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ANTI-OXIDANT AND ANTI-DIABETIC ENZYME INHIBITORY EFFECT OF PRUNASIN

Kanumuru Rahul Reddy[1], Dr. Abirami Arthanari[2]* and Dr. Parameshwari[3]

[1]Under Graduate Student, Saveetha Dental College, Saveetha Institute of Medical and Technical Science, Saveetha University, Chennai 600077, Tamil Nadu, India

[2]Senior Lecturer, Department of Forensic Odontolgy, Saveetha Dental College, Saveetha Institute of Medical and Technical Science, Saveetha University, Chennai 600077, Tamil Nadu, India

[3]Department of Pharmacology, Saveetha Dental College and Hospital, Saveetha Institute of medical and Technical Sciences, Saveetha University, Chennai -600077

ABSTRACT

INTRODUCTION:

Prunasin, a naturally occurring cyanogenic glycoside found in various plant sources, has attracted considerable attention for its potential health-promoting properties. This study aimed to investigate the antioxidant and anti-diabetic enzyme inhibitory effects of Prunasin, with the goal of understanding its therapeutic potential in combating oxidative stress and managing diabetes.The research assessed Prunasin's ability to scavenge free radicals and reduce oxidative damage, crucial factors in mitigating oxidative stress-related disorders. Additionally, the study explored Prunasin's inhibitory effect on key enzymes involved in glucose metabolism, which could contribute to regulating blood glucose levels and managing diabetes-related complications.

AIM:

This study aimed to investigate the antioxidant and anti-diabetic enzyme inhibitory effects of Prunasin.

MATERIALS AND METHODS :

Xanthine, α-glucosidase (1U/ml) from Saccharomyces cerevisiae, p-nitrophenyl- α -D-glucopyranoside (p-NPG), DPP-IV and synthetic substrate Gly-Pro-p-nitroanilide were procured from Sigma-aldrich, USA. Quercetin, Acarbose and Sitagliptin was purchased from TCI chemicals, India. All other chemicals, reagents and solvents used were of analytical grade and purchased from SRL chemicals, India.

RESULTS AND DISCUSSION:

Demonstrated significantly positive outcomes for Prunasin's antioxidant and anti-diabetic properties. It effectively scavenged free radicals, suggesting its role as a potent antioxidant agent capable of protecting cells and tissues from damage. Moreover, Prunasin showed promising inhibitory effects on key glucose-metabolising enzymes, providing potential avenues for diabetes management.These findings align with existing research on Prunasin's antioxidant and anti-diabetic potential, further supporting its potential as a therapeutic agent. However, more in-depth investigations are required to elucidate its underlying mechanisms of action and assess any long-term effects. In vivo studies and clinical trials are essential for validating Prunasin's efficacy and safety as a potential therapeutic agent for human use.

CONCLUSION:

This study contributes valuable insights into the health benefits of Prunasin, highlighting its potential in combating oxidative stress and managing diabetes. The findings present promising opportunities for future research and therapeutic applications, emphasizing the need for further exploration of this natural compound's potential in promoting human health and well-being.

Keywords: Prunasin, cyanogenic glycoside, Xanthine oxidase, α – glucosidase, DPP-IV inhibition assay

INTRODUCTION

Prunasin, a naturally occurring compound found in various plant sources, has garnered significant attention in recent years due to its potential health-promoting properties. Research into its biological activities has revealed

promising antioxidant and anti-diabetic enzyme inhibitory effects, making it a subject of interest in the field of nutrition and health.(1)(2)

As oxidative stress and diabetes continue to be major health challenges worldwide, the search for natural compounds with therapeutic potential has intensified. Prunasin, a cyanogenic glycoside commonly present in many fruits and vegetables, has emerged as a compelling candidate in this pursuit. Its ability to scavenge free radicals and reduce oxidative damage suggests a possible role in mitigating oxidative stress-related disorders.

Additionally, Prunasin has shown promise in inhibiting certain enzymes involved in glucose metabolism, making it a potential anti-diabetic agent. By modulating these key enzymes, Prunasin may help regulate blood glucose levels, thus presenting an exciting avenue for managing diabetes and its associated complications.(3)(4)(5)

This introductory exploration aims to provide an overview of the current scientific literature on the antioxidant and anti-diabetic enzyme inhibitory effects of Prunasin. By shedding light on its potential therapeutic applications, we hope to contribute to the growing body of knowledge surrounding natural compounds that hold promise in combating oxidative stress and diabetes. Understanding Prunasin's biological activities is crucial for unlocking its full potential as a valuable resource in promoting human health and well-being. (6)(7)(8)

Materials and Methods: **Chemicals and Reagents**

Xanthine, α-glucosidase (1U/ml) from *Saccharomyces cerevisiae*, p-nitrophenyl- α -D-glucopyranoside (p-NPG), DPP-IV and synthetic substrate Gly-Pro-p-nitroanilide were procured from Sigma-aldrich, USA. Quercetin, Acarbose and Sitagliptin was purchased from TCI chemicals, India. All other chemicals, reagents and solvents used were of analytical grade and purchased from SRL chemicals, India.

