On the other hand, K_i value for α-glycosidase was found as 30.16 nM. It has been observed that α-amylase and α-glycosidase enzymes can inhibit by resorcinol compound. It has the properties of inhibiting carbohydrate absorption by inhibiting α-glycosidase enzymes in the small intestine. For both metabolic enzymes, resorcinol may be preferred as an inhibitor. In this case, resorcinol may be used as an inhibitor against to healty problems like *Diabetes mellitus* and antiobesity.

***Keywords*:** Resorcinol, *α*-Glycosidase, *α*-Amylase, Enzyme inhibition, Antidiabetic.

Antidiyabetik Potansiyel: Resorsinol'ün α-Glukozidaz ve α-Amilaz Enzimleri Üzerine Etkisi

Özet. Biyomolekül sınıfında bulunan karbohidratların büyük çoğunluğu güneş ışığını kullanarak fotosentez sonucu oluşurlar. Bu sınıfın önemli enzimlerinden birisi ise *α*-Glikozidaz dır. Bu enzim, kompleks karbohidratları monosakkaritlere dönüştürür. Diğer taraftan bir başka önemli enzim türü ise α-amilaz enzimidir. Bu enzim ise nişasta ile doğrudan ilişkilidir. Nişasta, glukoz ünitelerinin birbirine glikozidik bağlarla C1 pozisyonunda yer alan oksijenden bağlanmasıyla oluşan bir polimer türüdür. α-Amilaz, nişastada bulunan bu glikozidik bağların hidrolizini katalizleyen enzimlerdir ve bu enzim çok çeşitli hayvan, bitki ve mikroorganizmalardan saflaştırılmaktadır. Bir fenol türevi olan resorsinol, birçok endüstriyel malzemelerin tekstil, ilaç, saç boyaları, plastik ve tarım ürünlerinin üretiminde önemli bir organik kimyasal maddedir. Fenol türevi olan resorsinol beyaz kristaller halinde bulunur ve resorsinol benzen halkasına bağlı iki hidroksil molekülünden oluşur. *α*-Glikozidaz ve *α*-amilaz inhibitörü olarak resorsinol kullanıldı. Buna göre α-amilaz ve α-glikosidaz, sırasıyla IC_{50} değerleri 1,34 μM ve 77,00 nM olarak hesaplandı. Öte yandan, α-glikosidaz için K_i değeri 30,16 nM olarak bulundu. *α*-Amilaz ve *α*-glikozidaz enzimlerini resorsinol bileşiği inhibe edebildiği

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gözlemlendi. İnce barsakta α-glukozidaz enzimlerini inhibe ederek karbohidrat emilimini geciktirme özelliklerine sahiptirler. Her iki metabolik enzim için resorsinol, bir inhibitör olarak tercih edilebilir. Bu durumda resorsinol, *Diyabet mellitus* ve antiobezite gibi sağlık sorunlarına karşı inhibitör olarak kullanılabilir.

***Anahtar Kelimeler*:** Resorsinol, α-Glikozidaz, α-Amilaz, Enzim Inhibisyon, Antidiyabetik.

1. INTRODUCTION

Diabetes mellitus is a life-long metabolic disease, which characterized by insulin deficiency or ineffectiveness, consequent chronic and acute complications. Diabetes can be diagnosed clinically by symptoms such as polydipsia, polyuria, polyphagia, purities, weight loss and complications such as disease-specific retinopathy, neuropathy and nephropathy [1].

Both insufficiency of insulin secretion and decrease of insulin sensitivity are mentioned in Type 2 DM. Due to increase in hepatic glucose production, increase in fasting plasma glucose, insulin secretion and peripheral use in the disorder results revealed an increase in postprandial glucose. Currently, available oral antidiabetics are effective in one or more of these pathophysiological disorders. In addition, there are different basic oral antihyperglycemic classes; enhancing insulin secretion, decreasing insulin resistance, and agents that alter the rate of glucose entry from the gastrointestinal tract [1, 2].

Starch is a polymer that occurs from glucose units linked together by glycosidic bonds. It is mainly found in seeds, roots and tubers of large plants. The plants produce starch as a result of photosynthesis. Starch synthesized in plasticizes is stored for use in respiration in plants during non-light periods. It is also synthesized as a long-term storage material in amyloplasts found in seeds, roots and tubers. Although many plants produce starch, only a few plants are important for industrial starch processes. These are potatoes, wheat, millet and tapioca [3].

