The effect of Walnut consumption on the serum levels of Glucose, Triglyceride, Cholesterol, HDL, VLDL, and LDL in rat’s serum with experimental diabetes

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ABSTRACT

Diabetes mellitus is an important problem in human and also it occurs in many animals, particularly in pet. This study was to evaluate walnuts effects on serum concentration of glucose, triglyceride, Cholesterol, VLDL, LDL and HDL in experimental diabetes mellitus in rat. 30 male Wistar rats with age of 8 weeks and weight of 200 ± 20 grams were selected. Then these rats were divided into five groups, 6 rats per group. Diabetic treatment group and diabetic control group received subcutaneous a single dose of Alloxan (100mg/kg BW) in saline solution. Pour control group and control group received subcutaneous saline normal (100 mg/kgBW). After observed diabetes symptoms (Polydipsia, Polyuria,Glucosuria and Hyperglycemia) in groups that were received Alloxan, we have initiated to feeding treatment group and diabetic treatment group in the ratio of equal with walnuts and pellet. These groups were fed for 10 days. Blood samples collected from whole groups at the end of 10day. Evaluation of the serum levels of triglyceride revealed 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 to the diabetic control group, but did not reveal statistically difference compared to the control group (P>0.05). Evaluation of the serum levels of HDL did not reveal statistically significant difference between groups (P>0.05). The walnut can be used as a natural fruit for prevent than diabetes mellitus disorders.

Key word: Diabetes, alloxan, walnut, serum parameters, rat

INTRODUCTION

Diabetes mellitus is syndrome occurs due to lack of insulin secretion or decrease in tissue sensility to insulin and results in disorder in carbohydrate, fat and protein metabolism [9].Regards to that diabetes is one of common diseases at world increasingly and there is no definite therapy by now,therefore only promising method includes proper care and controlled feeding and any overindulge may result in irretrievable consequences. In diabetes, in addition to serum levels of glucose, triglyceride, cholesterol, HDL, VLDL and LDL are increase significantly which each have related problems [8, 26]. The most important physiological event in diabetes mellitus includes hyperglycemia which occurs due to 3 cause: 1. Decrease in glucose arrival rate into different cells. 2. Decrease in glucose in different tissue. 3. Increase in glucose production by liver (gluconeogenesis) [9]. Main symptoms of diabetes mellitus include: polyuria, polydipsia and losing weight unlike sufficient feeding. Diabetes is 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)(9). The aim of this study is evaluating therapeutic effects of walnuts on biochemical tableau of experimental diabetes in rats so that to find whether walnuts can take role in decreasing effects of diabetes mellitus. 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. 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. The action of reactive oxygen species with a simultaneous
massive increase in cytosolic calcium concentration causes rapid destruction of B cells. Summing 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 [32].

Walnutis fruit from Juglandaceae family and Juglans Regia species contain a high percentage of carbohydrate & highfat (68%) with 8.3% omega 3 fats specially Poly Unsaturated Fatty Acids PUFA (70%) such as linoleic(12%) and linolenic acids(58%) and Ecosa Pentatonic Acid (EPA),Deca Hexanoic Acid (DHA) and Mono Unsaturated Fatty Acids [MUFA(18%)] and saturated fatty acids12% [2,3,4,5,10,15,16,24,29,30,33,34], high quality & high digestible protein (14.4%), Necessary Amino Acids (24,34) vitamins such as A, B1 (Thiamine), B6, B12, C, E, Folic acid, Nicotinic acid (Niacin), Pentatonic Acid, and a high percentage of dietary fibre (9.7%) [2,4,5,16,24,34]. Walnut contain many minerals such as Fe (0.21 gr/kg), K (3.32 gr/kg), Mg (1.34 gr/kg), P (3.5 gr/kg), Ca (0.89 gr/kg) and Zn, Cu, Co, Mn, Se & only Na (0.01%) and contain Tannins, Anti-oxidants, Anti Inflammatory agents, Anti Infectious agents and etc. [24,34].