Xanthine Oxidase Inhibitory Activity

The XO inhibitory activity was assayed spectrophotometrically under aerobic conditions, based on the procedure reported by Bustanji et al. 2011. The substrate and the enzyme solutions were freshly prepared. The assay mixture, consisting of 50µL of different concentrations Prunasin (10-320µM), 35µL of 0.1mM phosphate buffer (pH=7.5) and 30µL of enzyme solution (0.01units/ml of XO in 0.1mM phosphate buffer, pH=7.5), was prepared immediately before use. After 30mins of incubation at 25^{o}C, the reaction was initiated by the addition of 60µL of substrate solution (150mM of Xanthine in 0.1mM Phosphate buffer). The absorption at 295 nm, indicating the formation of uric acid at 25°C, was monitored and the initial rate was calculated. A blank was prepared in the same manner. One unit of XO was defined as the amount of enzyme required to produce 1 mmol of uric acid/minute at 25 °C. XO inhibitory activity is expressed as the percentage inhibition of XO in the above system, calculated as (1-B/A) × 100, where A and B are the activities of the enzyme without and with different concentrations of Prunasin. IC_{50} values were calculated from the mean values of data from three determinations. Quercetin was used as reference standard.

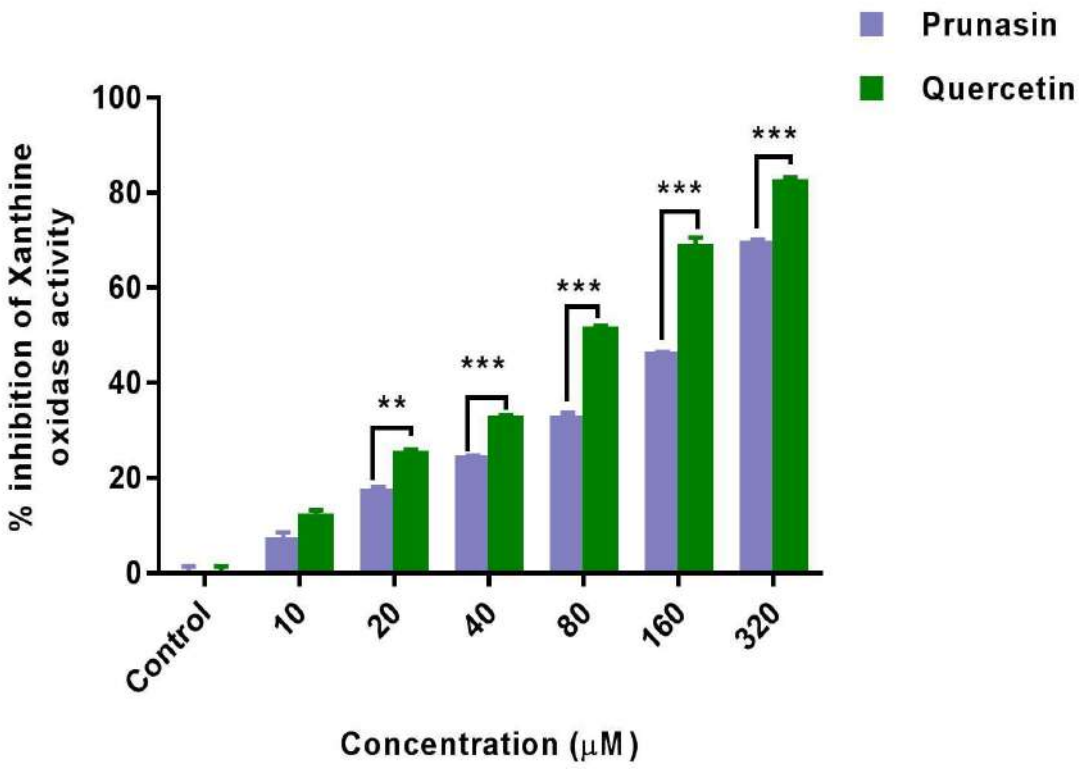
A – Glucosidase Inhibitory Activity

The enzyme inhibition activity for α-glucosidase was evaluated according to the method previously reported by Shruthi et al. (2011) with minor modifications. The reaction mixture consisted of 50µL of 0.1M phosphate buffer (with pH of 7.0), 25µL of 0.5mM 4-nitrophenyl α-D-glucopyranoside (dissolved in 0.1M phosphate buffer, with pH of 7.0), 10µL of different concentrations of Prunasin (10-320µM) and 25µL of α-glucosidase solution (a stock solution of 1mg/mL in 0.01M phosphate buffer, with pH of 7.0 was diluted to 0.1Unit/mL with the same buffer, with pH of 7.0 just before assay). This reaction mixture was then incubated at 37°C