Abstract

In Vitro and *in Silico* Studies of Polyphenols from *Vicia faba* Beans and its Evaluation of Antidiabetic activity

The aim of this research work was to explore the antidiabetic potential of polyphenols from faba beans by *in vitro* and *in silico* approach. Diabetes mellitus is a group of metabolic disorder of glucose metabolism. Excessive production of reactive oxygen species (ROS) is the result of oxidative stress and responsibility for the development of insulin resistance, β-cell dysfunction, impaired glucose tolerance, and type 2 diabetes. Free radical production is balanced by the anti-oxidative defense system. However, polyphenols (gallic-acid, ellagic-acid, catechin, epicatechin) have an inclination to neutralize excessive free radical generation due to antioxidant property. India is rich in valuable natural resources of fruits, vegetables, cereals, herbs, and medicinal plants many of which remain unexploited and are limited for regional or rural use only. *Vicia faba* beans are nutritionally rich and staple pulses of the less privileged society of India.

Recently, there has been a growing interest in natural polyphenols. Since the synthetic drugs available in the market have several side effects. Therefore, it would be important to explore natural dietary polyphenols of similar structure with inhibitory activity against α-amylase, α-glucosidase, xanthine oxidase in the treatment of diabetes mellitus. The present study on polyphenols derived from faba bean may be an alternatives strategies in overcoming hyperglycemia-induced chronic diseases, such as type 2 diabetes.

Phytochemical screening, chemo-profiling, FTIR and HPLC analysis confirmed the presence of phenolics compounds in *Vicia faba* beans. Acetone seed extract showed phenolics(polyphenols) compound like gallic acid (m/z [M−H]=169.0124,$C_7H_6O_5$) and flavonoids compounds ellagic acid, epigallocatechin (m/z[M−H=305.0644,$C_{15}H_{14}O_7$),

catechin (m/z[M−H]=289.0656,$C_{15}H_{14}O_6$), catechin derivatives epigallocatechin gallate (m/z [M−H]=457.0578,$C_{22}H_{18}O_{11}$) and epicatechin monogallate (m/z [M−H] =441.081 ,$C_{22}H_{18}O_{10}$). The protonated and fragmented behavior of acetone seed extract revealed the presence of gallic-acid (MS/MS ,m/z 169) and catechin (MS^n, m/z 288.3).

In vitro studies of the effect of phenolic extracts were made on key enzymes linked to type 2 diabetes mellitus (α-amylase and alpha-glucosidase). Different polar extract of faba seeds was evaluated and acetone extract was found to have the highest inhibitory potential against porcine α-amylase (IC_{50} value of 2.94 mg/mL). Kinetic analysis revealed that the acetone extract displayed a mixed mode of inhibition towards α-amylase. *In-silico* analysis was agreement with *in-vitro* studies in which phenolic compounds (catechin, epicatechin, gallic acid, and ellagic acid) showed more negative free energy against standard drug (acarbose) and bound with catalytic and nonresidues of α-amylase. Ethanol extract also showed the mixed mode of inhibition (Km, apparent = 0.59 ±0.09 mM and Vmax, apparent 0.152 ±0.022 mM/minute) on alpha-glucosidase. These results might be due to the synergistic action of constituents present in seed extract or acting separately. Molecular docking studies of gallic acid and catechin on α-glucosidase, alpha-amylase proposed productive binding modes with an effective number of hydrogen bonds and binding energy. Tyr 63, Arg197, Asp198, Glu 233, Asn324, Asp 326 of α-glucosidase participated in binding events with gallic acid and catechin. Gallic acid and catechin were demonstrated significant binding energy (-6.58 kcal/mol and -7.25 kcal/mol). Molecular dynamics simulation studies were performed for both complexes i.e. gal:α-glucosidase, cat: α-glucosidase and cat: α-amylase along with apo state of α-glucosidase, which revealed stable systems during simulation. Catechin displayed better binding robustness in comparison to gallic acid with α-glucosidase and α-amylase during simulation. Moreover, molecular docking and molecular dynamics simulation studies supply the

predicted residues that may hold favorable polyphenolic-specific interactions. Computational biology approaches might be influencing the effects of dietary polyphenols from faba bean on the modulation of metabolic diseases by testing the *in vitro* study.

