Anti-Diabetic mechanism of colchicum speciosum hydroalcoholic extract in vitro

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Abstract

Diabetes is a chronic metabolic disease, which has various treatments and recently Anti-Diabetic effects within herbal medicines and their mechanism have drawn the attention. This study was purposed to investigate the Anti-Diabetic effects of hydroalcoholic extracts of colchicum Speciosum in vitro conditions on three mechanisms for insulin release, glucose uptake and hemoglobin glycosylation. In preparing hydroalcoholic extract of the herb, soaking was used in concentrations 100,200,400,600,800, and 1000. The rate of releasing insulin from calf’s pancreas cells in presence and absence of extract was assayed by ELISA kit. The rate of glucose uptake was also measured on the plasma with red cells in the presence and absence of the extract during 3 hours, which was done in every 1 hour. The rate of hemoglobin glycosylation was assayed in the presence and absence of the extracts and the rate of glycosylation inhibition was calculated. The extracts of colchicum could decrease releasing insulin significantly (p-value ≤ 0.05). While the glucose uptake was raised significantly (p-value ≤ 0.05). Further, colchicum extract could enhance hemoglobin glycosylation inhibition. According the above results, colchicum Speciosum may have Anti-Diabetic properties and it can be used as an Anti-Diabetic medicine and as a nutritional supplement for diabetics. Surely, similar studies are necessary in this field.

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Introduction

Diabetes mellitus may be one of the most ancient diseases of humanity (Olokoba et al., 2012). Although, the causes of the disease is unknown, both genetics and environment factors such as obesity and sedentary life may increase the risk of developing diabetes (Beaulieu et al., 2003). In 2011, about 366 million people suffered from diabetes mellitus and this number maybe increased to 552 million to 2030 (Whiting et al., 2011). According to the statistics, prevalence of diabetes in Iran has been 4 to 4.5% in 1385, and in the average age above 30, it was more than 14%, in Yazd province has been increased to 14.2%, and in all other regions, it has been more prevalence among the women than the men (Baghiani Moghadam et al., 2007). Releasing insulin, glucose reuptake and hemoglobin glycosylation are of the most important mechanisms involved in diabetes.

Those materials cause to release insulin from pancreas are glucose, amino acids, free fatty acids, glucagon, secretin and effective medicines for curing diabetes. While rising the blood sugar, the insulin immediately decrease the blood sugar by spading glucose trans fusion to adipose and muscle tissue, and recycling glucose transfers (GLUT4) in the cell to the plasma membrane. Controlling the blood sugar at fixed level is one of the very sensitive regulations among all hemostatic mechanisms that liver and non-liver organs are interning in it (Murray et al., 2003). Glycolized hemoglobin is the consequence of linking covalent depended to glucose concentration with hemoglobin in erythrocyte (Dziedzic et al., 2011). Before knowing insulin and producing current medicines for diabetes, diabetics were cured by traditional treatments and using herbal medicines. So far, more than 1200 medicine herbals have been recognized in decreasing and controlling the blood sugar and its side effects and the positive effects of herbal medicines (Falah Hosseini et al., 2006).

Colchicum Speciosum is a medical herbal that its species is Colchicum and is from the Colchicaceae family. The most important compound of this plant is alkaloid colchicine e that its rate is different in each season (Alirezaie et al., 2013).

There are little studies on Anti-Diabetic effects of such plant. Giugliano et al. in 1981 recognized injections of colchicine cause to control insulin secretion from pancreas cells to respond to rising glucose in human. Experimental studies also have showed that some polyphenolic compounds may increase insulin dependent glucose uptake in muscle and adipocytes. Some polyphenols have potentials to increase glucose uptake (Bahadoran et al., 2013).

In this study, the effect of Colchicum Speciosum hydroalcoholic extract on insulin secretion, hemoglobin glycosylation and glucose uptake were studied.

Materials and methods

Preparing the Extract

The Colchicum Speciosum was prepared (local market of Isfahan) and the Soaking method was used for the extraction. Then, the dry extract was solved in alcohol 10% (Hamoon Co. Iran) and after labeling, it was kept for next steps of experiment.

The concentrations 100, 200, 400, 600, 800 and 1000 µg. ml\(^{-1}\) were prepared by alcohol 10%.

Glucose Uptake

In order to measure the rate of glucose uptake, plasma contains of red cell was prepared. Then, two series tubes were considered as evidence (without extract) and test (contains the extract with prepared concentrations). Afterwards, 1.5 ml plasma was added to all the tubes. The blood sugar in presence and absence of the extract was measured during 3 hours, that in every hour it was done by glucose kit (Pars Azmoon Co. Iran) in serum or plasma by photometric method and Hitachi (Hitachi-902; Japan-Germany) equipment. The experiment for the rate of glucose uptake was repeated in all concentrations.

Insulin Secretion

A suspension was made from the calf’s pancreas for assessing insulin secretion (Dickson and Suzangar,
The rate of insulin secretion in presence and absence of extract (as control) was measured by insulin kit (Monobind Co. Iran). The experiments for the rate of insulin secretion were repeated twice in all concentrations.

