Screening of In Vitro Antidiabetic Activity of Herbal Formulation Meshashringi

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Abstract

Diabetes is diagnosis by hyperglycemia due to absolute or relative deficiency of insulin. Today's lifestyle has been changed and sharp increase in the incidence and prevalence of diabetes mellitus has been perceived. The antidiabetic drugs reduce the gastrointestinal glucose production and absorption through the inhibition of carbohydrate digesting enzymes such as α-amylase and α-glucosidase. The present study was aimed to investigate in vitro antidiabetic activity of aqueous extract of Meshashringi formulation using enzyme inhibitory assays. The Meshashringi was evaluated against α-amylase, α-glucosidase and dipeptidyl peptidase-IV (DPP-IV). The Meshashringi revealed 50% inhibitory activity for α-amylase, α-glucosidase and DPP-IV enzyme at 106.00 μg/ml, 124.91 μg/ml and 160.67 μg/ml, respectively. Consequently, the standard drug Acarbose exhibited 50% inhibitory activity for α-amylase, α-glucosidase and DPP-IV enzyme at 37.80 μg/ml, 65.06 μg/ml and 48.22 μg/ml, respectively. The Meshashringi formulation produces dose dependent antidiabetic activity.

Keywords: Meshashringi, α-amylase, α-glucosidase, Dipeptidyl peptidase-IV

1 Introduction

Diabetes mellitus is defined as a chronic disorder characterized by elevated plasma glucose concentration resulting from insufficient amount of insulin and insulin resistance, or both along with disturbance in carbohydrate, fat and protein metabolism finally result a hyperglycemia. Diabetes mellitus is a global health problem and several year has becomes a major health problems worldwide. The number of diabetic people is expected to rise to 366 million in 2030\(^1\). The numerous synthetic drugs are available for the management of diabetes, and physician usually prescribed them\(^2\). The use of synthetic drugs often found to be associated with side effects. Hence people are more health conscious and approaching for alternative medicines. The Ayurvedic formulations are safe to use due to their minimum side effects, Ayurvedic medicine has curative properties due to the presence of many types of complex chemical substance of different composition\(^8\)\(^-\)\(^10\).

Diabetes mellitus for one therapeutic approach to decrease post-prandial hypoglycemia is to delay the digestion. The α-amylase and α-Glucosidase enzyme hydrolyzed complex polysaccharide to convert in oligosaccharide and disaccharide which are then hydrolyzed by α-Glucosidase enzyme to monosaccharide which are absorbed though the small intestinal in to hepatic portal vein. In vitro models are fairly based on a specific process, where in activity of an enzyme on a metabolic reaction or binding to a receptor. In vitro study is of considerable value in identifying the mechanism of action of a test material and more economical. In vitro assay is an ideal way of obtaining activity for crude extracts and later can be tested in vivo to confirm their effects\(^11\)\(^,\)\(^12\).

The Meshashringi, Ayurvedic medicine was selected for the present study. Meshashringi is listed in the Indian Pharmaceutical Codex and in Indian popular systems of traditional medicine, such as Sidha, Unani and Ayurveda and many type pharmacological actions. Consequently, it restrains the development of various diseases like ageing, carcinogenesis, obesity and diabetes\(^13\)\(^,\)\(^14\). Hence we planned to investigate the antidiabetic activity of Meshashringi formulation by in vitro enzyme inhibitory activity.

2 Materials and Methods

2.1. Material

The Meshashringi (Gymnema sylvestre) Ayurvedic medicine compound purchased from local shop Himalaya Herbal
Healthcare Drug Company and the calcium acetate, Gly-pro-p-nitroaniline, 3,5-dinitrosalicylic acid (DNS), p-nitrophenyl-α-D-glucoside purchased from sigma aldrich. All the chemicals used were of analytical grade.

2.2 Enzyme inhibitory activities by α-amylase

The assay mixture containing 200 μl of 0.02 M sodium phosphate buffer, 20 μl of enzyme and Meshashringi and Acarbose at different concentration (20-100 μg/ml), separately was incubated for 10 minutes at room temperature followed by addition of 200 μl of starch in all test tubes. The reaction was terminated with the addition of 400 μl DNS reagent and placed in boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water and absorbance was measured at 540 nm. The control samples were prepared without any test sample. The % inhibition was calculated according to the formula.