MATERIALS AND METHODS

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 maintained one week into the special cage and maintained at 23-25°C with a 12h dark and light. Average weight of all groups was 200±20 gr. At first day, one of groups were bled and blood serum sample were separated and analyzed after centrifuge. Alloxan monohydrate (by Fluka Co, in 10gr package) was used to induce type I diabetes mellitus. Diabetic treatment group and diabetic control group received subcutaneous a single dose of alloxan (100 mg/kg) in saline solution. Pour control group and control group received subcutaneous saline (100 mg/kg). Injection after 1 week in all groups was 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). Groups were fed by bottom stock after observing diabetic symptom:

Group 1: receiving physiological serum as a control group, was fed only with pellet 50±10 gr daily.
Group 2: receiving physiological serum as a treatment group was fed with (25±5 gr walnuts) + (25±5 gr pellet).
Group 3: receiving alloxan as a diabetic control group was fed only with 50±10 gr pellet.
Group 4: receiving alloxan as a diabetic treatment group was fed with (25±5 gr walnuts) + (25±5 gr pellet).

This groups were fed with this method twice a day. Whole groups were fed and maintained 10 days under above mentioned conditions and were controlled everyday on certain time, then remaining food was weighted and after defining amount of last day consumed food, fresh food was fed. Meanwhile, during day from consuming walnuts by treatment group and diabetic treatment group was assured. Blood samples collected from whole groups at the end of 10 day and was gathered test tubes, tubes lids were closed with Para film then were centrifuged for 10 minutes at 2500 turn/minute and serum were separated and analyzed. Whole rats were anesthesia by chloroform in glass jar then were bled by de-heading method and during bleeding care was performed to blood enter into the test tubes slowly and tangent with wall. Glucose, triglyceride, cholesterol and HDL serum levels were measured by enzymatic 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:

$$VLDL = \frac{\text{triglyceride}}{5}$$

LDL = cholesterol – (HDL + VLDL)

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

Table 1: Indicated serum levels of blood glucose (mg/dl) in pure control group, control group, diabetic control group, treatment group and diabetic treatment group.

<table>
<thead>
<tr>
<th></th>
<th>Pure Control group</th>
<th>Control group</th>
<th>Treatment group</th>
<th>Diabetic treatment group</th>
<th>Diabetic control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>86/63 ± 3/16</td>
<td>80/30 ± 8/90</td>
<td>80/76 ± 8/15</td>
<td>85/08 ± 14/38</td>
<td>162/50 ± 4/52</td>
</tr>
</tbody>
</table>

Similar letters in each row indicated no meaningful difference statistically (p> 0.05)
No similar letters in each row indicated meaningful difference statistically (p<0.05)
Evaluation of the serum levels of glucose revealed statistically significant differences between diabetic control group with control group and also between treatment group and diabetic treatment group with diabetic control group and control group. (P<0.05) Blood glucose level in diabetic control group that receiving alloxan had meaningful increase as to pure control group and control group but in diabetic treatment group and treatment group revealed decrease statistically significant as to pure control group and control group (P<0.05).

Table 2: Indicated serum levels of triglyceride (mg/dl) in pure control group, control group, diabetic control group, treatment group and diabetic treatment group.

<table>
<thead>
<tr>
<th></th>
<th>Pure Control group</th>
<th>Control group</th>
<th>Treatment group</th>
<th>Diabetic treatment group</th>
<th>Diabetic control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>77/90 ± 9/73</td>
<td>82/30 ± 13/07</td>
<td>89/90 ± 6/40</td>
<td>90/80 ± 5/15</td>
<td>97/25 ± 4/62</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>a</td>
<td>c</td>
<td>c</td>
<td>b</td>
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</table>

Similar letters in each row indicated no meaningful difference statistically (p> 0.05)
No similar letters in each row indicated meaningful difference statistically (p<0.05)

Evaluation of the serum levels of triglyceride revealed 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) Blood levels of triglyceride in diabetic treatment group and treatment group revealed decrease statistically significant as to diabetic control group and revealed increase statistically significant as to control group (P<0.05).

Table 3: Indicated average serum levels of cholesterol (mg/dl) in pure control group, control group, diabetic control group, treatment group and diabetic treatment group.

