Insulin mimetic potential of *Hylocereus undatus* from extracted myo-inositol and proteins

Porshia Sharma[1], Pragya Rathore[2], Sarvesh Seth[2], Lubaina Kaba[2], Neha Bhamawat[2] & Palak Jain[2]

[1] Institute for Innovative Learning and Research (IILR) Academy, Indore, India

[2] Softvision Institute of Biotechnology and Science, Indore, India

Correspondence: Porshia Sharma, Institute for Innovative Learning and Research (IILR) Academy, Indore, India. E-mail: venice12051999@gmail.com

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Abstract

Diseases are spreading like a trend and victimising every other individual globally. Here, we are referring to one such most common disease that has not even spared young lives i.e., diabetes. Annually, several people lose their lives and loved ones because of this dangerous disease. This has compelled the researchers to think and work on some life saving treatment. People suffering from hyperglycaemic conditions have insulin resistance which can be improved by intake of myo-inositol. Myo-inositol has potential to regulate this insulin hormone which can prevent and control diabetes mellitus. In this research, we have used a natural source (fruit) *Hylocereus undatus*; it has proved to be a good source of myo-inositol and some proteins that help in insulin regulation naturally. Several techniques and tests were performed such as extraction, purification, crystallisation, proteolytic activity assay, protein estimation, etc. Positive results of myo-inositol were observed through crystallisation together with decent amount of protein concentrations by *Folin Lowry* test and SDS-PAGE analysis.

Keywords: myo-inositol, *D*-chiro inositol, diabetes, insulin resistance, *Hylocereus undatus*.

Potencial mimético da insulina de *Hylocereus undatus* extraída de mio-inositol e de proteínas

Resumo

As doenças estão se espalhando como uma tendência e vitimando todos os outros indivíduos globalmente. Aqui, estamos nos referindo a uma dessas doenças mais comuns que nem mesmo poupou vidas jovens, ou seja, diabetes. Anualmente, várias pessoas perdem suas vidas e entes queridos por causa dessa doença perigosa. Isso obrigou os pesquisadores a pensar e trabalhar em algum tratamento para salvar vidas. As pessoas que sofrem de condições hiperglicêmicas têm resistência à insulina, que pode ser melhorada pela ingestão de mio-inositol. O mio-inositol tem potencial para regular esse hormônio insulina que pode prevenir e controlar o diabetes mellitus. Nesta pesquisa, usamos uma fonte natural (fruto) de *Hylocereus undatus*, que provou ser uma boa fonte de mio-inositol e algumas proteínas, que ajudam na regulação da insulina naturalmente. Várias técnicas e testes foram realizados, como extração, purificação, cristalização, ensaio de atividade proteolítica, estimativa de proteína etc. Resultados positivos de mio-inositol foram observados através da cristalização juntamente com quantidades decentes de concentrações de proteína pelo teste *Folin Lowry* e análise SDS-PAGE.

Palavras-chave: mio-inositol, *D*-chiro inositol, diabetes, resistência à insulina, *Hylocereus undatus*.

1. Introduction

Inositol is a type of sugar made in the body and found naturally in foods, which can be found in nine forms in nature. Inositol greatly refers to a group of molecules that shares structural similarity to glucose and is involved in cellular signaling. In terms of chemistry, Inositols are polyols that consists of six-carbon ring structure where each carbon is hydroxylated. Among these sugar-alcohol isomers many are in biologically active forms, of which myo-inositol (MI) forms the most common one. Inositol plays a crucial role as a component of membrane

phospholipids and also in mediating osmoregulation (Majumder; Biswas, 2006), whereas its phosphorylated derivatives act as second messengers in the signal transduction pathways (Berridge, 2009) and also mediate phosphorylation of proteins (Saiardi et al., 2004). Apart from this inositol is also involved in various regulatory and metabolic processes andhas potential to balance certain chemicals in the body inorder to help with mental conditions such as depression, panic disorder and obsessive compulsive disorder (OCD) and it might also help inbetter working of insulin.