for 30 min. Then, the reaction was terminated by the addition of 100µL of 0.2M sodium carbonate solution. The enzymatic hydrolysis of substrate was monitored by the amount of p-nitrophenol released in the reaction mixture at 410nm using microplate reader. Individual blanks were prepared for correcting the background absorbance, where the enzymes were replaced with buffer. Controls were conducted in an identical manner replacing the plant extracts with methanol. Acarbose was used as positive control. All experiments were carried out in triplicates.

In vitro DPP-IV inhibition assay (Hamendra Singh Parmar et al., 2012)

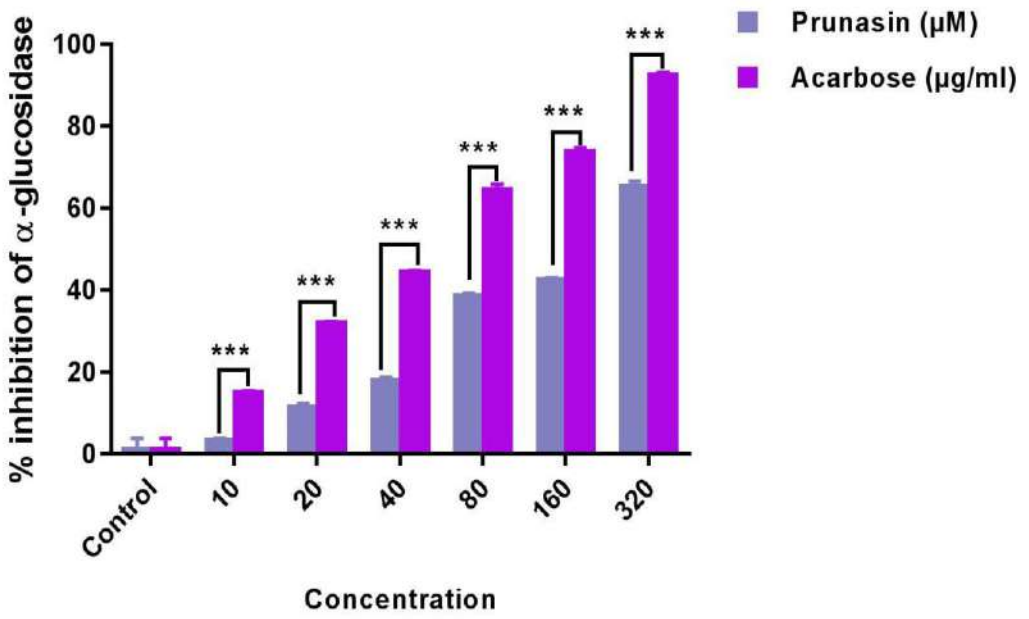
Different sets of tubes each in triplicate were used for DPP-IV control; DPP-IV + different concentrations of sitagliptin (10-320nM), DPP-IV + Prunasin (10-320µM). Briefly, the sitagliptin phosphate monohydrate and Prunasin were dissolved in dimethyl sulphoxide (DMSO) and diluted with tris buffer (pH 8.0, 50mM) to achieve the concentration the highest concentration of 1000µM. From the stock concentration of test compound and standard, the working dilutions of 10, 20, 40, 80, 160, 320µM and 10, 20, 40, 80, 160, 320nM respectively was prepared. The assay was conducted using DPP-IV enzyme diluted with Tris buffer (0.05 U/ml) and pipette 50µl into clear microplate wells. Subsequently, 10µl of tris buffer or sitagliptin phosphate monohydrate or Prunasin was also added and incubated at 37°C for 20 min. Finally a 50µl of (0.20mM prepared in tris buffer) chromogenic substrate Gly-Prop-nitroanilide (Sigma–Aldrich, USA) was added into each well. The hydrolysis of the substrate was monitored at 405nm wavelength using a Microplate plate reader from Synergy Biotek, USA. Activity was expressed as ΔA_{405} nm/min. The experiment was performed in triplicate and compared with negative control (enzymatic solution without inhibition), while standard DPP-IV inhibitor drug sitagliptin employed as positive control.(9)(10)(11)

