Effect of locally produced honey on serum levels of glucose, triglyceride, cholesterol, HDL, VLDL and LDL in alloxanized diabetic rats

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Abstract

Diabetes mellitus is a metabolic disorder in human and animal, particularly in pets. This study is conducted to evaluate the effect of Honey on serum levels of glucose, triglyceride, cholesterol, VLDL, LDL and HDL in experimental diabetes mellitus in rat. Male Wistar rats randomly allocated in four groups: normal, honey control, diabetic control and diabetic rats receiving honey. For creation of diabetes, a single dose of Alloxan (100 mg/kg) injected subcutaneously. After observing diabetes symptoms in groups received Alloxan, we have initiated to feed treatment group and diabetic treatment group with the equal ratio of honey and pellet. The diabetic control and control group fed only with pellet. These groups fed for 10 days. Blood samples collected from whole groups at the end of 10th day. Evaluation of the serum levels of glucose, cholesterol and LDL significantly increased between diabetic group with control group and also between treatment group and diabetic treatment group with diabetic Evaluation of the serum levels of triglyceride did not reveal statistically significant differences in diabetic treatment group and treatment group with control group, but decreased significantly compared to the diabetic control group (P<0.05). Evaluation of the serum Levels of VLDL decreased significantly in diabetic treatment group and treatment group compared with the diabetic control group (P<0.05), but did not reveal statistically difference compared with the control group. Evaluation of the serum levels of HDL did not reveal statistically significant difference between groups. Honey could be used as a natural drug for preventing diabetes mellitus disorders.

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**Introduction**

Diabetes mellitus is a syndrome occurring due to the lack of insulin secretion or decreasing in tissue sensitivity to insulin, so results in disordering in carbohydrate, fat and protein metabolism (Kim *et al.* 1994). With regarding to that, diabetes is one of the common diseases in the world increasingly and there is no definite therapy by now, therefore only promising method includes proper care, controlled feeding and any overindulging may result in irretrievable consequences. In diabetes, in addition to serum levels of glucose, triglyceride, cholesterol, VLDL and LDL are increasing significantly, which have related problems. The most important physiological event in diabetes mellitus includes hyperglycemia that occurs due to 3 causes: 1. Decreasing in glucose arrival rate into different cells. 2. Decreasing in glucose in different tissue. 3. Increasing in glucose produced by liver (gluconeogenesis) (Kim *et al.*1994). Main symptoms of diabetes mellitus include: polyuria, polydipsia and losing weight unlike sufficient feeding. Diabetes divided into two groups totally: 1. Diabetes type I or insulin-dependent diabetes mellitus (IDDM). 2. Diabetes type II or non-insulin-dependent diabetes mellitus (NIDDM) (Kim *et al.*1994). The induction of experimental diabetes in the rat using chemicals, which selectively destroy pancreatic B cells, is very convenient and simple to use. The most usual substances to induce diabetes in the rat are Alloxan and streptozotocin. Alloxan are widely used to induce experimental diabetes in animals. The mechanism of the effect of this drug is studied comprehensively in pancreatic B cells, so the way of the functioning is known for us these days.

Cytosolic action of this drug, that causes diabetes, is due to the action of reactive oxygen species.

Alloxan and the product of its reduction, Dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. After that, the function of the hydroxyls production is done rapidly. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of B cells (Kim *et al.*1994; Szkudelski .2001). To sum up, the toxic action of Alloxan on pancreatic B cells are the sum of several processes such as oxidation of essential -SH groups, inhibition of glucokinase, generation of free radicals and disturbances in intracellular calcium homeostasis (Szkudelski . 2001).

Honey is one of the oldest and basic sweetened which has been used till now .It includes sugars such as Glucose , Fructose, and minerals like Magnesium, Potassium, Calcium, Chlorine sodium, Sulfur Iron, Phosphate. In addition, some vitamins such as C, B6, B3, B5, B1, B2 with regarding to their quality and type of honey are found among various portions. Besides those mentioned, Copper, Iodine, Zinc, and Manages, are found in honey even if with low portion. Honeybee adds a sort of Diastase enzyme called Anortase to the nectar, that forms four fifth of its weight with saccharin elements and starch. This is the chief factor of the crystallization. The belief that “Unnatural honey is crystallized” is wholly inaccurate. On the contrary, the reason of crystallization, is because of the Diastase and Carbohydrates presence (Stefan *et al.* 2000). The Diastase activity (DN) considered as a qualitative factor which effects on the durability of the honey and the degree of the temperature. Besides, it is the sign of the freshness and the sign of the being heat. The standard minimum degree of the activity of the Diastase is 8 (Stefan *et al.* 2000). The more the Glucose was founding in the honey, the more quickly the crystallization be done. This virtue is peculiar to the natural and unfabricated honey, since only natural honey contains Diastase. Per 100gm of honey produces 330c energy. In addition, honey has the nutritive and energetic value that melts the lipids surrounded around the heart by its Diastases. The molecules of sugar found in the honey, could be changed to the other type of sugar. Therefore, even the most sensitive stomachs are able to digest it easily. Honey is the rich source of Anti Oxidant. Since consisting high degree of Fructose, honey has 25% more sweetness than sugar does (Stefan *et al.*2000).
Materials and methods