Myo-Inositol is a cyclitol which present in all three domains of life (Michell, 2011; Croze; Soulage, 2013) and naturally occurs in animal and plant cells either in its free form or in bound state with phospholipids or inositol phosphate derivatives. The myo-inositol form is used as a majority of inositol supplementation, as it is the most abundant isomer of inositol present in the body. Hence, myo-inositol and *D*-chiro-inositol (DCI) forms the most common in supplements available. Although, both myo-inositol and *D*-chiro-inositol are the most common supplements, but all forms are interchangeably referred to as inositol and are best known for their effects on insulin resistance. Myo-inositol is also known by its chemical name as 1,2,3,5-*Trans*-4,6-cyclohexanehexolis which is the predominant isomeric form of inositol that we can easily find in nature and in alsoin our food.

The role of myo-inositol as a second messenger is believed to improve insulin sensitivity and increase the intracellular glucose uptake (Regidor; Schindler, 2016) and their use as dietary supplements displayed insulin-mimetic effects (Croze; Soulage, 2013). The researchers suggest that myo-inositol may provide a wide variety of benefits as a dietary supplement whereas; DCI is synthesized by an insulin dependent epimerase that is responsible for the conversion of MI into DCI. Both MI and DCI are involved in an array of cellular functions and abnormalities in their metabolism and are actively involved in particular into development of insulin resistance and various diabetic complications.

Hyperglycemia and hyperlipidemia causes endothelial dysfunction that is an early feature of diabetes (Nacimento et al., 2006) to which Inositol phosphor-glycans (IPGs) are generated rapidly as a response to insulin and shows insulin-like effect in-vivo and *in-vitro* (Huang et al., 1999). Normally, In human urine the level of chiro-inositol is found absent or if present then negligible, while the MI content increases in the urine of diabetic patients. The no or low urinary chiro-inositol is inversely correlated to insulin resistance, the administration of DCI in diabetic patients effectively decrease hyper-glycaemia and hyper-triglyceridemia (Larner, 2002). In type 2 diabetes, the higher levels of MI and the lower levels of DCI are referred to as inositol imbalance. Thus, chiro-inositol deficiency and imbalance together with myo-inositol are directly related to insulin resistance (Larner et al., 2010).

Also on the other hand, digestive enzyme also helps the body in breaking down of nutrients in food for proper absorption and is used by the body. Diet rich in proteins that are broken down by the protease enzyme helps lowering blood glucose post-prandially in people with type-2 diabetes and improves the overall glucose control in the body. Researchers proved that the dietary protein plays a vital role in management of both pre-diabetes and diabetes mellitus (Campbell; Rains, 2015), this is because ideal protein focuses on both reducing carbohydrates and increasing the amount of healthy proteins in the body which is not only safe but also help preventing and even reversing of diabetes mellitus.

It has been reported that Dragon fruit has medicinal properties and also have potential for the treatment for diabetes mellitus (Omidizadeh et al., 2014). It has been seen in some conducted studies that in some animals dragon fruit produces anti-diabetic effect by regenerating the pancreatic beta cells. Thus, high amounts of fruit consumption helps in reduction of blood glucose level as it protects against insulin resistance and fatty liver problems.

So, here we have extracted myo-inositol, checked protein content and protease enzyme activity of; all these together can contribute in controlling and prevention of diabetes.

2. Materials and Methods

2.1 Graphic summary of the experimental study

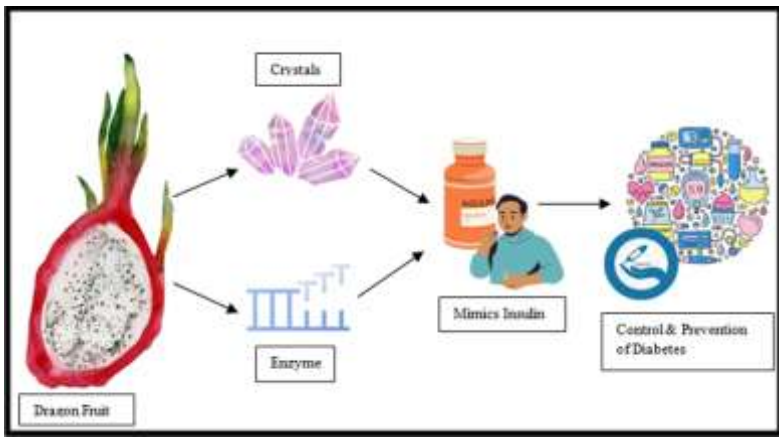

Figure 1. *Hylocereus undatus* fruit, crystal production extraction and enzyme for drug production. Source: Authors, 2023.

