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Research Article

α -AMYLASE, α -GLUCOSIDASE AND ALDOSE REDUCTASE INHIBITORY POTENTIAL OF BETANIN FOR THE MANAGEMENT OF DIABETES AND ITS COMPLICATIONS

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ABSTRACT

Diabetes is an important human ailment that affects many countries. It's proving to be a major health problem in India, especially in urban areas. Herbal formulations are used to reduce the ill effects of diabetes and its complications. The present study demonstrates the potential of Betanin; a natural compound obtained from *Beta Vulgaris,* against the enzymes aldose reductase, α-glucosidase, and α-amylase, involved in diabetes and its complications by *in vitro* evaluation. Aldose reductase assay was performed by using NADPH as starting material and DL-Glyceraldehyde as a substrate. Ranirestat was used as standard drug. DNS method was used for alpha amylase inhibitory activity and for alpha glucosidase inhibitory activity, p-nitrophenyl glucopyranoside (pNPG) was used as substrate. Acarbose was used as a standard drug for alpha amylase and alpha glucosidase enzymes. Betanin shows potent inhibitory effect against aldose reductase (IC_{50}:4.245±0.068µg/ml), α-glucosidase (IC_{50}:2.85±0.0219µg/ml) and α-amylase (IC_{50}:1.75±0033µg/ml), respectively. From the results it was concluded that Betanin potentially inhibit all the three enzymes more than their standard drugs and it will be helpful in the management of diabetes and its complications.

Keywords: Diabetes, aldose reductase, α -amylase, α -glucosidase, Betanin

1. INTRODUCTION

One of the most severe endocrine metabolic disorders, Diabetes mellitus has considerable importance in both, the condition of being diseased and the state of being subject to death due to microvascular and macrovascular complications [1]. Diabetes is a chronic metabolic disorder that develops when the body fails to produce or use adequate insulin. In this condition, the individual can produce insulin but becomes resistant to it. High blood glucose levels are due to insulin resistance and insulin deficiency [2]. In Type 1 diabetes, β-pancreatic cells fail to secret insulin. On the other hand, Type 2 diabetes is specified by an insulin resistance [3].

Alpha-amylase enzyme is involved in breaking down of the large polysaccharides like starch into absorbable molecules and is present in the pancreatic juice and saliva. α-glucosidase is another carbohydrate metabolising enzyme present in the mucosal brush border of the small intestine and catalyzes the end step of digestion of starch and disaccharides that are abundant in human diet. α-amylase and α-glucosidase inhibitors delay the breaking down of carbohydrates in the small intestine and reduce the postprandial blood glucoselevel [4]. The medicinal plants or natural products by inhibiting the carbohydrate hydrolyzing enzymes retard the absorption of glucose [5]. Acarbose, miglitol and voglibose are the drugs which find application in the clinical practice for management of diabetes. But they have many gastrointestinal side effects such as abdominal pain, flatulence and diarrhea in the patients. So, there is a need to identify the inhibitors from natural sources having fewer side effects [6].

Diabetic complications are leading causes of morbidity and death in diabetic patients, including retinopathy,

neuropathy, nephropathy, and cataracts [7]. Aldose reductase is the polyol pathway's first and rate-limiting enzyme and reduces glucose to sorbitol using NADPH as a cofactor. Sorbitol is then metabolized by sorbitol dehydrogenase into fructose. In the diabetic state, sorbitol does not easily diffuse across cell membranes, due to which, accumulation of sorbitol occurs, this leads to microvascular complications of diabetes such as peripheral neuropathy, nephropathy, retinopathy, and cataracts [8].

Indian medicinal systems make extensive use of herbal medicines to treat diabetes. As herbal medicines are known to be safer hence there is a much interest in them in the international scenario [9].

The aim of this research is to explore the effects of Betanin on some identified targets such as α-amylase, α-glucosidase and aldose reductase enzymes involved in diabetes and its complications.

2. MATERIALS AND METHODS

Chemicals and enzymes used in the present work were purchased from Loba Chemie (India) and Sigma-Aldrich (India). Betanin and standards were purchased from commercial suppliers.

2.1. Biological Evaluation

2.1.1. Aldose reductase (are) inhibitory activity

2.1.1.1. Preparation of Enzyme

Fresh goat eyeballs were obtained from local slaughter house and the lenses were quickly removed from it. In a homogenizer, lenses (100-200 g) were taken and homogenized in 3 volumes of cold distilled water. Afterwards it was centrifuged for 15 min. at 10, 400 RPM at 0-4°C. To the supernatant fluid, saturated ammonium sulphate was added to 40% saturation and then it was allowed to stand for 15 min with stirring. It was centrifuged and then precipitate was discarded. The same procedure was repeated by increasing the ammonium sulphate concentration to 50% and then 75% saturation by centrifuging the mixture. Aldose reductase was then precipitated. The precipitate obtained was used for the enzymatic assay [10].