RESULTS



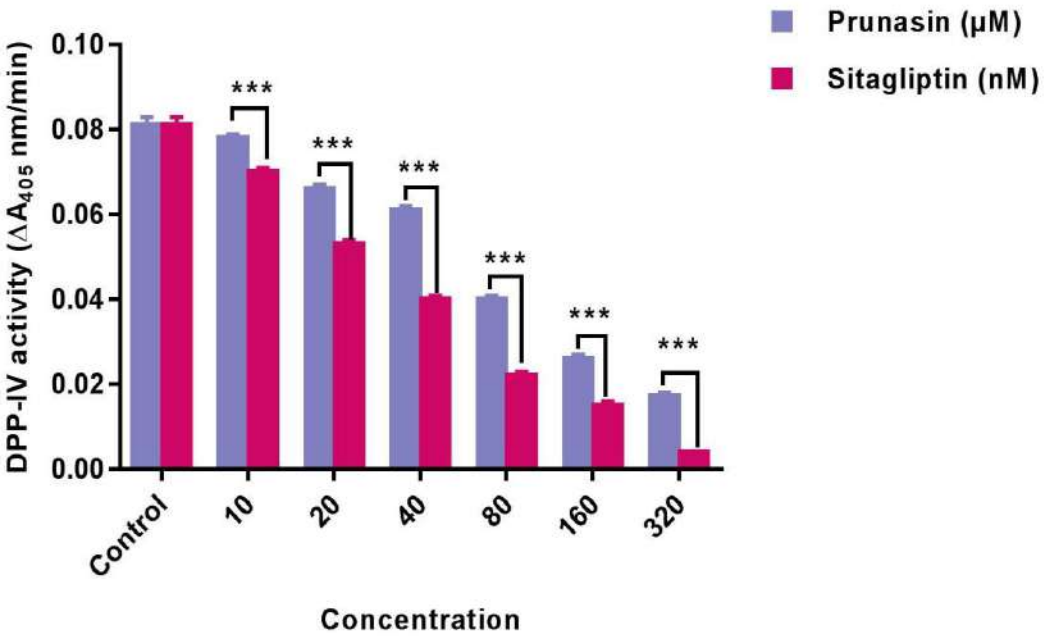
Graph 1: Xanthine oxidase inhibitory activity

The standard taken here for the comparison is quercetin. Our sample exhibited similar inhibition of xanthine oxidase as of that of standard. This indicates that our sample has scavenging properties.



Graph 2: α – glucosidase inhibitory activity

The standard taken here for the comparison is acarbose. Our sample shows increased inhibition activity of α – glucosidase with the increase in concentration. This indicates that our sample possesses α – glucosidase inhibitory activity.



Graph 3: In vitro DPP-IV inhibition assay

The standard taken here for comparison is sitagliptin. Our sample shows similar activity of that of standard indicating the presence of DPP-IV inhibition.

DISCUSSION:

Our study investigated the antioxidant and anti-diabetic effects of Prunasin, a natural cyanogenic glycoside found in fruits and vegetables. The results showed significant positive outcomes, suggesting its potential as a therapeutic agent for combating oxidative stress and managing diabetes. Prunasin effectively scavenged free radicals, indicating its role as a potent antioxidant that could protect cells from damage. Moreover, it exhibited an inhibitory effect on key glucose-metabolizing enzymes, potentially regulating blood glucose levels crucial for diabetes management.(12)

These findings align with previous research highlighting Prunasin's antioxidant properties and anti-diabetic potential. Nevertheless, further research is necessary to understand its mechanisms of action and long-term effects. In vivo studies and clinical trials are essential for validating Prunasin's efficacy and safety as a potential therapeutic agent in humans. Overall, this study contributes valuable insights into Prunasin's health benefits, presenting promising opportunities for future exploration in promoting human health.(13)

CONCLUSION:

This study provides valuable insights into the antioxidant and anti-diabetic enzyme inhibitory effect of Prunasin. The significantly positive results support the notion that Prunasin holds promise as a natural compound for addressing oxidative stress and diabetes-related complications. By contributing to the growing body of knowledge surrounding Prunasin’s bioactivity, this research opens up new avenues for future exploration and potential therapeutic applications. The pursuit of harnessing the full potential of Prunasin in promoting human health remains an exciting area of research with promising prospects for improving the well-being of individuals worldwide.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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ETHICAL CLEARANCE

In vitro study. Not needed

DURATION OF STUDY

3 months

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