2.2 Fruit processing

Hylocereus undatus was thoroughly cleaned with tap water in order to remove impurities such as dust particles and grit. After washing, the fruit was then peeled, and the pulp was separated and cut into pieces (to increase surface area which will increase extraction efficiency) weighing around 250 g. This was followed by extraction, purification, and crystallisation processes.

2.3 Extraction & purification

Hylocereus undatus pulp of 250 g was soaked in 250 mL 60% methanol for around 3 h at room temperature. The sample was then subjected to water bath (60-70 °C) for 20 min after which the sample was filtered, and the obtained filtrate was ready for further experiments. For obtaining slurry syrup the filtrate was concentrated by evaporation at 40-50 °C with a help of magnetic stirrer and a vacuum pump for around 4 h. The obtained slurry was then rinsed with 50 mL ether followed by 100% 50 mL ethanol. The solvent was then decanted, and the insoluble protein was dissolved in 15 mL (*d/w*) following filtering of sample; then again add 15 mL ethanol and keep at 4-6 °C for 3-4 h, filter it and the filtrate was subjected to evaporation for obtaining concentrated sample.

2.4 Crystallisation

The resulting mass was dissolved in 15 mL cold water and 100% 25 mL ethanol. The sample was left at 4-6 °C for 7 days for crystallization. The resulting crystals were removed from solution through filtration followed by air drying and werestored carefully in a glass vial.

2.5 Protease extraction & purification

The freshly chopped pieces of *H. undatus* pulp was given a quick blend with sodium acetate buffer having 4:1 ratio at 3 °C temperature and pH 5. The mixwas then filtered and the filtrate was centrifuged at 7000 rpm for 5 min at 5 °C and the supernatant obtained is the crude enzyme. This crude enzyme was used further for purification process. The crude enzyme was saturated to 20% by adding powdered ammonium sulphate gradually accompanied by gentle stirring for 1 h. Through centrifugation precipitate was removed and the enzyme was dissolved in Tris-HCL buffer. The supernatant was saturated using 40%, 60% and 80% ammonium sulphate whereas the precipitate obtained in each step was dissolved in Tris-HCL buffer of pH 8 and was dialyzed against pH 5 Tris-HCL buffer. After dialysis, the ammonium sulphate precipitate was subjected to cation exchange chromatography on SP-Sepharose fast flow column. Further, the column washing was done with the buffer until proteins were extracted out using linear gradient ranging between 0-1 M. The fractions obtained were examined for proteolytic activity, protein concentration and protein separation using SDS-PAGE analysis (Zanphorlin et al., 2011).

2.6 Proteolytic activity assay

For determining the proteolytic activity, the reaction mixture containing azo-casein (1 mL of 5% *w/v*) was prepared in Tris-HCL buffer of pH 8 and 0.1 mL of enzyme. This reaction mixture was then incubated in water bath between 75-80 °C for 60 min and 0.4 mL TCA (Trichloroacetic acid) (10% *w/v*) was added in order to stop the reaction; this was further centrifuged at 9,000 rpm for 10 min after which the optical density of TCA-soluble supernatant was determined using UV-*Vis* spectrophotometer at 430 nm.

2.7 Protein concentration

Protein concentration was determined using *Folin Lowry* method with BSA (Bovine Serum Albumin) as standards with 10 min incubation at room temperature and adding *Folin Ciocalteu* reagent with incubating the prepared samples at room temperature for 30 min and recording the optical density at 670 nm. The density observed was 0.07, 0.14, 0.24, 0.31 and 0.38 for 40 µL, 80 µL, 120 µL, 160 µL and 200 µL respectively. The protein concentration of the test sample was determined using standard graph and straight line equation.

