EFFECT OF SALEP (EULOPHIA CAMPESTRIS) ON GLYCATION OF IgG IN-VITRO CONDITION

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ABSTRACT

Protein glycation takes place when elevated levels of reduced sugars react with amino groups in proteins, reaction known as Maillard reaction. If this process continues, it will lead to the formation of complex, often unstable, irreversible and reactive compounds Advanced Glycation End-Products (AGEs) a process that may take weeks or even months to accomplish. Plant extracts have their own importance and now being studied extensively due to having little or no side effects. In present study salep was selected and used to check the glycation inhibitory activity. Various combinations of glucose, protein and salep extracts were made under in vitro conditions and their activity was monitored with Trichloro acetic acid treatment method at 350 nm. Glycated products’ AGEs were more with high glucose and high protein concentration and these were decreased by highest concentration of salep extract i.e. 25 mg/mL or 250 μL. Lower concentrations of plant extract produced either no or least response against Maillard reaction.

Keywords: Maillard reaction, AGEs, Eulophia Campestris, plant extract, TCA treatment.

INTRODUCTION

Non-enzymatic glycosylation of proteins in serum and tissues is a pathophysiological consequence of hyperglycemia in diabetes mellitus, and also correlates with ageing (Brownee M et. al. 1998, Beisswenger PJ et. al. 2001). There is an increasing evidence that chronic hyperglycemia is the major cause of secondary complications of diabetes such as microangiopathy, retinopathy, euroopathy, and nephropathy (Brownee M et. al. 1984, Gugliucci A. 2000, Sheetz MJ and King GL 2003). Discovery of glucose dependent chemical modification of various proteins suggested that they could induce functional abnormalities in these proteins, and thereby lead to the pathophysiology of diabetes (Means GE and Chang MK. 1982) properties include decrease solubility and elasticity. (Kohn RR. et. al. 1984). There is evidence for in vitro and in vivo glycation of IgG (Dolhofer-Bliesener R. and Gerbitz KD. 1990, Danze PM. et. al. 1987). Treatment of purified human IgG with glucose induced its glycation and this process was time and pH dependent. Kenedy et al. 1994 showed that the level of glycated IgG, IgM, and IgA increased in diabetic patients. Although reduction of the ability of glycated IgG for complement fixation has been reported, another group of investigators believe that glycated IgG can stimulate complement activity higher than non-glycated (Davin JC. et. al. 1997).

In 1912, Louis Camille Maillard described the browning of proteins in food and called it as Maillard reaction. This is also known as non-enzymatic glycation of proteins, or a process which links chronic hyperglycemia to a series of physiopathological alterations considered important in the development of chronic complications of different diseases like diabetes (Takeuchi et al., 2004). These glycated proteins further rearrange and give rise to a stable Amadori product that degrades into a variety of compounds which, more reactive than the sugars from which they are derived (Wautier and Schmidt, 2004). These propagators again form yellow-brown, often fluorescent (some are non fluorescent), irreversible compounds, usually called Advanced Glycation End-Products (AGEs) or Maillard products. Candidate active AGE compounds include N-(carboxymethyl)-L-lysine (CML) pyrraline, pentosidine and their crosslinks (Kaysen G. 2001). Plants have been the major source of drugs in the world and in sub-continent system of medicinal therapy. Information on such plants in sub-continent has been systematically organized (Satyavati et al., 1987). It is known that medicinal plants have little or no side effects. Some of them are being used in traditional systems of medicine from hundreds of years in many countries of the world (Eshrat and Hussain, 2002).