<table>
<thead>
<tr>
<th></th>
<th>Pure Control group</th>
<th>Control group</th>
<th>Treatment group</th>
<th>Diabetic treatment group</th>
<th>Diabetic control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>100/5 ± 6/93</td>
<td>98/68 ± 5/45</td>
<td>90/73 ± 2/14</td>
<td>87/88 ± 11/18</td>
<td>120/45 ± 5/95</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>a</td>
<td>b</td>
<td>b</td>
<td>c</td>
</tr>
</tbody>
</table>

Similar letters in each row indicated no meaningful difference statistically (p> 0.05)
No similar letters in each row indicated meaningful difference statistically (p<0.05)

Evaluation of the serum levels of cholesterol revealed statistically significant differences between diabetic control group with control group and also between treatment group and diabetic treatment group with diabetic control group and control group (P<0.05). Blood Cholesterol level in diabetic control group that receiving alloxan had meaningful increase as to pure control group and control group but in diabetic treatment group and treatment group revealed decrease statistically significant as to diabetic control group and pure control group and control group (P<0.05).

Table 4: Indicated serum levels of VLDL (mg/dl) in pure control group, control group, diabetic control group, treatment group and diabetic treatment group.

<table>
<thead>
<tr>
<th></th>
<th>Pure Control group</th>
<th>Control group</th>
<th>Treatment group</th>
<th>Diabetic treatment group</th>
<th>Diabetic control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL (mg/dl)</td>
<td>15/56 ± 1/94</td>
<td>19/90 ± 2/61</td>
<td>21/40 ± 7/48</td>
<td>27/76 ± 7/03</td>
<td>29/45 ± 2/92</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>a</td>
<td>b</td>
<td>b</td>
<td>b</td>
</tr>
</tbody>
</table>

Similar letters in each row indicated no meaningful difference statistically (p> 0.05)
No similar letters in each row indicated meaningful difference statistically (p<0.05)

Evaluation of the serum Levels of VLDL decreased significantly in diabetic treatment group and treatment group compared to the diabetic control group, but did not reveal statistically difference compared to the control group (P<0.05).

Table 5) Serum levels of LDL (mg/dl)

<table>
<thead>
<tr>
<th></th>
<th>Pure Control group</th>
<th>Control group</th>
<th>Treatment group</th>
<th>Diabetic treatment group</th>
<th>Diabetic control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL (mg/dl)</td>
<td>49/67 ± 3/43</td>
<td>46/20 ± 0/14</td>
<td>41/03 ± 4/70</td>
<td>35/66 ± 14/03</td>
<td>65/40 ± 11/33</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>a</td>
<td>a</td>
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<td>b</td>
</tr>
</tbody>
</table>

Similar letters in each row indicated no meaningful difference statistically (p> 0.05)
No similar letters in each row indicated meaningful difference statistically (p<0.05)
Table 5: Indicated serum levels of LDL (mg/dl) in pure control group, control group, treatment group, diabetic treatment group and diabetic control group.

Evaluation of the serum levels of LDL revealed statistically significant differences between diabetic control group with control group and also between treatment group and diabetic treatment group with diabetic control group and control group (P<0.05). Blood LDL level in diabetic control group that receiving alloxan had meaningful increase as to pure control group and control group but in diabetic treatment group and treatment group revealed decrease statistically significant as to diabetic control group and pure control group and control group (P<0.05).

Table 6: Indicated serum levels of HDL (mg/dl) in pure control group, control group, treatment group, diabetic treatment group and diabetic control group.

Table 6) Serum levels of HDL (mg/dl)

<table>
<thead>
<tr>
<th>Group</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic control group</td>
<td>23/30 ± 0/53</td>
</tr>
<tr>
<td>Diabetic treatment group</td>
<td>25/24 ± 1/00</td>
</tr>
<tr>
<td>Treatment group</td>
<td>28/26 ± 0/68</td>
</tr>
<tr>
<td>Control group</td>
<td>24/46 ± 0/67</td>
</tr>
<tr>
<td>Pure Control group</td>
<td>22/30 ± 0/53</td>
</tr>
</tbody>
</table>

Similar letters in each row indicated no meaningful difference statistically (p>0.05)
No similar letters in each row indicated meaningful difference statistically (p<0.05)

Evaluation of the serum levels of HDL did not reveal statistically significant difference between groups (P>0.05).

DISCUSSION

Today diabetes mellitus is one of the important problems in human and animal society. Also, in veterinary diabetes mellitus occurs in most animal specially in pets and to encourage animals to do different works, using chocolate and sweetness is current [8]. What is important in diabetes mellitus is increasing of blood glucose level and change in insulin level or insulin receptors? As result, lack of consuming blood glucose results in sets of metabolic changes in body that can make significant changes including glyco genesis, lipolysis and gluconeogenesis. When consuming blood glucose does not occur. Glucagon hormone increases and cause to above changes, as result, stored glycogen level decrease and it is synthesis is lowered due to inhibitor of glycogen synthesis enzyme [8, 26].