α-Amylase (E.C. 3.2.1.1) is a secretory enzyme widely found in nature. They are monomeric enzymes commonly found in animals, plants and microorganisms. Amylases are enzymes that degrade glycoside bonds in starch by hydrolysis. They are divided into two groups, α-amylases breaking starch α-1,4 glycoside bonds and β-amylases breaking α-1,4 bonds on non-reducing ends [4].

Glucose syrup is obtained by successive effects of α-amylase and glycoamylase on starch or by mixing these enzymes. This commonly used process brings the indicated enzymes to an important commercial position. Nowadays amylase enzymes have great importance in applications in biotechnology, food, textile and paper industries. Amylases are derived from a variety of sources such as plants, animals, and microorganisms [4,5].

Complex carbohydrates are cleaved by oligosaccharides by amylase in the small intestine. Oligosaccharides need to be disassociated with monosaccharides before they are absorbed. Oligosaccharides are cleaved by monosaccharides by α-glycosidase enzymes (glycoamylase, maltase, sucrose, isomaltase, and dextrinase). Normally, carbohydrates are absorbed quickly from the distal duodenum and proximal jejunum as primers. α-glycosidase inhibitors competitively reversibly bind to the enzyme, delaying carbohydrate absorption and allowing it to continue along the gastrointestinal tract. Thus, both type 1 and type 2 DM cause a decrease in postprandial plasma glucose. The mechanism of action of different alpha glycosidase inhibitors is similar. α-glycosidase inhibitors do not affect the absorption of glucose and shift the site of absorption to the more distant in the gastrointestinal tract. The most important advantage of α-glucose inhibitors is the local effect in the intestine and the absence of systemic effects. For this reason, it is particularly suitable for advanced diabetic patients. They do not lead to weight gain and hypoglycemia [2, 6].

Recently, it has been reported that there is an inverse relationship between the consumption of

fruits and vegetables and the risk of developing chronic diseases such as cancer, diabetes, cardiovascular diseases, especially in the diet. It was found that this situation is mainly caused by secondary metabolites and that phenolic compounds have a more active role than other compounds. Phenolic compounds contain one or more hydroxyl groups attached to the aromatic ring and they are divided into four basic classes as flavonoids, phenolic acids, stilbenes and lignans [7]. Resorcinol, a phenolic compound, was also used in this study.

Resorcinol is a compound, which is attached to an aromatic hydrocarbon group having a benzene ring structure and is formed from a hydroxyl functional group (-OH) (Figure 1) [8]. This compound and its derivatives have utility in different areas. Particularly, these compounds are used in many areas such as antibacterial, anti-inflammatory, antitumor, anticonvulsants and antioxidants [8-11]. Also, the isomers of di-hydroxyl-benzenes are used in the paint, cosmetic, pharmaceutical and chemical industries [9, 10]. Resorcinol is also used in the treatment of skin diseases such as eczema, psoriasis and warts in the pharmaceutical industry. In our study, it was aimed to prevent diabetes by delaying diagnosis and delaying complications.

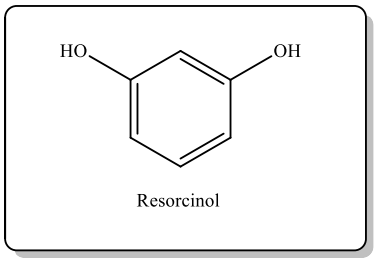

Figure 1. Chemical structure of Resorcinol.

2. MATERIALS AND METHODS

In this paper, *p*-nitrophenyl *α*-D-glycopiranoside (*p*-NPG), the enzymes including *α*-amylase, *α*-glycosidase, and starch were obtained from Sigma-Aldrich G5003 (St. Louis, MO, USA).

2.1. α-Glycosidase activity

When α-glycosidase enzyme activity was studied, *p*-NPG was used as the substrate [12]. The samples were prepared by dissolving 10 mg in 10 mL ethanol. First, 100 µL of phosphate buffer, pH 7.4 of phosphate buffer, 10 µL of enzyme solution was mixed, and samples of 50-100 µL were run. Then it was preincubated at 35°C for 10 min by adding the *p*-NPG to the initiation of the reaction. In addition, 50 µL of *p*-NPG in pH 7.4 of NaH_2PO_4 (5 mM) after preincubation was added and the incubation was again carried out at 35°C. IC_{50} and K_i values were evaluated with the obtained data. Acarbose compound was used as a positive control. Absorbances were spectrophotometrically measured at 405 nm.

2.2. α-Amylase activity

α-Amylase activity and inhibition study of this enzyme was measured according to procedure recorded by Xiao et al. [13], and measured at 580 nm spectrophotometrically using starch substrate conforming to previous studies.

