Future Journal of Pharmaceutical Sciences xxx (2017) 1–5

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Contents lists available at ScienceDirect

Future Journal of Pharmaceutical Sciences

journal homepage: http://www.journals.elsevier.com/future-journal-of-pharmaceutical-sciences/

# Glycolytic enzyme inhibitory and antiglycation potential of rutin

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ARTICLE INFO

*Article history:*
Received 3 December 2015
Received in revised form
10 April 2017
Accepted 21 May 2017
Available online xxx

*Keywords:*
α-amylase
α-glucosidase
Antiglycation
Docking
Rutin

ABSTRACT

The aim of the present was to determine the mechanism for the antidiabetic potential exerted by rutin by studying its effect on the glycolytic enzyme inhibitory and antiglycation potential. For the determination of glycolytic enzyme inhibitory potential of rutin, porcine pancreatic amylase inhibitory assay and α-glucosidase inhibitory assay were performed. The antiglycation potential was determined by glycation of bovine serum albumin followed by antiglycation estimation of fructosamines adducts, protein carbonyls, protein thiols, congo red absorbance. Rutin showed a significant inhibition of α-amylase ($p < 0.001$; 53.66%) and α-glucosidase ($p < 0.001$; 52.56%). To study antiglycation potential, various parameters were determined and fructosamine inhibition was found to be 35.55%, protein carbonyls were inhibited up to 13.49%. Protein thiols were inhibited up to 80.27%. In the present study, it was concluded that rutin showed glycolytic enzyme inhibitory and antiglycation potential.



## 1. Introduction

Diabetes mellitus (DM) is a metabolic disorder observed by inefficiency of the body to oxidize carbohydrate due to disruption in insulin function [1]. Diabetes mellitus causes a long-term damage in various body functions which may also cause failure of various organ functions [2]. The present estimation says that about 150 million people worldwide are suffering from diabetes and this number will increase to 220 million by 2010 and 300 million by 2050 [3]. 90% of the global population is affected by type II diabetes [4]. Different pharmacological methods have been initiated for improving the diabetic conditions [4]. The enzymes involved in the breakdown of polysaccharides which have shown have remarkable results in order to control blood glucose level in type II diabetes mellitus [5–7]. α-amylase is one of the major enzymes involved in such process which catalyses the breakdown of starch to maltose which finally gets converted to glucose, that is the only sugar which can be utilized by the body [8]. Other important enzyme that comes into light is intestinal α-glucosidase located at the epithelium of the small intestine which acts as a key enzyme for the digestion of carbohydrate. Both α-glucosidase and α-amylase are now regarded as a therapeutic target for the modulation abnormality that occurs in Type II DM [9]. Thus inhibition of these enzymes causes a decrease in glucose level. Various studies have revealed that inhibition of these enzymes causes a marked effect on the level of glucose [5]. In recent years, plants and their constitutions have received much attention in the treatment of diabetes for various reasons and many researchers have focused on hypoglycemic agents from medicinal plants [4]. Some studies reveal the presence of α-amylase and α-glucosidase inhibitors in various plants [10,11], and owing to the results obtained it would not be surprising that researchers have focused their view on the natural source for potential antidiabetic drugs. Rutin is of flavonoid origin which has shown antidiabetic potential. Thus the aim of present study was to investigate the α-amylase and α-glucosidase inhibitory along with antiglycation potential of rutin.

## 2. Material and method

### 2.1. Chemicals

The various chemicals used in this study includes porcein

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Peer review under responsibility of Future University.

http://dx.doi.org/10.1016/j.fjps.2017.05.005

pancreatic amylase (PPA), gallic acid (SRL, Mumbai), acarbose (Bayer Pharmaceutical), rutin (CDH, Delhi), trichloro acetic acid, ascorbic acid(Merck, Mumbai), ammonium molybdate (Qualigens, Mumbai) and 3,5-dinitrosalicylic acid (Loba Chemie, Mumbai).

### 2.2. Glycolytic enzyme inhibition studies

#### 2.2.1. α-amylase inhibitory assay

Porcine pancreatic amylase (PPA) inhibitory assay was performed as per the method reported by Hansawasdi et al. [12]. 2 mg of starch was suspended in each of the tubes containing 0.2 ml of 0.5 M Tris-HCl buffer pH 6.9 and 0.01 M $CaCl_2$. The tubes containing the substrate solution were boiled for 5 min and were then incubated at 37 °C for 5 min. 0.2 ml of rutin was taken in each tube containing different concentrations (10, 20, 40, 60, 80 and 100 μg/ml) of dimethyl sulfoxide. PPA was dissolved in Tris-HCl buffer to form a concentration of 2 units/ml and 0.1 ml of this enzyme solution were added to each of the above mentioned tubes. The reaction was carried out at 37 °C for 10 min and was stopped by adding 0.5 ml of 50% acetic acid in each tube. The reaction mixture was centrifuged at 3000 rpm for 5 min at 4 °C. The absorbance of the resulting supernatant was measured at 595 nm using a spectrophotometer (Shimadzu 1700 spectrometer). The α-amylase inhibitory activity was calculated as follows:

$$= ((\mathrm{Ac}+) - (\mathrm{Ac}-)) - ((\mathrm{As} - \mathrm{Ab}))/((\mathrm{Ac}+) - (\mathrm{Ac}-)) \times 100$$

where Ac+, Ac−, As and Ab are defined as the absorbance of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme activity), a test sample (with enzyme) and a blank (a test sample without enzyme), respectively.