2.1.1.2. Aldose reductase inhibition assay

A mixture of 0.75 ml of 0.1 M phosphate buffer (pH 6.2), 0.5 ml of 0.104 mM NADPH, 0.3 ml of lens supernatant and 0.1 ml of Betanin (2-10 µg/ml) was taken in a sample cuvette, incubated at 30°C for 10 min. After this 0.75 ml of 10 mM DL-glyceraldehyde as a substrate was added and the absorbance was recorded at 340 nm. Same assay was performed for reference blank cuvette containing all the compounds except the substrate and standard drug ranirestat. The procedure was repeated three times. Percentage inhibitions and IC_{50} value were calculated [11].

2.1.2. α-glucosidase (AGE) inhibition assay

Various concentrations (2-10 µg/ml) of Betanin (50 µl) was preincubated with α-glucosidase (100 µl) for10 min. 3.0 mM pNPG (50 µl) solution was added to start the reaction, which was prepared in Phosphate buffer (20 mM , pH 6.9). To stop the reaction, 0.1 M Na_2CO_3 (2 ml) solution was added and then were incubated at 37°C for 20 min and assessed for p-nitrophenol release from pNPG at 405 nm. Acarbose was used as a standard drug [12]. The enzyme inhibition rate expressed as a percentage of inhibition was calculated using the following formula:

Percentage Inhibition of α- glucosidase activity = (Abs C - Abs S)/Abs C)x100

Where Abs C is the absorbance of the control (100 % enzyme activity) and Abs S is the absorbance of the tested sample (Betanin or acarbose).

2.1.3. α-amylase (AAE) Inhibition Assay

Half (0.5) ml of the betanin at various concentrations (2, 4, 6, 8 and 10 µg/ml) and 0.5ml of alpha amylase (0.5 mg/ml), which was prepared in 20mM phosphate buffer (6.9),were mixed together and incubated at 25°C for 10min. After this, 0.5ml of starch (1%) solution was added to the mixture and further incubated at 25°C for 10min. The reaction was then stopped by adding 1ml of dinitrosalicylic acid (DNS) reagent and the reaction mixture was kept on boiling water bath for 5min. and cooled. The solution was made upto 10 ml with distilled water and the absorbance was measured at 540 nm. Acarbose was used as a standard drug.

Absorbance was calculated by using following formula:

Percentage inhibition of α-Amylase Activity = $[(Ac^+)–(Ac^-) – (As-Ab)/(Ac^+)–(Ac^-)] \times 100$

Where, Ac^+, Ac^-, As, Ab are absorbance of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme), a test sample (with enzyme) and a blank (a test sample without enzyme)respectively [13, 14].

2.2. Statistical Analysis

All the results were expressed as mean±SEM for triplicate determinations.

3. RESULTS

In the present study, the inhibitory property of Betanin (2-10 µg/ml) (Fig.1) on aldose reductase, α-glucosidase and α-amylase was evaluated (Table 1, Fig. 2). It shows potent inhibitory activity against aldose reductase (IC_{50}: 02.914±0.133µg/ml), α-glucosidase (IC_{50}:3.55±0.0176 µg/ml) and α-amylase (IC_{50}:1.40±0.0754µg/ml), respectively.

The IC_{50} values for standard drugs are found to be: Ranirestat (IC_{50}:09.261±0.107 µg/ml) for aldose reductase, Acarbose (IC_{50}:03.577±0.064 µg/ml) and (IC50:05.1±0.064 µg/ml) for α-glucosidase and α-amylase, respectively.

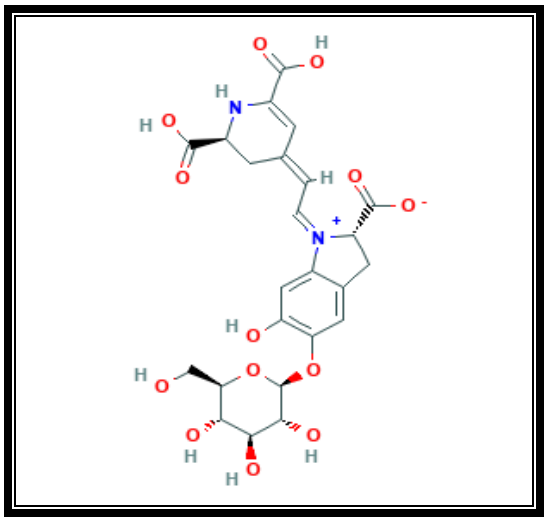
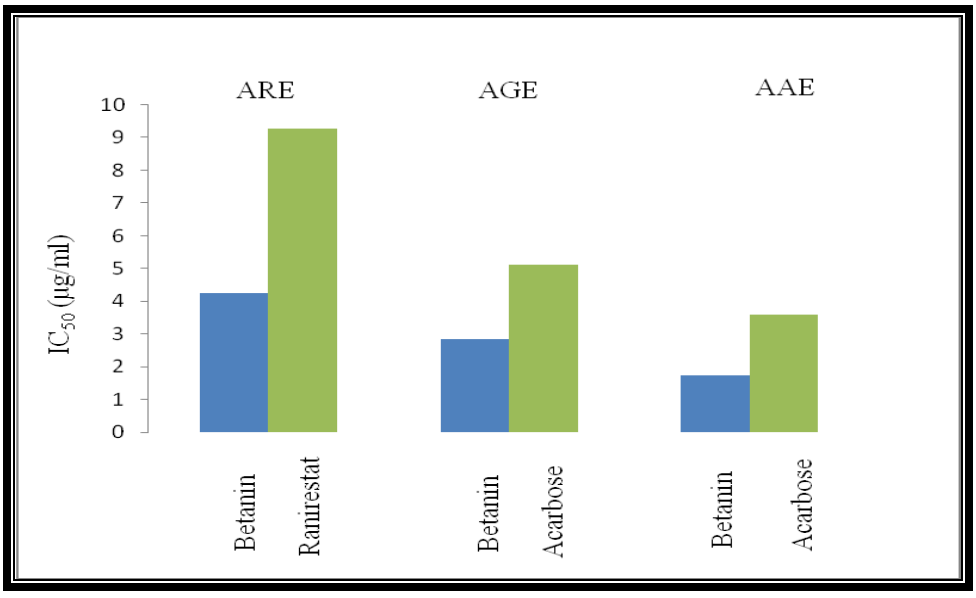


