Evaluation of antidiabetic and antihyperlipidemic activity of Kefir in alloxan induced diabetes mellitus rat

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ABSTRACT
Objective: The present study was undertaken to investigate the antihyperglycemic and antihyperlipidemic effects of skimmed milk Kefir in alloxan-induced diabetic rats.

Methods: Diabetes was induced in male Wister rats by single intraperitoneal administration of alloxan monohydrate (150 mg/kg). Animals were divided into four groups (n=12) receiving different treatments. Skimmed milk Kefir (3.6ml/200gm) was studied in alloxan-induced diabetic rats for a period of 20 and 40 days. Glibenclamide (600 μg/kg) was used as a reference drug in alloxan-induced diabetic rats.

Results: The daily oral treatment with skimmed milk Kefir showed a significant reduction in blood glucose (p>0.05) with respect to the control group. Besides, administration of Kefir for 20 and 40 days was significantly (p>0.05) decreased serum contents of total cholesterol, triglycerides, low density lipoprotein LDL and very low density lipoprotein VLDL whereas HDL-cholesterol was effectively increased. This Study showed clearly that skimmed milk Kefir has hypoglycemic and antihyperlipidemic effects.

Conclusion: In this study and depend on the results it is possible to observe that skimmed milk kefir products has antidiabetic and antihyperlipidemic effect in induced diabetic rats which showed elevated in serum glucose, hyperlipidemia and decrease in HDL. Consumption of skimmed milk kefir products in (20) and (40) days during the period of study was very useful. The beneficial effects of Kefir products were found to be decrease serum glucose and other lipid picture but there is increase in HDL. Therefore skimmed milk kefir products are considered to be a useful bioactive natural food, which have a hypolipidemic and hypoglycemic effect and one of the important dietary choices for patients with diabetes who need safe and useful food products to fight the dangerous effects associated with the disease.

INTRODUCTION

Kefir grains or kefiran are cauliflower like, lobed, irregularly shape, white to yellowish white in colour, and have a slimy but firm texture a slimy a polysaccharide–protein matrix embedded by lactic acid bacteria and yeasts, and microbial action of a wide community of microorganisms presented in kefir grains on milk which produce a culture milk beverage, and many studies shows that the frequent consumption of milk fermented by these microbes my treat many cases, inhibition of tumor growth. Antimicrobial properties.
Cholesterol metabolism. Probiotic bacteria should be able to resistant and bearing the sever environment conditions of the gastrointestinal tract, including stomach pH conditions and the effect of bile salts and digestive enzymes; the specific characteristic mixture of kefir is unique which makes it different from other probiotics because it has specific complex component. Many researches proved that kefir considered a complex probiotic and its in different of other probiotic cause it has large number and types of different bacteria and yeast, the isolation and identification of these species individually is difficult because these microbial population appears many similar characteristics and its could be benefit for animal because kefir increases the beneficial bacteria of the gastrointestinal tract such as Bifidobacterium and Lactobacillus and this lead to the reduction of harmful microbes such as Clostridium perfringens. But identification of these microorganisms will be easy by using of advanced molecular and biological techniques. Kefir has many different sources and the synergistic interaction of the bacteria and other microbial component in kefir grains effect on its production and the differences of ingredients that produced by those microbial components of kefir give the special texture and taste. There is evidence shows that metabolism, digestion and immune function in human affected by kefir consumption and influences in their action.

The microbial community of Tibetan kefir grains depends primarily on their source. It has been reported that Tibetan kefir grains contain Lactobacilli, Lactococci and yeast, and sometimes acetic acid bacteria, depending on the source of origin.

Hyperglycemia the characteristic signs that appear during the dangerous metabolic disorder Diabetes mellitus, number of diabetic people are increase every year, the type 2 diabetes prevalence increased yearly, it will be affecting on of diabetes complications, lower quality of human healthy and morbidity and mortality affected by the diabetes mellitus. Water kefir is a less cost bioactive compound which found by less time consuming act as hypoglycemic and hypolipidimic treatment. Water kefir can be useful consumption beneficial nutrients to control glucose in diabetes and control lipid levels potentially.

Maintaining in serum triglyceride and antidiabetic are improve results which made by regular consumption of kefir products, and by improving in pancreatic β-cells lead to decreasing in plasma glucose and increasing in glutathione peroxidase activity.