Study Design

30 male Wistar rats with age of 8 weeks were selected. Weighted by true digital balance and divided into 5 groups, so that there were 6 rats per group. In order to get used to environment, first they were kept for one week in the special cage and maintained at 23-25 °C with a 12 h dark and light. Average weight of all groups was 200±20 gr. On first day, one of groups was bled and blood serum samples were separated and analyzed after centrifuge.

Diabetes Induction

Alloxan monohydrate (by Fluka Co, in 10gr package) was used to induce type-I DM. Diabetic treatment group and diabetic control group received subcutaneous a single dose of Alloxan (100 mg/kg WB) in salin solution. Pour control group and control group received subcutaneous salin normal (100 mg/kg BW). Injections after 1 week in all groups were repeated. After second injection, groups that received Alloxan, showed diabetic symptoms including: polydipsia, polyuria, glucosuria and hyperglycemia, which blood glucose was measured in fasting mood by digital glucometer one day after second injection, showed hyperglycemia(162.50±4.52 mg/dl) to healthy rats(86.6±3.16 mg/dl), and glucosuria was confirmed with human urine tapes(by Manchereg-Nagel Co).

Nutrition program

Natural unprocessed honey, dark yellow in color and of multifloral origin, was used for the trial. Honey locally produced by khoy city beekeepers in Iran. Biochemical tests performed in Food quality control-analysis Lab (Islamic Azad University-Tabriz Branch, Tabriz, Iran) and the honey was stored at room temperature for use in the study. The composition of the selected honey presented in Table1 (Table 1). Participants carried out no procedure on the honey before consumption.

Groups fed by bottom stock after observing diabetic symptom: Group 1: receiving physiological serum as a control group fed only with pellet 50±10 gr daily.

Group 2: receiving physiological serum as a treatment group fed with (25±5 gr honey) + (25±5 gr pellet).

Group 3: receiving Alloxan as a diabetic control group fed only with 50±10 gr pellet. Group 4: receiving Alloxan as a diabetic treatment group fed with (25±5 gr honey) + (25±5 gr pellet).

These groups fed with this method twice per day. Whole groups fed and maintained 10 days under above-mentioned conditions and controlled every day at certain time, then the remaining food was weighted and after defining amount of last day consumed food, fresh food replaced. Meanwhile, during the day from consuming honey by treatment group and diabetic treatment group assured. Blood samples collected from whole groups at the end of 10 day and gathered in test tubes, tubes lids closed with parafilm, then they centrifuged for 10 minutes at 2500 turn/minute and serums separated and analyzed.

Biochemical Examination

Whole rats were anesthesia by chloroform in glass jar then were bled by deheading method and during bleeding carefully it is done to prevent from blood enter into the test tubes slowly and tangent with wall. Glucose, triglyceride, cholesterol and HDL serum levels were measured by enzyme method with commercial kits built in biochemistry factory by producer Co, because of proposed waves with spectrophotometer BIOWAVE model F2100 built in England, and serum values of LDL and VLDL were calculated according to follow formula: Chol - HDL x TG/5 = LDL.

After obtaining results, comparing average obtained parameters were measured statistical experiment ANOVAs and Paired student’s t-Test by software SPSS. The design completely randomized.

Results

Indicated serum levels of blood glucose (mg/dl) measured in pure control group, control group, diabetic control group, treatment group, and diabetic treatment group. Statistical comparing between serum levels of glucose among different groups,
revealed statistically meaningful difference between diabetic control group with pure control group and control group, and diabetic treatment group with pure control group and control group and diabetic control group (P<0.05). Blood glucose level in diabetic control group that received Alloxan had meaningful increase as to pure control group and control group. In diabetic treatment group infected with experimental diabetes by drug and received honey, revealed meaningful decrease compared with diabetic control group but showed increase compared with pure control group and control group (Table 2).