Hemoglobin Glycosylation Inhibition
To measure inhibition rate of hemoglobin glycosylation, at first, a blood sample was prepared from a healthy volunteer (non-diabetic with normal lipid profile, non-suffering from kidney disease and not using medicine), and citrate was used as non-clotting of blood. Then, red cells were washed three times by NaCl (Merck Co. Germany) 14% solution (Van Kampen and Zijistra, 1965). Then, the hemoglobin 5% gr was prepared. Hemoglobin glycosylation was measured in presence and absence of herbal extract and was measured with spectrophotometer (Shimadzu- uv 3100; Japan) on wavelength 443 nm (Asgari et al., 2002). The experiments of controlling percent for hemoglobin glycosylation were repeated twice in all concentrations.

Statistical Analysis
For analyzing the data, SPSS software (Chicago, IL, version 15.0) was used. The data were expressed as a mean ± SD. T-Test, Kruskal-Wallis and ANOVA Test were used to comparing the result. P≤0.05 was considered statistically significant.

Results
Insulin Secretion
In the experiment of insulin secretion rate on the calf’s pancreas, it was recognized that hydroalcoholic extract of Colchicum Speciosum significantly cause to decrease insulin secretion (p-value ≤ 0.05) from Beta cells of calf’s Islets of Langerhans (Table 1).

Hemoglobin glycosylation
An experiment was done to study controlling effects of the extract on glycosylation, and it was recognized that the hydroalcoholic extract of Colchicum Speciosum has significant effects on glycosylation inhibition. This effect was started in concentration 100 µg.ml⁻¹ from 21%, it was increase, and it reached to 59.07% in 1000 µg.ml⁻¹ concentration (Table 3).

Discussion
In this study, it was recognized that hydroalcoholic extract of Colchicum Speciosum has significant effects on involved mechanisms in diabetes. Rising glucose

### Table 1. Effect of different concentration of hydroalcoholic extract of Colchicum Speciosum on insulin resealing rate.

<table>
<thead>
<tr>
<th>Concentration of hydroalcoholic extract of Colchicum Speciosum (µg.ml⁻¹)</th>
<th>Released insulin</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>93.3</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>64.25±2.8*</td>
<td>0.01</td>
</tr>
<tr>
<td>200</td>
<td>69.10±0.84</td>
<td>0.01</td>
</tr>
<tr>
<td>400</td>
<td>76.40±2.50</td>
<td>0.02</td>
</tr>
<tr>
<td>600</td>
<td>64.85±4.10</td>
<td>0.03</td>
</tr>
<tr>
<td>800</td>
<td>66.50±1.50</td>
<td>0.004</td>
</tr>
<tr>
<td>1000</td>
<td>90.50±1.50</td>
<td>0.22</td>
</tr>
</tbody>
</table>

* Mean ± SD, for all such values.  
† P-values <0.05 are statistically significant.
uptake was significant by Colchicum Speciosum extract in each 3 hours. Hydroalcoholic extract of Colchicum Speciosum caused to decrease insulin secretion significantly, which was recognized by the insulin secretion from the calf’s pancreas. It was also showed that the hydroalcoholic extract of Colchicum Speciosum caused to raise glycosylation inhibition significantly.

### Table 2. Function of different concentration of of hydroalcoholic extract of Colchicum Speciosum on glucose uptake rate.

<table>
<thead>
<tr>
<th>Concentration of hydroalcoholic extract of Colchicum Speciosum (µg.ml⁻¹)</th>
<th>Function in first hour</th>
<th>Function in second hour</th>
<th>Function in third hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>8.66±1.15*</td>
<td>1.00±1.00</td>
<td>6.99 ± 0.57</td>
</tr>
<tr>
<td>200</td>
<td>9.99±0.57</td>
<td>6.00±1.00</td>
<td>9.99 ± 2.08</td>
</tr>
<tr>
<td>400</td>
<td>9.66±0.57</td>
<td>11.60±0.57</td>
<td>9.66 ± 0.57</td>
</tr>
<tr>
<td>600</td>
<td>10.60±0.57</td>
<td>13.60±0.57</td>
<td>10.9 ± 0.57</td>
</tr>
<tr>
<td>800</td>
<td>10.90±0.57</td>
<td>14.00±1.0</td>
<td>10.6 ± 0.57</td>
</tr>
<tr>
<td>1000</td>
<td>11.90±0.57‡</td>
<td>14.30±0.57‡</td>
<td>11.9 ± 0.57‡</td>
</tr>
</tbody>
</table>

* Mean ± SD, for all such values.
† P-values <0.05 are statistically significant.
‡ The best function compared to other concentration.

### Insulin secretion

Alkaloid colchicine is the best-known medical compounds of Colchicum Speciosum (Alirezaie et al., 2013). The results have showed that injection of colchicine in normal people, has decreased the insulin fast respond to glucose injection and the glucose tolerance is being critical. The negative intervention of colchicine on glucose tolerance was seen after oral use of the herb. These observations make possible the special effects. On other hand, In oral use, the interventions by digestive system and increasing non special digestive factors (GIP) which are able to change the glucose tolerance can’t be disregarded.