**MATERIALS AND METHODS**

**Experimental Design:** Forty eight male rats were included in this experiment, weighed 200-250gm and aged 12-14 weeks. Rats put in the animal house of College of Veterinary Medicine, University of Kerbala. The Skimmed Milk-kefir were prepared using (10 % w/v) kefir grains immersed in sterilized (121°C for 15 min) skimmed milk, before being incubated for 20h at 20°C then kefir grains removed by using cheesecloth layers and kept at 4°C until used. The rats were fed with standard pellet and water ad libitum. Temperature and light were provided to animals within standard condition. Rats were introduced into four groups (12 rat/each) and animals of first group drenched normal saline as control and second group were injected intraperitoneally alloxan (single dose) to induce diabetes mellitus and orally drenched with normal saline orally as diabetic control group, while animals of third and fourth group were intraperitoneally injected with 150 mg/kg of alloxan (single dose) to induce diabetes mellitus and orally drenched with skimmed milk Kefir(3.6ml/200gm) and. Gliben clamide (600 μg/kg) respectively.

Blood samples were collected via heart puncture from six animals of each group at Day 20 and at Day 40 of experiment. spectrophotometric analysis was used to measure Random blood glucose, Total cholesterol (TC), triglycerides(TG), low density lipoprotein LDL and very low density lipoprotein VLDL whereas HDL-cholesterol were measured, by using kits of SPECTRUM Company, Egypt. Serum LDL- and VLDL Concentration were calculated by following formulas LDL-C = TC – HDL-C – TAG/5 and VLDL = TAG / 5 consequently.

The data were statistical analyses by using Statistical Program for Social Sciences. Comparisons between groups were done via analysis of variance. Difference between groups was determined according to calculated least significant different test (LSD)

**RESULTS**

Data of glucose levels in the present study confirmed induction of diabetes mellitus in all groups that were administered alloxan. Alloxan result in a significant increase (p ≤ 0.05) in the glucose levels of treated rats compared with normal control. Skimmed milk Kefir received rats exhibit significant reduction in level of blood glucose, same that caused by Gliben clamide (reference drug) in compare with untreated diabetic rats. However, these reductions don’t reach to glucose value in normal control group at Day 20 and at Day 40 of experiment.

On the other hand, Tables 1 and 2 also revealed the effect of skimmed milk kefir and Gliben clamide in lipid profile in diabetic’s male rats at 20th day and sacrificing day. Significant increase in the serum concentration of total cholesterol is showed by diabetic rats which also exhibited significant increase in triglycerides and low density lipoproteins, while there was significant decrease in the high density lipoproteins concentration when compared with normal rats and those rats with diabetes that have been treated. In diabetic groups treated with skimmed milk kefir, all above mentioned parameters were significantly decreased except high density lipoproteins concentration was increased in pattern resemble to that recorded in Gliben clamide group.
The synthesis of proinsulin and its cts were found to be decrease lactobacillus acidophilus were also. Lactobacillus casei was found to have a hypoglycemic activity. Milk kefir contain of yeast and bacteria that have anti diabetic and antihyperlipidemic effect in induced diabetes. Different Capital letters refer to presence significant difference at (p≤0.05) between groups.

Table 1: Show effect of skimmed milk kefir on lipid profile in diabetic rat at Day 20 of experiment (Means ± Standard error).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>TC. mg/dl.</th>
<th>TG. mg/dl.</th>
<th>HDL. mg/dl.</th>
<th>LDL. mg/dl.</th>
<th>VLDL. mg/dl.</th>
<th>Glucose g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Control )</td>
<td></td>
<td>72.20 ± 2.11</td>
<td>56.60 ± 1.76</td>
<td>44.08 ± 1.48</td>
<td>16.81 ± 3.02</td>
<td>11.31 ± 0.28</td>
<td>97.58 ± 2.14</td>
</tr>
<tr>
<td>Group 2 (DM)</td>
<td></td>
<td>101.45 ± 2.73</td>
<td>102.21 ± 3.67</td>
<td>34.71 ± 2.47</td>
<td>46.33 ± 3.45</td>
<td>20.44 ± 1.09</td>
<td>256.89 ± 25.89</td>
</tr>
<tr>
<td>Group3(induced DM + Kefir)</td>
<td></td>
<td>83.88 ± 2.39</td>
<td>62.47 ± 2.45</td>
<td>44.52 ± 2.55</td>
<td>26.85 ± 2.11</td>
<td>12.50 ± 1.05</td>
<td>190.69 ± 13.8</td>
</tr>
<tr>
<td>Group 4(DM + Gliben clamide)</td>
<td></td>
<td>86.56 ± 2.97</td>
<td>60.19 ±2.76</td>
<td>38.48 ±1.72</td>
<td>36.05 ±3.76</td>
<td>12.04 ±0.32</td>
<td>188.30 ±21.62</td>
</tr>
</tbody>
</table>