**Table 1.** Composition of the selected honey.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reductive sugars</td>
<td>66 g %</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5.6 g %</td>
</tr>
<tr>
<td>Glucose</td>
<td>24 g %</td>
</tr>
<tr>
<td>Fructose</td>
<td>42 g %</td>
</tr>
<tr>
<td>Moisture</td>
<td>16.2 g %</td>
</tr>
<tr>
<td>Ash</td>
<td>0.04 g %</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.092 mg %</td>
</tr>
<tr>
<td>Calcium</td>
<td>11 mg %</td>
</tr>
<tr>
<td>Iron</td>
<td>0.784 mg %</td>
</tr>
</tbody>
</table>

*Source: Food quality control- analysis Lab., Islamic azad university of Tabriz.

Indicated serum levels of Triglyceride (mg/dl) measured in pure control group, control group, diabetic control group, treatment group, and diabetic treatment group. Statistical comparing of triglyceride among different groups, revealed statistical meaningful difference among diabetic control group with control groups, and treatment group and diabetic treatment group (P<0.05). Serum levels of triglyceride which was made by drug revealed statistical meaningful increase in diabetic control group compared with control groups (P<0.05). Nevertheless, in diabetic treatment group triglyceride level revealed statistical meaningful decrease compared with diabetic control group (P<0.05) (Table 3).

**Table 2. Studying the serum levels of glucose in blood (mg/dl) among different groups.**

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Pure control group 1st day</th>
<th>Pure control group last day</th>
<th>Pure treatment</th>
<th>Treatment diabetic</th>
<th>Control diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3/16 ± 86/63</td>
<td>8/90 ± 80/30</td>
<td>8/40 ± 80/46</td>
<td>11/24 ± 87/22</td>
<td>4/52 ± 162/50</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>b</td>
</tr>
</tbody>
</table>

Indicated average serum levels of cholesterol (mg/dl) measured in pure control group, control group, diabetic control group, treatment group and diabetic treatment group. Statistical evaluation of cholesterol among groups revealed statistically meaningful difference among cholesterol of diabetic control group with control groups, and also treatment group and diabetic treatment group with diabetic control group (P<0.05). Serum levels of cholesterol in diabetic control group had statistically meaningful increase compared with diabetic control group (P<0.05). Serum levels of cholesterol revealed also statistically meaningful decrease in diabetic treatment group compared with the control groups (P<0.05), (Table 4).

Indicated serum levels of VLDL (mg/dl) measured in pure control group, control group, diabetic control
group, treatment group, and diabetic treatment group. Statistical evaluating of serum levels of VLDL among groups revealed statistically meaningful difference among diabetic control group with other groups (P<0.05). Serum levels of VLDL reveal meaningful increase in diabetic control group compared with other groups, (Table 5).

Table 3. Studying the serum levels of triglyceride (mg/dl) among different groups.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Control treatment group</th>
<th>Pure treatment</th>
<th>Treatment diabetic</th>
<th>Control diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride</td>
<td>9/73±77/90</td>
<td>13/07±82/30</td>
<td>11/54±90/66</td>
<td>8/50±92/40</td>
<td>14/62±97/25</td>
</tr>
</tbody>
</table>

Indicated serum levels of LDL (mg/dl) measured in pure control group, control group, treatment group, diabetic treatment group and diabetic control group. Statistical comparing between serum levels of LDL among groups shows statistically meaningful difference between diabetic control group with pure control group and control group and diabetic treatment group and treatment group. (P<0.05).

Table 4. Studying the serum levels of cholesterol (mg/dl) among different groups.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Control treatment group</th>
<th>Pure treatment</th>
<th>Treatment diabetic</th>
<th>Control diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>6/93±100/5</td>
<td>5/45±98/68</td>
<td>4/58±94/36</td>
<td>8/98±90/60</td>
<td>5/95±120/45</td>
</tr>
</tbody>
</table>

Indicated serum levels of HDL (mg/dl) measured in pure control group, control group, treatment group, diabetic treatment group, and diabetic control group. Statistical evaluating of average HDL among groups revealed no meaningful difference statistically (Table 7).

Discussion

Today diabetes mellitus is one of the important problems in human and animal society. In addition, in veterinary diabetes mellitus occurs in most animal especially in pet and animals that encouraged to do different works, using chocolate and sweetness is current. The important point in diabetes mellitus is increasing of blood glucose level and changing in insulin level or insulin receptors. As result, lack of consuming blood glucose results in sets of metabolic change in body that can make significant changes including glycogenesis, lipolysis and gluconeogenesis. When consuming blood glucose do not occur. Glucagon hormone increases and causes to above changes, as result, stored glycogen level decrease and its synthesis lowered due to inhibitor of glycogen synthesis enzyme. As result of gluconeogenesis, body proteins decomposed and blood glucose increases, ammonia, and urea will be produce from protein metabolism to supply energy.