As result of gluconeogenesis, body proteins decomposed and blood glucose increase and ammonia and urea will be produced from protein metabolism to supply energy. On the other hand, body's fats will be decomposed due to sensitive of lipase enzyme to hormone, then it cause to increase of lipoproteins, triglyceride and cholesterol. Acetyl-coA resulted from metabolism must be reacted with oxaloacetate (C4) and enter to Krebs cycle as citric acid (C6) but cannot be used because of lack of oxaloacetate [8, 26]. As result have two ways:

A) 3 molecules of Acetyl-CoA combined together then gives cholesterol molecule. B) Chooses ketogenesis path gives rise ketone bodies (beta-hydro butyric acid, acetone, acetooacetic acid) [26]. There are 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) [11, 21], inhibitory drugs α-glycosidase (control of compound carbohydrate being decomposed and latency in monosaccharide’s absorption from digestive system) [25,31], and Troglitazone (A mechanism including skeletal muscles in absorbing and taking glucose and increasing sensitivity to insulin).

The aim of study is evaluating therapeutic effects of dates on biochemical tableau of experimental diabetes in rats to find whether dates can take role in decreasing diabetes mellitus measuring blood glucose showed that after alloxan prescribing serum level of glucose has meaning increase in diabetic control group compared to control group. Alloxan influences on β-islets Langerhans cells and causes to cell decomposition which it is symptoms are polyuria and polydipsia. Alloxan effects 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 (1986) [32], Kim et al. (1994) [17], Kiliber et al. (1996) [19] and Weaver et al. (1978) [35].

Walnut prescribed in Diabetic treatment group and Treatment group decreased blood glucose levels compared with Diabetic control group was almost a blood glucose level in the control group. Mechanism that could explain the effect of walnut on lowering blood sugar is unknown. But it may be concluded was too low Walnut glucose level (10%), and it can be glucose penetration to tissues in diabetic animals. [22] Koohi (1386) has done similar results with the use of the Date in diabetic rats gained is that the results are consistent. Alizadeh (1386) Effect of honey consumption in the diabetic, has been reported to reduce blood sugar, which is consistent with this study.
On the other hand, fiber level is high in dates and this can prevent from diabetes mellitus spread. Mechanism is not clear but it can say that fiber prevents from fast increase of glucose by causing latency in stomach empty and cause to latency in hungry feeling. It also, can be decomposed into colon by micro-flora and give rise to short chain fatty acids, this fermentation cause to liver glucose production decrease and by this way cause to decrease glucose after meal. Obtained results from this study are accordance with other results of researchers [12, 36] Jue et al. (2003) have reported effect of dietary with high fiber as factor in decreasing blood sugar [13]. As walnut contain as higher fiber, can perform this influence.

Also Mg as element that it is lack is observed in diabetes mellitus and cardiovascular disease and in is resulted from urine excretion, decrease in absorption from intestine, glucosuria and urea excretion. Mg takes important role in carbohydrate metabolism and insulin function and as cofactor that acts in transport, Jointing and glucose secretion. Insulin cause to increasing of Mg arrival into the cell while this problem is observed in diabetes type I (IDMM). Mg level is relative high at dates, thus can interfere with carbohydrate metabolism and other mentioned cases adjusts increased level of glucose. Results of this research are in agreement with that of Chetan et al. (2002) [6]. Koohi(2008) evaluated effect of Date on induced diabetes by Alloxan which are in agreement with results of this study [20].

Alizadeh (2008) evaluated effect of honey on induced diabetes by Alloxan which is in accordance with these results [1]. Zn is one essential element for insulin metabolism, and usually decrease in diabetes type I. As dates contains Zn, can be effective in preventing β-cells decomposition [7]. Miller et al. (2003)[31] Serum levels of cholesterol after prescribing Alloxan in diabetic control group reveal meaningful increase. In diabetes occurred, glucose cannot supply energy, β-cells of pancreases destroyed, insulin lowered in turn glucagon is increased. As result this increasing of lipolysis occurs. On one hand produced Acetyl-CoA cannot enter to Krebs cycle and from combining 3 molecules, cholesterol is produced [8, 14 and 26].