The previous studies about the effects of colchicine on extracted cells from the mouse pancreas in vitro conditions, the significant controlling effects were observed in induced insulin secretion by glucose by using 10.5 molar colchicine. The next investigations showed that acute consumption (500 µg per Kg) and chronic (200 µg per Kg) and after 10 days use, decreased the releasing insulin and glucose tolerance. Also, it was showed that acute prescribing the colchicine has caused the first and second phases of insulin glucose in respond to the maximum glucose stimulation (Bahadoran et al., 2013).

The hidden controlling effects of colchicine on insulin secretion are disputable. Regarding to the important role of microtubules in releasing insulin, the controlling effects of colchicine can be attributed to the low performance of microtubules. The other suggested mechanism for controlling can be PGE (Endogenous Prostaglandin E), which controls the induced insulin secretion by glucose dependently. The evidences show that colchicine may increase the endogenous PGE synthesis. The colchicine effects are replaced completely by LAS (lysine acetylsalicylate). LAS is the controller of PGE synthesis, which increases the stimulated insulin responses by glucose and speeds up cleaning the blood from glucose. These observations make possible the second hypothesis (Giugliano et al., 1981).

It was recognized that some polyphenol compounds can decrease the induced chronic stimulation on the activity and pressure on Beta cells and as a result, the destruction of Islets of Langerhans and diabetes progression is postponed. It seems this effect is the result of decreasing the hyperglycemia stimulation effects on insulin secretion by inducing some metabolic variations in Beta cells (Bahadoran et al., 2013).
Glucose uptake
Some investigations have demonstrated that poly phenol compounds can control glucose after meal. Some poly phenols can regulate the key paths of carbohydrates and glucose liver hemostasis, including Glycolysis, Glycogenesis and Gluconeogenesis that may be failure in diabetes. The experimental studies have showed that some polyphenolic compounds increased glucose reuptake by recycling GLUT4 to plasma membrane that generally is done by inducing protein kinase depended to AMP, which is considered as an important sensor for cell energy condition and plays an important role in metabolic control. Developing this procedure as a new therapy for obesity, diabetes type 2 and metabolic syndrome and the main aim of Anti-diabetic medicines including Metformin is considered. It was shown that poly phenol effects in activating the protein kinase is more than 50-200 times compared to Metformin. Some poly phenols have the induction potential of Phosphatidylinositol 3-kinase (PI3K) are as a key signal path for glucose absorption in general, many results showed that the herbs containing polyphenols effect on many metabolic dysfunction induced by diabetes and they interfere in carbohydrates metabolism, glucose Homeostasis, insulin secretion and insulin resistance (Bahadoran et al., 2013).

Table 3. Effect of different concentration of hydroalcoholic extract of Colchicum Speciosum on hemoglobin glycosylation percent.

<table>
<thead>
<tr>
<th>Concentration of hydroalcoholic extract of Colchicum Speciosum (µg.ml⁻¹)</th>
<th>Glycation Inhibition percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>21.10±0.84*</td>
</tr>
<tr>
<td>200</td>
<td>24.04±0.05</td>
</tr>
<tr>
<td>400</td>
<td>41.03±0.02</td>
</tr>
<tr>
<td>600</td>
<td>45.02±0.05</td>
</tr>
<tr>
<td>800</td>
<td>58.08±0.02</td>
</tr>
<tr>
<td>1000</td>
<td>59.07±0.03</td>
</tr>
</tbody>
</table>

* Mean ± SD, for all such values.

Hemoglobin glycosylation
Colchicum Speciosum had a significant effect on glycosylation inhibition in vitro. The inhibitory effects were considerable and in 1000 µg.ml⁻¹ concentration was as much as 60%. The inhibition effects of food stuff and herbal medicine on hemoglobin glycosylation were studied before. Proposed mechanism were consisted of: scavenging hydroxyl radicals and superoxide radicals to alleviate oxidative stress; blocking the carbonyl or dicarbonyl groups; metal ion chelation; Inhibiting the formation of late-stage Amadori products (a relatively stable ketoamine); breaking the crosslinking structures; Blocking RAGEs (AGE-specific receptors) (Wu et al., 2011). The hydroalcoholic extract of Colchicum Speciosum can inhibit glycosylation by any of these mechanisms. Further studies are surly necessary to investigate the exact mechanism.

Conclusion
Respect to the mentioned effects from colchicine, the effective material of colchicum Speciosum and the effects in poly phenol compounds, it is possible that this herb by decreasing insulin secretion, reduces the induced pressure on Beta cells and it prevents destructing the cells and progressing diabetes.

Although, because the nature of diabetes as a systemic disease, this investigation in vitro is considered as a limitation. However, the procedure of effectiveness of herbal medicines can be studied in this way. Also, studying the Colchicum Speciosum without analyzing the effective components is another limitation of plan which reflects the general effect of this herb. It is suggested to investigate the systematic effects of the herb extracts on human (or rats or cultured cell). So it is advised the effective
components in all part of the herb were separated and analyzed by chromatography method.

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