Table 5. Studying the serum levels of VLDL (mg/dl) among different groups.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Control treatment group</th>
<th>Pure treatment</th>
<th>Treatment diabetic</th>
<th>Control diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL</td>
<td>1/94±15/56</td>
<td>2/61±19/90</td>
<td>2/30±21/33</td>
<td>1/70±18/88</td>
<td>2/92±29/45</td>
</tr>
</tbody>
</table>

Yegani et al.
On the other hand, body's fats will be decomposing due to sensitivity of lipase enzyme to hormone, and then it causes to increase of lipoproteins, Triglyceride, and cholesterol. Acetyle-CoA resulted from metabolism must be reacted with oxaloacetate (C4) and enter to krebs cycle as sitric acid (C6) but can't be used because of lack of oxaloacetate. As result, have two ways: A) 3 molecules of Acetyle-CoA combined together then gives cholesterol molecule. B) Chooses ketogenesis path gives rise to keton bodies (beta-hydroxybutyric acid, acetone, acetoacetic acid). There is different therapeutic methods in order to diabetes treatment including Glibenclamide (increase of insulin effect), Metformin (decrease of liver glucose exit and decrease of insulin strength)(Melchior and Jaber 1996). Inhibitory drugs α-glocosidaze (control of compound carbohydrate being decomposed and latency in monosaccharides absorption from digestive system) (Weaver et al. 1978), and Troglitazone (a mechanism inducing skeleton muscles in absorbing and taking glucose and increasing sensitivity to insulin)(Scheen 1997; Sparano and Seaton 1998).

Table 6. Studying the serum levels of LDL (mg/dl) among different groups.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Control treatment group</th>
<th>Pure treatment</th>
<th>Treatment diabetic</th>
<th>Control diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL</td>
<td>3/43 ± 49/67</td>
<td>0/14 ± 46/20</td>
<td>6/91 ± 41/53</td>
<td>8/99 ± 26/22</td>
<td>11/33 ± 65/40</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>a</td>
<td>a</td>
<td>c</td>
<td>b</td>
</tr>
</tbody>
</table>

Measuring blood glucose showed that after alloxan prescribing serum level of glucose has meaningful increase in diabetic control group compared to control group. Alloxan influences on β-islets langerhans cells and causes to cell decomposition that its symptoms are polyuria and polydipsia. Alloxan effect in giving rise to diabetes mellitus is resulted from free radical production (superoxide and hydroxyl) and on the other hand, it causes to these cells decomposition by increasing intracellular calcium concentration. Results of this study are accordance with research result of Szkudelski (2001), Kim et al. (1994), Kliber et al.(1994) and Weaver et al.(1978) (Szkudelski 2001; Kim et al.1994; Kliber et al.1994; Weaver et al. 1978).

Table 7. Studying the serum levels of HDL (mg/dl) in different groups.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Control treatment group</th>
<th>Pure treatment</th>
<th>Treatment diabetic</th>
<th>Control diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL</td>
<td>0/53 ± 23/30</td>
<td>1/00 ± 25/24</td>
<td>0/50 ± 31/50</td>
<td>0/50 ± 43/50</td>
<td>0/53 ± 22/30</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>a</td>
</tr>
</tbody>
</table>

*In All Tables: Similar letters show nonexistence meaningful statistical differences( P> 0/05)
*In All Tables: Dissimilar letters show the existence of meaningful statistical difference (P<0/05).

Prescribing honey to pure treatment group, treatment diabetic group causes deduction in the serum level of glucose comparing with pure diabetic that is meaningful from the viewpoint of statistic. The deduction in the serum level of glucose can be attribute to the high degree of Fructose available in honey, that due to disusing of Glucose, Fructose taken into account as a source for energy and it enters into the cells, and then produce energy. After absorbed from intestine, Fructose enters into liver via blood and during the enzymal process, first it changed into Fructose1Phosphate through Fructokinase accompanying by an ATP molecule, next Fructose turn into Fructose1-phosphate, while this material converted to dihydroxyacetone phosphate and glyceraldehyde by aldolase. Finally glyceraldehyde phosphorylate at ATP present by phosphoglyceraldehyde kinase and converge to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate that are compound resulted from glycolysis and convert into Krebs cycle. On the other hand during lack of glucose, liver tissue cells,
muscles, brain and blood tissue cells can covert fructose to fructose 6-phosphate by certain hexokinase to supply energy. Therefore, it can be resulted that using no glucose on one hand, and providing fructose and supplying energy by fructose, is a factor for decreasing blood glucose.