3. RESULTS AND DISCUSSION

DM is a serious disease that is still growing all over the world and in our country, despite all the precautions taken, threatening the health of the community. The relative decrease in insulin resistance, insulin secretion, which leads to the onset of the disease in Type 2 diabetic patients, leads to high plasma glucose levels during both fasting and satiety periods. DM is a widespread disease that seriously affects the health of the community and has a very high economic cost leading to adverse outcomes. It has been known clinically since ancient times [14].

The human environment is constantly exposed to carcinogenic and mutagenic agents. And the removal of these factors from the environment presents a rather challenging and difficult picture. Recently, plants and some herbal metabolites are considered to be the main sources of compounds with antimutagenic potential. Even some secondary herbal metabolites have a protective effect against genotoxic agents [7].

Acarbose was used as a positive control, which discovered for the first time in 1977 and moved to the market in 1990. Acarbose is an oral antidiabetic that is a reversible inhibitor of the enzyme α-glycosidase in the small intestine. Acarbose is a reversible inhibitor of the competent α-glycosidase enzyme of the small intestine brushy-edge β-glycosidase enzyme. These inhibitors delay carbohydrate digestion [14].

Table 1. IC_{50} and K_i of Resorcinol and Acarbose against *α*-Amylase and *α*-Glycosidase Enzymes.

Compounds	α-Amylase IC_{50}	r^2	α-Glycosidase IC_{50}	r^2	α-Glycosidase K_i
Resorcinol	1.34 μM	0.9208	77.00 nM	0.8670	30.16 nM
Acarbose[b]	10.00 μM	-	22.80 μM	-	12.60 μM

[a]K_i values could not be determined for *α*-amylase enzyme.
[b]It was used as positive control for α-amylase and α-glycosidase enzymes and determined as *μ*M levels, which obtained from references [15] and [16].

α-Glycosidase enzyme inhibitors are used for the treatment of metabolic disorders such as non-insulin dependent hyperglycemia, type II, DM and obesity. α-Glycosidase inhibitors are oral anti-diabetic drugs used to regulate carbohydrate digestion, which is converted into monosaccharides and absorbed in the gut. α-Amylase inhibitors are used in diabetes treatment and in the food sector in various fields.

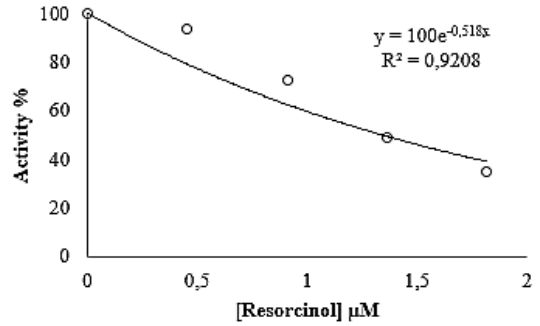

Figure 2. The IC_{50} graphs of Resorcinol against α-Amylase enzyme.

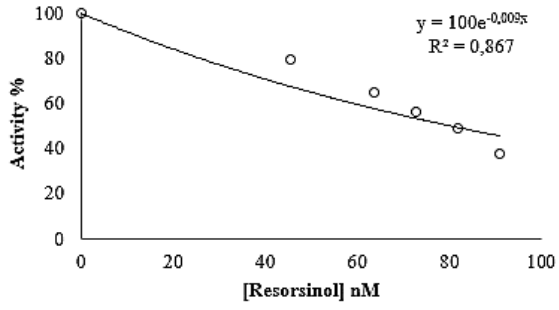

Figure 3. The IC_{50} graphs of Resorcinol against α- α-Glycosidase enzyme.

At the end of the runs, graphs were plotted to calculate IC_{50} and K_i values. These values are given in Table 1, Figure 2 and Figure 3. According to this, very effective results of resorcinol have been obtained. It is thought that resorcinol can be seen as an alternative for people with allergic reactions especially against acarbose. For both enzymes, resorcinol may be preferred as an inhibitor. In this case Resorcinol can be preferred as an inhibitor by offering DM and antiobesity treatment.

4. CONCLUSION

In this study, inhibition properties of the resorcinol phenolics on α-amylase and α-glucosidase enzymes were studied. Accordingly, inhibition was observed at nanomolar level for α-glucosidase when it was at micromolar level for α-amylase. Considering these results of this study, it was determined that resorcinol may be an important compound for the diabetes problem. As a result, it is thought that resorcinol will illuminate pharmacological studies.

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