\[
\% \text{Inhibition} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

2.3 Enzyme inhibitory activities by α-glucosidase

The α-glucosidase inhibitory activity was determined by measuring the release of 4-nitrophenol from p-nitrophenyl α-D-glucopyranoside. The assay mixtures for these experiments contained 0.3 ml of 10 mM p-nitrophenyl α-D-glucopyranoside, 1.0 ml of potassium phosphate (0.1M, pH: 6.8), 0.2 ml of enzyme solution and 0.2 ml of inhibitor Meshashringi and Acarbose at different concentration (20-100 μg/ml), separately, all in a final volume of 1.7 ml. Following an incubation time of 30 min at 37º C, the reaction was terminated by the addition of 2.0 ml of 100 mM sodium carbonate. The liberated p-nitrophenol was determined at 400 nm using spectrophotometer. The % inhibition rates were calculated using the formula.

\[
\% \text{Inhibition} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

2.4 Enzyme inhibitory activities by Dipeptidyl peptidase IV (DPP-IV)

DPP-IV inhibitory activity (IC50) was determined using a modified method of Konrad (2014). DPP-IV from goat intestine was resuspended in 0.1 M/L Tris–HCl buffer, pH 8.0. The Meshashringi (25μL) was pre-incubated with the equal volume of the substrate Gly-Pro-p-nitroanilide (1.6 mM) at 37º C for 10 min. Afterwards, 50μL of DPP-IV (0.01 U/mL, in 0.1 M/L Tris–HCl buffer, pH 8.0) was added and the mixture was incubated at 37º C for 60 min. The reaction was stopped by the addition of 100μL of 1 M/L sodium acetate buffer, pH 4.0. The released p-nitroanilide as a hydrolysis product was measured at 405 nm. The % inhibition was calculated according to the formula.

\[
\% \text{Inhibition} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

2.5 Statistical analysis

The values of results were expressed as mean value ± S.E.M. the variation in a set of data has been estimated by performing analysis of variance (ANOVA). Individual comparison of group means values were done by using Graph pad prism 7.01.

3 Results

3.1 Inhibitory activity of Meshashringi on α-amylase enzyme

The finding of inhibitory activity of Meshashringi is represented in the table 1. The IC50 value of Meshashringi was 106.0 μg/ml for on α-amylase enzyme (Fig 1). The standard drug Acarbose exhibited 50% inhibition on α-amylase enzyme at 37.80 μg/ml (Fig 2). The Meshashringi revealed dose dependent inhibitory property for α-amylase enzyme.

### Table 1: Percentage inhibition of wheat alpha-amylase Meshashringi and Acarbose

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>% inhibition by Mesh.</th>
<th>IC50 (µg/ml)</th>
<th>% inhibition by Acarb.</th>
<th>IC50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>33.33±0.5</td>
<td>55.55±0.5</td>
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<tr>
<td>40</td>
<td>44.44±0.8</td>
<td>70.37±0.5</td>
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<tr>
<td>60</td>
<td>66.66±0.2</td>
<td>96.29±0.3</td>
<td>37.80</td>
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</tr>
<tr>
<td>80</td>
<td>81.48±0.6</td>
<td>118.5±0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>96.29±0.2</td>
<td>129.6±0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM of three determinations

Figure 1: Percentage inhibition of wheat α-amylase enzyme by Meshashringi

3.2 Inhibitory activity of Meshashringi on α-glucosidase enzyme

Table 2 demonstrated the outcomes of inhibitory activity of Meshashringi for α-glucosidase enzyme. The Meshashringi
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demonstrated 50% inhibitory activity for α-glucosidase enzyme at 124.91 μg/ml (Fig 3).

Meshashringi produces lower inhibitory activity for α-glucosidase enzyme compared to α-amylase enzyme.

Figure 2: Percentage inhibition of wheat α-amylase enzyme by Acarbose

Table 2: Percentage inhibition of α-glucosidase enzyme by Meshashringi and Acarbose

<table>
<thead>
<tr>
<th>Conc. (μg/ml)</th>
<th>% inhibition by Mesh. IC50 (μg/ml)</th>
<th>% inhibition by Acarb. IC50 (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>17.42±0.2</td>
<td>37.12±0.6</td>
</tr>
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<td>40</td>
<td>42.42±0.05</td>
<td>62.87±0.3</td>
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<tr>
<td>60</td>
<td>52.27±0.4</td>
<td>97.72±0.6</td>
</tr>
<tr>
<td>80</td>
<td>92.42±0.9</td>
<td>108.3±0.4</td>
</tr>
<tr>
<td>100</td>
<td>99.24±0.6</td>
<td>115.1±0.8</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of three determinations

Figure 3: Percentage inhibition of α-glucosidase enzyme by Meshashringi

The standard drug Acarbose exhibited 50% inhibition on α-amylase enzyme at 65.06 μg/ml (Fig 4). The findings indicate that The Meshashringi produces dose dependent inhibitory property for α-glucosidase enzyme. In addition, the standard drug Acarbose displayed 50% inhibition on DPP-IV enzyme at 48.22 μg/ml (Fig 6). The findings indicate that Meshashringi revealed dose dependent inhibitory property for DPP-IV enzyme. Consequently, the Meshashringi produces lower inhibitory activity for DPP-IV enzyme compared to α-glucosidase enzyme and α-amylase enzyme.