Fig.1: Chemical structure of betanin

Table 1: Percent inhibitory activity of Betanin on aldose reductase, α-glucosidase and α-amylase enzymes

Concentration (µg/ml)	Percent Inhibitory Activity					
	Betanin			Ranirestat	Acarbose	
	ARE	AGE	AAE	ARE	AGE	AAE
2	31.2 ±1.243	44.54±0.1819	51.41±0.1155	21.48 ±1.05	31.41±0.524	39.54 ±0.469
4	51.53 ±0.904	57.33±0.3791	63.32±0.0914	32.06 ±1.12	43.95 ±0.37	52.32 ±0.384
6	63.05 ±1.016	71.17±0.2759	74.9±0.2082	39.48±0.712	55.59±0.454	66.42 ±0.411
8	77.7 ±0.508	84.44±0.4564	87.47±0.1484	44.2 ±0.777	67.61±0.154	79.72±0.206
10	86.07 ±0.601	98.56±0.1852	98.68±0.0.0577	52.57±0.692	77.53±0.272	91.76±0.293
IC50	04.245±0.068	2.85±0.0219	1.75±0033	09.261±0.107	05.1±0.064	03.577±0.064

ARE: Aldose Reductase Enzyme, AGE:α-Gulcosidase Enzyme, AAE: α-amylase Enzyme



ARE: Aldose Reductase Enzyme, AGE: α- Gulcosidase Enzyme, AAE: α-amylase Enzyme

Fig. 2: IC_{50} value of Betanin and standard drugs on aldose reductase , α-glucosidase and α-amylase enzyme

4. DISCUSSION

α-amylase and α-glucosidase are carbohydrate hydrolyzing enzymes that are responsible for postprandial hyperglycemia. α-amylase hydrolyses the 1, 4-glycosidic linkages of polysaccharides (starch, glycogen) to disaccharides and the work of α-glucosidase is to catalyze the disaccharides to monosaccharides, due to which postprandial hyperglycemia occurs. Inhibitors of these enzymes delays carbohydrate digestion and are useful in the control of hyperglycemia which reduces the postprandial plasma glucose level.

Since the polyol pathway is known to cause diabetic microvascular complications, there is a greater interest in aldose reductase inhibition which is involved in this pathway. Phytoconstious as Aldose reductase inhibitors are currently the most widely used oral agents for their strong penetration through cell membranes and quick sorbitol metabolism by sorbitol dehydrogenase. They are considered important for treatment of diabetic complications such as retinopathy and cataract.

The result shows that Betanin efficiently inhibited all the three enzymes *in vitro* and there was a dose-dependent increase in percentage inhibitory activity against the enzymes. From the result, it was observed that the plant isolate shows a high percentage of inhibition than the standard drugs.

5. CONCLUSION

Despite the availability of modern medicines on the market for the management of diabetes mellitus, plant-derived medicinal products have gained substantial expertise in the treatment of diabetes mellitus and its complications. In the present study the potential of Betanin to inhibit the target enzymes was investigated. From the study, it was concluded that Betanin is more potent inhibitor of α -amylase, aldose reductase and α-glucosidase as compared to standard drugs ranirestat and acarbose. From *in vitro* analysis it was observed that Betanin can control hyperglycemia by inhibiting the carbohydrate metabolizing enzymes and the polyol pathway due to which diabetes complications like cataractogenesis occurs. Further, *in vivo* researches are required to confirm the present results and there is a need to prepare health supplementary herbal product by using Betanin for the treatment of diabetes and its complications.

6. DECLARATIONS

Conflict of interest: *The author declares that there is no conflict of interest.*

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