3. Results and Discussion

3.1 Crystallisation

The crystals of myo-inositol were obtained after 7 days from *H. undatus*, and the crystals were visible to naked eyes however it is observed under microscope. The crystals were then weighed, and it was found to be 1.20 mg. The amount is quite significant in order to proceed further studies of bio-transformation.

3.2 SDS-PAGE analysis

The obtained purified protease enzyme was subjected to Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis for separation of enzymes according to their molecular weights. Here, ***L***-protein ladder, ***A*** - crude enzyme, ***B*** - $(NH_4)_2SO_4$ precipitated enzyme, ***C*** - SP-Sepharose (purified enzyme), ***D*** - purified enzyme (Figure 2). The obtained separated purified enzyme is protease of molecular weight 28 kDa.

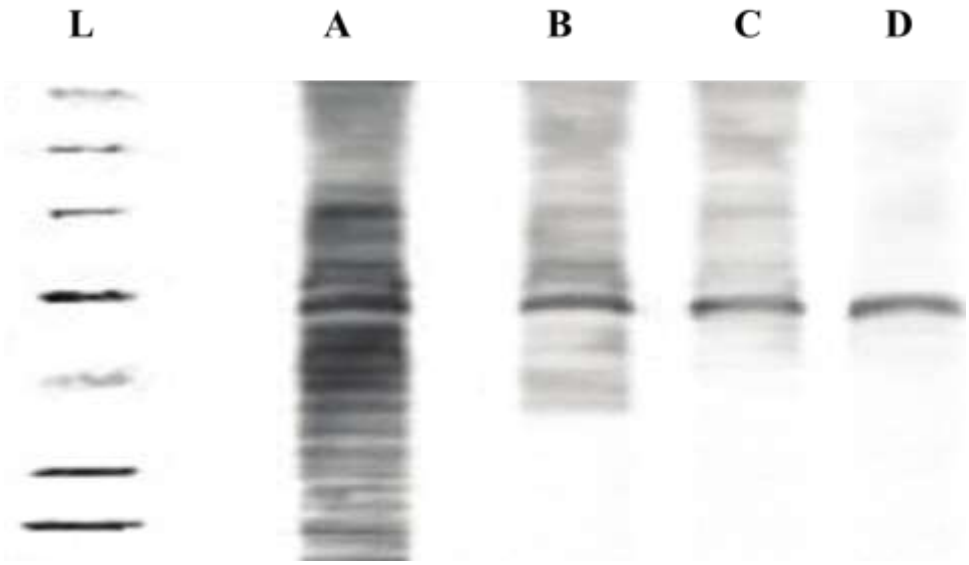

Figure 2. SDS-PAGE of purified enzyme (protease). Note: L- protein ladder, (**A**) crude enzyme. (**B**) $(NH_4)_2SO_4$ precipitated enzyme. (**C**) SP-Sepharose (purified enzyme). (**D**) purified enzyme. Source: Authors, 2023.

3.3 Protein concentration

The protein concentration estimated using Folin Lowry method at 670 nm was observed to be 6.19 mg/g^{-1}, (Figure 3). The utility of myo-inositol in insulin pathway is significantly high. The major issue arises in the pathway when there is an imbalance in between the ratio of myo-inositol and *D*-chiro inositol. Myo-inositol is the precursor of *D*-chiro inositol and is converted into DCI with the help of epimerase enzyme. There are

possibilities that some of the diabetic patients lack epimerase enzyme in that case insulin metabolism is affected due to lack of DCI and thus this mark up the starting of insulin resistance of the body.

The balance between the MI and DCI may also results in coping up of insulin resistance. Thus, intake of *H. undatus* in decent amounts can help in insulin regulation and maintain the blood glucose levels in the body through myo-inositol and protease enzyme. Accordong to a review (Loewus; Murthy, 2000); myo-inositol is highly involved in metabolism of plants and also in the following few processes such as; fertilization, seed dessication, nutrient storage, osmoregulation, auxin physiology, membrane biogenesis, senescence and in the synthesis of cell wall uronosyl and pentose units.