#### 2.2.2. α-Glucosidase inhibitory activity

The α-glucosidase inhibitory activity was determined using the method described by Sutedja [13]. The enzyme solution was prepared by dissolving 0.5 mg α-glucosidase in 10 ml phosphate buffer (pH 7.0) containing 20 mg bovine serum albumin. It was diluted further to 1:10 with phosphate buffer just before use. Sample solutions were prepared by dissolving 4 mg sample extract in 400 μl DMSO. Five concentrations; 50, 100, 150, 200 and 250 μg/ml were prepared and 5 μl each of the sample solutions or DMSO (sample blank) was then added to 250 μl of 20 mM p-nitrophenyl-α-D glucopyranoside and 495 μl of 100 mM phosphate buffer (pH 7.0). It was pre-incubated at 37 °C for 5 min and the reaction started by addition of 250 μl of the enzyme solution, after which it was incubated at 37 °C for exactly 15 min. 250 μl of phosphate buffer was added instead of enzyme for blank. The reaction was then stopped by addition of 1000 μl of 200 mM $Na_2CO_3$ solution and the amount of p-nitrophenol released was measured by reading the absorbance of sample against sample blank (containing DMSO with no sample) at 400 nm using UV-visible spectrophotometer.

### 2.3. Antiglycation potential

#### 2.3.1. Glycation of bovine serum albumin

Albumin glycation was performed with certain modifications [14]. Glycated BSA samples were prepared with BSA (10 mg/ml) fructose (250 mM) in potassium phosphate buffer (200 mM, pH 7.4 containing 0.02% sodium azide) with rutin. These were incubated in dark for 4 days at 37 °C in sealed tubes. Positive control (BSA+fructose) was also maintained in similar conditions. All the incubates were in triplicates. The unbound form of fructose in the solution was obtained by dialysis against the phosphate buffer (200 mM, pH 7.4) and was stored at 4 °C. The resultant obtained was used for determination of antiglycation activity of rutin by estimation of fructosamines adducts, protein carbonyls, protein thiols and congo red absorbance.

### 2.4. Estimation of fructosamine

Nitrobluetetrazolium assay was used for the determination of fructosamine [15]. 0.8 ml of nitrobluetetrazolium (0.75 mM) in sodium carbonate buffer (100 mM, pH 10.35) was added to the aliquots of glycated samples and positive control (40 ml) and these were incubated at 37 °C for 30 min. After incubation the absorbance was taken at 530 nm and percent inhibition of fructosamines by rutin was calculated by following equation.

$$\text{Inhibitory activity}(\%) = [(A_0 - A_1)/A_0] \times 100$$

where $A_0$ is the absorbance value of the positive control and A1 is absorbance of the glycated albumin samples co incubated with rutin.

### 2.5. Carbonyl group estimation

For protein carbonyls, absorbance was taken at 365 nm and the concentration was calculated by molar extinction coefficient [16]. The results obtained were given as % inhibition and was calculated by the formula used in estimation of fructosamine.

### 2.6. Protein thiol estimation

The estimation of thiol groups of glycated albumin samples and positive control was performed by DTNB [17]. In this assay, 250 μl samples and control were incubated with three volumes of 0·5 mM-DTNB (750 μl) for about 15 min and then the absorbance was taken at 410 nm. The free thiol concentration in the solution was taken by the standard curve of various BSA concentrations (0·8 to 4 mg/ml) as nM thiols/mg protein. The % protection was calculated by the formula used in estimation of fructosamine.

### 2.7. Binding of Congo red

Congo red binding was measured by taking the absorbance at 530 nm [18]. For this assay, the samples (500 μl) were incubated with 100 μl of congo red (100 μM) in PBS with 10% (v/v) ethanol for 20 min at RT. The absorbance was recorded for both congo red incubated samples as well as for congo red background. The results were expressed as % inhibition calculated by the same formula used in estimation of fructosamine.

### 2.8. Docking studies

Molecular docking was performed on rutin against α-amylase and α-glucosidase. A Lamarckian genetic algorithm method implemented in the program AutoDock 4.2 was employed to determine the orientation of ferulic acid with the active site of gastric proton pump. Ferulic acid was drawn using Mervin Sketch. Protein structure files (PBD ID: 3OLD for α-amylase and PBD ID: 3A4A for α-glucosidase) were taken from PDB (www.rcsb.org/pdb) and edited by removing the hetero atoms with simultaneous adding of C terminal oxygen. For docking calculations, Gasteigere-Marsili partial charges were assigned to the ligands, non-polar hydrogen atoms were merged and all torsions were allowed to

Please cite this article in press as: S. Dubey, et al., Glycolytic enzyme inhibitory and antiglycation potential of rutin, Future Journal of Pharmaceutical Sciences (2017), http://dx.doi.org/10.1016/j.fjps.2017.05.005