Acetone extract of faba bean (*Vicia faba* L.) was found to be highest total phenol and flavonoid content among all extracts. Antioxidant activity for inhibition percentage (free radical scavenging activity) had 86.47 % for acetone extract, and 97.36% for ascorbic acid respectively. IC_{50} value of ascorbic acid and acetone extract was found to be 9.0 µg/mL±0.20 and 30.0 µg/mL±0.21 respectively. Faba bean seeds contains catechin, epicatechin, gallic acid, and ellagic acid which on molecular docking study revealed that it binds effectively with xanthine oxidase by binding energy of -7.78 kcal/mol, -6.11 kcal/mol,-6.39 kcal/mol,-5.78 kcal/mol respectively compared to standard allopurinol drug with binding energy of -4.94 kcal/mol. It was found that gallic acid, ellagic acid, catechin, epicatechin (polyphenols) and allopurinol bind other than catalytic residues (Glu-1261) of xanthine oxidase. The probable binding modes of the gallic-acid and catechin from the present study may extend the knowledge of the XO-polyphenols interactions and offer a way to design the analogs of gallic acid and catechin with minimizing oxidative stress. *In vitro* and *in silico* analysis showed that mode of enzyme inhibition was the mixed type.

2-Deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose (2-NBDG) can be transported into *S. cerevisiae* through glucose transporters and that glucose uptake activity in yeast can be directly evaluated by measuring the incorporation of 2-NBDG into the cell as fluorescence intensity. Acetone extract showed a significant effect on glucose uptake rate in yeast cells by 77.28±2.42% and least was found in chloroform seed extract by 52.36± 2.06% %. Flow cytometry and confocal microscopy techniques

revealed that acetone seed extract was reducing oxidative stress with respect to control in yeast cells. AFM microscopy revealed that at 3% hydrogen peroxide concentration roughness was found to be maximum (441±6.7 nm), when 3% H_20_2 was used in combination with acetone seed extract on yeast cells, surface roughness diminished to 251±6.2 nm. Propidium iodide and DAPI staining showed apoptotic ratio significantly high as 0.40 (40 ± 1.18%, $P<0.05$) and 0.42 (42 ± 1.16%, $P<0.05$) respectively in hydrogen peroxide-treated cell as compared to other untreated cells. MTT assay showed that acetone extract had maximum survival rate (82.067%, $P<0.05$) and least survival rate was found to be in the case of chloroform extract (70.48%, $P<0.05$).

Flow cytometry study revealed the consequence of H_2O_2 on cell line as increased cell death was observed from 9.72% to 41.66% as compared to the control. AFM, SEM and ROS measurement also confirmed the protective effect of polyphenols in 3T3-L1 cell lines. Propidium iodide and DAPI staining showed that apoptotic ratio as 0.35 ± 2.62% ($P<0.05$) and 30 ± 2.54 in hydrogen peroxide-treated the cell as compared to control. The observations of flow cytometry and confocal microscopy marked the effect of seed extract (0.86±0.031, 3.52 ±0.52, $P<0.05$), on glucose uptake in cells through the better relative fluorescence intensity than control. Synergistic effect of polyphenols might be responsible for the reduction of oxidative stress and hypoglycaemic potential in the yeast cell and 3T3-L1 cell line. Hypoglycemic and anti-oxidative potential of faba bean may be due to the synergistic effect of polyphenols present in seed extract or acting independently. Dietary faba bean polyphenols would act as a lead compound to drug discovery and development process. The findings of the present study may give an insight into the further development of the novel antidiabetic drug from the seeds of *Vicia faba*.

Keywords: ***Antidiabetic activity; Antioxidant activity; Vicia faba; Polyphenols; Synergistic effect; Saccharomyces cerevisiae; 3T3-L1 cell line***.

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