This shows that myo-inositol is a crucial compound in regulating both plant biochemistry and physiology. Moreover, it has also been found concentrated in cerebrospinal fluid ranging between 100–500 μM and it increases to 10 mM or more in the cells of brain (Harwood, 2005). The reason given to this phenomenon is probably that myo-inositol participates actively in the synthesis of membrane phospholipids that affects the neuronal plasticity and also synapse formation in neuron cells (Deranieh; Greenberg, 2009).

The possible connection between depletion of inositol in the frontal temporal lobes and patients having bipolar disorder where depletion in inositol level would result in dysfunctioning of mitochondria that causes a decrease in oxidative phosphorylation, a decrease in intra-cellular pH and an increase in the levels of lactate in brains causing neural related disorders (Shimon, 1997; Silverston, 2005). The other roles of inositol and its common derivative myo-inositol include working as potent regulators for large number of hormones, growth factors and neurotransmitters.

As a result of its significance myo-inositol has also been used as part of treatment for diabetes mellitus (Clements; Reynertson, 1977); status epilepticus (Solomonia et al., 2010); obsessive-compulsive disorder (OCD) (Fux et al., 1996); psoriasis and eczema; and recently a report said that myo-inositol may improve metabolic syndromes in post-menopausal women (Giordano et al., 2011). Further studies in regular uptake of *H. undatus* monitoring can confirm the maintaing of blood glucose levels in humans on a mass level is required.

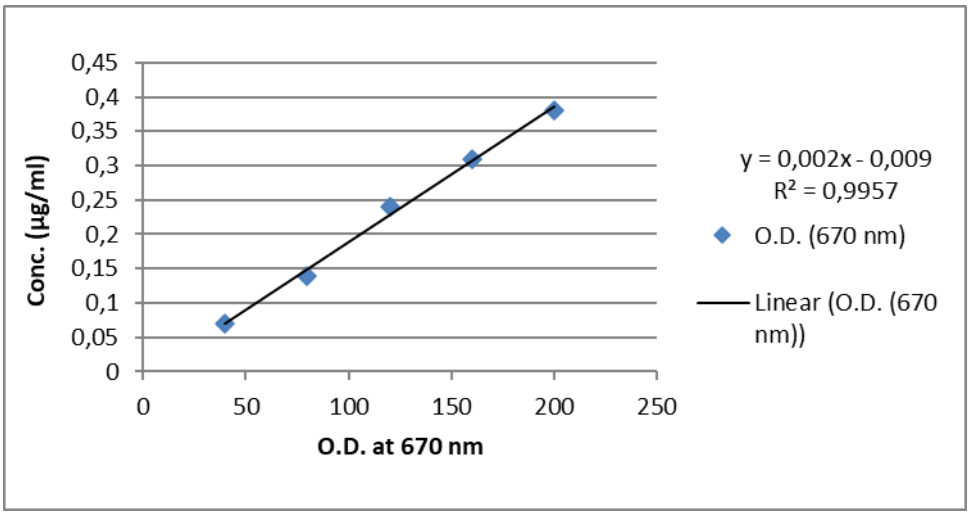

Figure 3. Representation of protein concentration. Source: Authors, 2023.

4. Conclusions

In the present work, *Hylocereus undatus* a species of "Dragon fruit" is studied for it's benefits in controlling and preventing diabetes mellitus. Overall, to sustain a healthy life in the world full of toxic and harmful environment the need of some natural treatment is the need of an hour. In this fast growing world, the diseases are also growing day by day, some of which are becoming threat to human race and also kills. This concern to public health makes us to think in a critical manner and produce a solution for such one of the problems i.e., diabetes mellitus.

The consumption of *H. undatus* can be a potential source of myo-inositol and also has proteins which together can prove to be a healthy option and a natural source for control and prevention of hyperglycaemia. Thus, intake of this fruit can be a good alternative rather than taking artificial/man-made drugs in the form of tablets and

injectables.

5. Acknowledgments

Not applicable.

6. Auhors' Contributions

Porshia Sharma: performed analysis, wrote the paper. *Pragya Rathod*: designed the analysis. *Sarvesh Seth*: collected data. *Lubaina Kaba*: performed analysis. *Neha Bhamawat*: contributed data. *Palak Jain*: analysis tools.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Not applicable.

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