Samantha Et al (1985) examined the effect of the honey on Diabetes type I and type II, therefore they concluded that honey is the best substitute for sugar and it decreases Glucose. In addition, Samantha’s study accompanies with the result of this survey (Samantha et al. 1985). Moreover Onate et al (1991) also goes along with the effect of honey on reduction of Glucose in blood, which this study wanted to say (Onate et al. 1991). Nuray et al (2005) reported the effect of mad honey on the reduction of Glucose too. Nuray et al (2005) believed that in some types of honey, which originated from special species of flowers, produce substance called Girayanatoksin. Girayanatoksin function by stimulating M2 Moskarini receivers and Parasapatic system (Nuray et al. 2005).

Therefore, it enforces the stimulation of insulin excretion in the pancreas. Serum level of Cholesterol in Diabetic pure group shows statistically meaningful increase comparing with Pure groups while in Treatment diabetic group and Pure treatment group shows statistically meaningful decrease comparing with Diabetic treatment(p<0/05). However, the amount of the Cholesterol is as the same as amount in Pure group. Increasing of Cholesterol after Alloxan prescription and affecting by Diabetes are due to not entering of the astil ko enzyme A, produced via various paths to Caris cycle. 3 of them are combined to produce Cholesterol. But after prescribing honey, the assumption amount of the astil ko enzyme produced through Fructose usage decreases because of: 1) honey lacks Cholesterol and its pre requisite 2) supplying energy through using Fructose .These results accompany with Nuray’s(2005), Onate’s(1991) and Samantha’s(1985)( Nuray et al. 2005; Onate et al. 1991; Samantha et al. 1985).

The serum level of triglyceride increased after Alloxan prescription and affecting by Diabetes in Diabetic pure group due to the effect of Glokagon hormone of the process of Lipoliz and releasing of fat sources from stored tissues such as liver and fat tissue. However, after using honey in pure treatment group and Diabetic pure shows meaningful decrease comparing with Diabetic pure group (p<0/05). Honeybee adds a sort of Diastase enzyme to the nectar that forms 4/5 of the weight of honey along with starch and saccharin ingredients. (Lotfy et al. 2007; Stefan et al. 2000) This enzyme can cause the lipid to be dissolving. Then, it can be decrease of Triglyceride (Lotfy et al. 2007). So for that reason it is suggeste to the old and patients suffering from heart disease. Besides, Girayano found in some natural honeys is a factor that has a role in stimulating the pancreas to excrete insulin, so this is itself an effective cause in decreasing Triglyceride. Moreover because the amount of fat and Triglyceride are low, then maybe it is a reason for decrease of Triglyceride (Stefan et al. 2000). This funding goes along with other researcher’s funding such as Samantha (1985), Nuray (2005), and Onate (1991) (Samantha et al. 1985; Nuray et al. 2005; Onate et al. 1991).

The serum level of VLDL after Alloxan prescription in Diabetic pure group shows meaningful decrease that is because of the fat stimulation, Triglyceride decrease, and VLDL Sentez decrease by liver. However, after using honey by bees this amount decreases and reaches to the pure group’s degree that decrease is because of the Triglyceride decrease that is due to the usage of honey. The result of this study accompany with the Nuray’s funding (2005) (Nuray et al. 2005). The serum level of LDL after Alloxan prescription and affecting by Diabetes shows a meaningful decrease that is because of the Glokagon hormone effect and it is normal phenomena. However, after honey prescription the serum amount of Lipoporotein decreases and even less than pure groups, so it can be consider this as a positive point.

The reason of Triglyceride decrease after using honey can be relate to decrease of the serum level of
Cholesterol and LDL Santez holding in liver by honey. There is no available research in this area and it is for first time that serum level of LDL in honey surveyed. On the other hand, Grayanotoksin found in honey that stimulates Beta cells and produces insulin can be effective in LDL(Samantha et al.1985). The serum level of the HDL after affecting by Diabetes in diabetic pure group comparing with pure groups does not show meaningful difference, but in treatment diabetic and pure treatment shows statistically meaningful increase in HDL serum level. The reason of this decrease is unknown and is a suggested area for examination. Maybe the reason for decreasing of serum level of LDL and Cholesterol is due to the decrease in serum level of HDL after honey using.

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