Table 3: Percentage inhibition of DPP-IV enzyme inhibition by Meshashringi and Acarbose

<table>
<thead>
<tr>
<th>Conc. (μg/ml)</th>
<th>% inhibition by Mesh. IC50 (μg/ml)</th>
<th>% inhibition by Acarb. IC50 (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>32.13±0.6</td>
<td>52.23±0.3</td>
</tr>
<tr>
<td>40</td>
<td>40.41±0.1</td>
<td>68.22±0.4</td>
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<tr>
<td>60</td>
<td>48.26±0.6</td>
<td>88.56±0.5</td>
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<tr>
<td>80</td>
<td>56.51±0.8</td>
<td>108.23±0.2</td>
</tr>
<tr>
<td>100</td>
<td>64.81±0.9</td>
<td>128.25±0.8</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of three determinations

4 Discussions

Diabetes patients are identified by abnormal postprandial increase of blood glucose level. The α-amylase and α-glucosidase enzyme play chief role to enhance the glucose level in blood by catalyzing the release of α-glucose from the non-reducing end of the substrate1. The increment of blood sugar following carbohydrates meal can be reduced by
inhibiting the α-amylase and α-glucosidase enzyme\textsuperscript{18}. These enzymes are present in epithelium of the small intestine, and enzyme enable the absorption of glucose by the small intestine by catalyzing the hydrolytic cleavage of oligosaccharides into absorbable monosaccharides\textsuperscript{19}. Consequently, on inhibiting the α-amylase and α-glucosidase enzyme in the small intestine, it decrease conversion rate of hydrolytic cleavage of oligosaccharide and the process of carbohydrate digestion spreads to the lower part of small intestine. This digestion process of carbohydrate delays the total absorption rate of glucose and declines the postprandial blood glucose peak in diabetic patients\textsuperscript{20-22}. The findings of present study, Meshashringi demonstrated inhibition against both α-amylase and α-glucosidase enzyme. The results implies that the extract of Meshashringi were potent inhibitors of α-amylase and α-glucosidase enzyme. Moreover, the IC\textsubscript{50} values of Meshashringi are nearer to IC\textsubscript{50} values of Acarbose and therefore can be potentially useful as an effective therapy for postprandial hyperglycemia with minimal side effects. The study supports the data of Picot et al\textsuperscript{23} stated natural α-glucosidase inhibitors from plants to have strong inhibition towards the activity of the enzyme compared to Acarbose.

The glucagon like peptide-1 (GLP-1), a potent insulinotropic peptide and capable to control the blood glucose level in Type 2 diabetes. GLP-1 can stimulate the release of insulin or suppressed the release of glucagon. DPP-IV enzyme inhibits the stimulation of GLP-1 and enhances the blood sugar level\textsuperscript{24}. The DPP-IV inhibition is an approach to extend the circulating half-life of GLP-1, thus making DPP-IV inhibitors a promising target for the treatment of type 2 diabetes. The outcomes of study exhibited inhibitory effect of Meshashringi on DPP-IV enzyme, and effective approach to treat type 2 diabetes mellitus by potentiating insulin secretion.

Hence the findings of present study exhibited that the Meshashringi incorporating potent α-glucosidase, α-amylase and DPP-IV inhibitors, and were effective for suppressing postprandial hyperglycemia. The Meshashringi should be given as food supplement for management of diabetes.

5 Conclusion
The present study demonstrated that the Meshashringi formulation produces α-amylase, α-glucosidase, and DPP-IV inhibitory activity. It is predicted that Meshashringi suppress the glycemic response in diabetic conditions. The antidiabetic activity of Meshashringi formulation can also be attributed to the intestinal α-amylase, α-glucosidase and DPP-IV inhibitory activity. Further in vitro antidiabetic and cell line study are require to confirm the exact mechanism of Meshashringi.

6 Conflicts of Interests
The authors hereby declare that there are no conflicts of interests.

7 Author’s contributions
PKS and PP carried out data analysis/interpretation and manuscript preparation. AR worked on the final document approval. PKS carried out the research conception/design and data acquisition.

8 References