Metformin is the only ethical drug approved for the treatment of non insulin dependent diabetes mellitus (NIDDM) patients (Beisswenger et al., 1999), which is derived from a medicinal plant Galega officinalis and historically used for treatment of diabetes (Oubre et al., 1970). There are many anti-diabetic plants, which might provide useful sources for the development of drugs, in the treatment of diabetes mellitus. The literature on medicinal plants with hypoglycemic activity is vast. As many of these plants were used for many centuries and sometimes as regular constituents of the diet, it is assumed that they do not have many side effects (Shankar et al., 1980). Synthetic inhibitors and inhibitors from plant extracts have their own importance and now are studied extensively. There are reports of some natural substances isolated from plants with AGE-inhibitory effects. One such compound is curcumin isolated from Curcuma longa (Turmeric), commonly known as Haldi. Ginger (Zingiber officinale Rosc.) is another spice useful for diabetic therapy (Broadhurst, 2000). When type 2 diabetic rats are fed ginger, they show hypoglycemic activity, thus improving their diabetic condition (Kar et al., 2003). Now it is the demand of time to develop some new compounds either from plants or synthetically to control diabetes and other age accelerating diseases. As plants have fewer side effects so these should be preferred to study. In this study salep (Eulophia campestris) extract was used to study its effect on glycation and Maillard products. The major object of this study was to investigate the effect of salep as inhibitor of AGE or Maillard reaction.
under in vitro conditions and measure its activity against AGE production or inhibition.

**MATERIALS AND METHODS**

**Preparation of salep (Eulophia campestris) extract**

Dried and ground salep plant (10 g) was extracted with 60 mL of 50% ethanol at 37 °C for 10 days and then filtered and stored at 4 °C.

**Sample recovery**

Recovering of sample was done by evaporating the ethanol using Rotary Evaporator. Dried sample was dissolved in 25 mL of phosphate buffer saline and stored at 4°C for further use.

**In vitro glycation inhibition with salep (Eulophia campestris) extract**

Conditions and concentrations selection for salep (Eulophia campestris) extract

To measure glycation inhibition with salep extract, different concentrations of protein (BSA) and glucose (two of each) and three concentrations or volumes of salep extract were used. These are given in table 1

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Components of reaction</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B : Buffer (PBS)</td>
<td>0.1M</td>
</tr>
<tr>
<td>2</td>
<td>P1 : Protein (BSA)</td>
<td>15 mg/mL</td>
</tr>
<tr>
<td>3</td>
<td>P2 : Protein (BSA)</td>
<td>7.5 mg/mL</td>
</tr>
<tr>
<td>4</td>
<td>G1 : Glucose</td>
<td>250 mM</td>
</tr>
<tr>
<td>5</td>
<td>G2 : Glucose</td>
<td>5.5 mM</td>
</tr>
<tr>
<td>6</td>
<td>PF1 : Plant Filtrate</td>
<td>300 μL</td>
</tr>
<tr>
<td>7</td>
<td>PF2 : Plant Filtrate</td>
<td>150 μL</td>
</tr>
<tr>
<td>8</td>
<td>PF3 : Plant Filtrate</td>
<td>75 μL</td>
</tr>
</tbody>
</table>

**Table 1: Concentration of different components used to study glycation inhibition under in vitro conditions with salep (Eulophia campestris) extract.**

**In vitro glycation of BSA with salep (Eulophia campestris) extract**

Glucose, BSA with or without inhibitor (plant extracts in PBS pH 7.4) were prepared and their mixture was incubated at 37°C and 50°C for 5 weeks. During this, samples were drawn for glycation inhibition activity after 1st, 3rd and 5th week of incubation. The samples kept at 4°C until analysis.

**Trichloracetic acid (TCA) method for Maillard reaction inhibitory activity of salep (Eulophia campestris) extract**

This method is also known as TCA treatment method described by Matsuura et al. (2002) was followed with some modification.

**RESULTS AND DISCUSSIONS**

Salep plants were dried in hot air oven at 37°C. After drying, ground to powder form and kept in 50% ethanol for extraction. Samples were recovered by Rotary Evaporator. Other method like Heat and liquid nitrogen method were also tried but Rotary evaporator was cheaper, less time consuming and simple to perform. Samples were drawn for glycation inhibition activity after 1st, 3rd and 5th week of incubation. Glucose concentration 250 mM and 5.5 mM was selected as it was cleared that 250 mM is a hyperglycaemia condition (Rahbar and Figarola, 2003) and 5.5 mM is a normal glucose level (human body). Bovine serum albumin (BSA) was used as protein for glycation and its concentration was 0.15 mg/mL and 7.5 mg/mL. Salep extracts (300 μL, 150 μL and 75 μL) in phosphate buffer were prepared. Each extract along with glucose and BSA were incubated at 37°C and 50°C for five weeks to monitored glycation and Millard reaction inhibitory activity. TCA treatment method described by Matsuura et al. (2002) was adopted with modifications. The absorbance change based on Schiff base formation was measured by spectrophotometer at 350 nm. This gave the apparent inhibitory activity. Real inhibitory activity was estimated by subtracting the quenching effect from the apparent inhibitory activity. The results obtained by this method are explained below.