At diabetic treatment group and treatment group also serum level of cholesterol reveal meaningful decreased compared to diabetic control group, pure control group and control group. Also decrease is high in diabetic treatment group and cholesterol level is lower compared to control group. Cause for cholesterol decreasing can be due to low level of cholesterol in dates [1,15].

On the other hand, due to the essential fatty acids found in walnut is especially linoleic acid and linolenic acid.JK.chan et al 1991 [5] in their study of the effect of dietary linolenic acid and linoleic and oleic containing serum cholesterol levels were performed on serum cholesterol levels have been observed to show a significant decrease. Because of Walnut contain high levels of unsaturated fatty acids, especially linoleic and linolenic can bedecrease cholesterol levels.

Cholesterol into the body to be transported by lipoproteins or Esterified by fatty acids. If Esterification do by polysaturated fatty acids especially necessary fatty acids, it can easily be done Cholesterol metabolism, cropping and placement in cell membrane, that it can easily be done Cholesterol metabolism that is evidence cholesterol-lowering. It to the other side polysaturated fatty acids can increase the activity of carrier protein of transferred cholesterol ester in the body that as a result of the transfer of cholesterol to simplicity [2].

Serum level of triglyceride in diabetic control group have increased after prescribing Alloxan which is resulted from effect of Alloxan on β-cells of pancreases and decrease of insulin serum that follow increasing glucagon and induced lipolysis process [8,14,26].

But despite the increase triglyceride levels is in the normal range of reference sources. But to increase this parameter does not seem like a lack of glucose consumption causes release of reserves triglyceride storage places, including the liver and the adipose tissue [9, 28].

Walnut consumption decrease triglycerides in diabetic group and Treatment group. Triglycerides levels in mentioned groups less than Diabetic control group but is higerthan control group. Amounts of essential fatty acids, especially polysaturated and necessary fatty acids in walnuts is high and after attracting effect on triglyceride metabolism and decreased liver triglyceride synthetase activity. Triglyceride levels will result decrease. Presence of unsaturated fatty acid particularly ω-3 prevent from increasing lipids, and synthesis of fatty acid and triglyceride on liver, that these result are in agreement with that of Kinsell (1987) [18]Effect of unsaturated fatty acids on cardiovascular disease is reviewed.Showing that consumption of these fatty acids can decrease triglyceride levels. Walnut have high amount of linoleic and linolenic acids that due better transfer of lipids by lipoproteins and will be decrease triglycerides.
Triglycerides levels in Diabetic treatment group and Treatment group compared with the control group, revealed significant increased that is caused by diabetes [9, 27] but despite the significant increase in the mean normal range is referenced. Increasetriglyceride due to increased VLDL levels that are created as a result of diabetes. The body needs to be transfer of triglycerides are the major lipoprotein carrier of triglycerides is VLDL. (9) The results with the results Howard et al (1987)and Saxena et al (1992)is consistent [3,33].

Triglycerides levels in Diabetic group compared with the control group, revealed significant increased. While in Diabetic treatment group and Treatment group compared with Diabetic control group and control group revealed significant decreased. Increase of cholesterol levels in Diabetic control group stimulates synthesis carry cholesterol lipoprotein (LDL). Therefore, it will increase in the serum, whereas the two groups mentioned Walnut reduce serum cholesterol levels due to the reasons listed and results in decreased serum LDL levels. Metabolized of plasma lipoprotein by multiple enzyme and receptor done and the LDL called lipoprotein refer can be metabolized by the hepatic LDL receptor. The client is willing to saturated fatty acids. If saturated fatty acids, replace with unsaturated, LDL levels will decrease. Walnut also contain unsaturated fatty acids is the result of lowering LDL caused by cholesterol reduction and also due to the unsaturated fatty acids. These results are consistent with the findings of other investigators [2, 4, 5, 15, 16, 24, 29].

Serum HDL levels in diabetic control group and diabetic treatment group and treatment group don’t show statistical difference with the control groups. Or about diabetes indicated and Walnut consumption has noteffect on serum levels of this parameter.

The overall conclusion of the results of research, Walnut consumption in normal range in healthy subjects and in diabetic subjects due to decreased serum levels of glucose, cholesterol, triglycerides, VLDL, LDL can be useful. But how much of walnuts a day can be used? May need to be further investigated.

Suggests:
1) To be investigated in humans.
2) Other hormones especially pancreatic hormones after administration of walnuts to review.

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