**Effect of salep extraction on maillard reaction**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Different Combinations of reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>G1+P1</td>
</tr>
<tr>
<td>2</td>
<td>G1+P1+PF1</td>
</tr>
<tr>
<td>3</td>
<td>G1+P1+PF2</td>
</tr>
<tr>
<td>4</td>
<td>G1+P1+PF3</td>
</tr>
<tr>
<td>5</td>
<td>G1+P2</td>
</tr>
<tr>
<td>6</td>
<td>G1+P2+PF1</td>
</tr>
<tr>
<td>7</td>
<td>G1+P2+PF2</td>
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<tr>
<td>8</td>
<td>G1+P2+PF3</td>
</tr>
<tr>
<td>9</td>
<td>G2+P1</td>
</tr>
<tr>
<td>10</td>
<td>G2+P1+PF1</td>
</tr>
<tr>
<td>11</td>
<td>G2+P1+PF2</td>
</tr>
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<td>12</td>
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<td>15</td>
<td>G2+P2+PF2</td>
</tr>
<tr>
<td>16</td>
<td>G2+P2+PF3</td>
</tr>
</tbody>
</table>

**Maillard reaction inhibitory activity by salep (Eulophia campestris) extract**

The results obtained from salep (Eulophia campestris) extract after TCA treatment at 37°C and 50°C. At temperature 37°C (Figure 1), G1 P1 produced more Maillard products 0.1 after 5th week, while G2 P2 produced minimum products 0.03 after 1st week at same temperature.

![Figure 1: Maillard Reaction inhibitory Activity by salep (Eulophia campestris) with TCA treatment method under in vitro conditions measured at 37°C temperature.](image)

G1 P2 and G2 P1 produce moderate level of products. Salep extract followed the pattern from PF1 to PF3 as PF1 (300 μL) generated maximum response while PF 3 (75 μL) generated least or no response at 37°C. BSA (15mg/mL, 7.5mg/mL), glucose (250mM, 5.5mM) with or without salep (Eulophia campestris) extract (300 μL, 150 μL and 75μL) in phosphate buffer (0.1M), pH 7.4 and mixture was incubated at 37°C for five weeks.

Samples were drawn for glycation inhibition activity after 1st, 3rd and 5th week of incubation. Absorbance was recorded at 350nm. At 50°C (Figure 2) G1 P1 produced more Maillard products (0.109) after 5th week, while G2 P2 produced minimum products 0.037 at same temperature after 1st week of incubation. Here it was also seen that G2 P2
generated maximum products at 3rd week (0.048) in spite of 5th week at 50°C. PF1 (300 μL) generated maximum response while PF3 (75 μL) generated least response at 50°C and minimum inhibitory activity was observed. At both temperatures PF2 (150 μL) produced moderate response against Maillard products inhibition.

![Image](image-url)

Figure 2: Maillard Reaction inhibitory Activity by salep (Eulophia campestris) with TCA treatment method under in vitro conditions measured at 50°C temperature.

It was also observed from both figures that high temperature facilitate the production of Maillard products. Moreover, high temperature did not affect the activity of salep extract i.e. their trend towards inhibition is same. Onion and garlic have significant blood sugar lowering action. Our results are supported by Demerdash et al. 2005

CONCLUSION

Our studies concluded that salep has ability to inhibit Maillard products that ultimately lead to AGEs production. It was also cleared that 15mg/mL concentration of BSA was more active towards glycation of glucose. However, PF3 (75 μL) concentration of salep extract was almost unable to produce any effect at 37°C. So it is suggested that more concentration of Salep extract should be used to stop or decrease glycation in human body level in case of diabetes mellitus and persistent hyperglycaemia.

ACKNOWLEDGMENT

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Abbreviations

AGEs, Advanced Glycation End-Products; NIDDM; Non-insulin dependent diabetes mellitus; BSA; Bovine serum albumin PBS; Phosphate buffer saline; TCA, Tri-chloro acetic acid.

REFERENCES


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