Different Capital letters refer to presence significant difference at (p≤0.05) between groups.

Table 2: Show effect of skimmed milk kefir on glucose and lipid profile in diabetic rat at Day 40 of experiment (Means ± Standard error).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>TC. mg/dl.</th>
<th>TG. mg/dl.</th>
<th>HDL. mg/dl.</th>
<th>LDL. mg/dl.</th>
<th>VLDL. mg/dl.</th>
<th>Glucose g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Control )</td>
<td></td>
<td>76.21 ± 1.94</td>
<td>51.70 ± 1.76</td>
<td>46.65 ± 1.38</td>
<td>16.23 ± 2.62</td>
<td>12.17 ± 0.19</td>
<td>78.23 ± 1.04</td>
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<tr>
<td>Group 2 (DM)</td>
<td></td>
<td>104.23 ± 3.09</td>
<td>106.21 ± 3.11</td>
<td>33.51 ± 2.07</td>
<td>43.03 ± 2.95</td>
<td>21.42 ± 1.31</td>
<td>423.12 ± 39.13</td>
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<tr>
<td>Group3(induced DM + Kefir)</td>
<td></td>
<td>84.81 ± 2.19</td>
<td>65.01 ±3.75</td>
<td>45.53 ±2.98</td>
<td>28.05 ±1.91</td>
<td>11.59 ±1.19</td>
<td>182.84 ±15.02</td>
</tr>
<tr>
<td>Group 4(DM + Gliben clamide)</td>
<td></td>
<td>82.52 ± 2.22</td>
<td>61.49 ±3.06</td>
<td>40.18 ±1.92</td>
<td>32.25 ±2.76</td>
<td>12.24 ±0.42</td>
<td>184.32 ±31.42</td>
</tr>
</tbody>
</table>

Different Capital letters refer to presence significant difference at (p≤0.05) between groups.

Discussion
The basic mechanism that caused increase glucose level in diabetes mellitus includes the high glucose production due to rise rate glycogenolysis and gluconeogenesis in liver and or reduction tissues glucose utilization. Mechanism of action of alloxan which occur in the beta cells is the effect of reactive oxygen system on the DNA as well as a fragmentation of DNA due to exposure to alloxan material which causes DNA damage, which stimulates poly ADP-riboseylation, which considered a process take part in DNA repair. Antioxidants like superoxide dismutase, catalase and the non-enzymatic scavengers of hydroxyl radicals have been found to protect against alloxan toxicity. Decrease of blood glucose level in diabetic rats treated with skimmed milk kefir may be resulted from skimmed milk kefir contain of yeast and bacteria that have hypoglycemic activity. Lactobacillus acidophilus and Lactococcus casei were significantly limited the rate of glucose intolerance, hyperglycemia, hyperinsulinemia and dyslipidemia onset. Abnormal lipid metabolism due to insulin deficiency in the body caused by the damage to pancreatic beta cells in diabetic rat. Insulin has ability to activate lipoprotein lipase therefore decrease of activity of these enzyme lead to increases the levels of lipoprotein in the blood. An increase in plasma cholesterol, triglycerides, LDL and VLDL and decreases in HDL, were previously reported related to increase of glucose level in induced diabetes.

Diabetes mellitus one of the many diseases that characterized by prolonged elevation concentration of VLDL, IDL, chylomicron remnants, or LDL occur in the blood is often coincidence with premature or more severe atherosclerosis. HDL2 concentrations had an inverse correlation with coronary heart disease. Rats with induced diabetes by streptozotocin were also showed elevation in cholesterol and triglyceride levels in the blood. The synthesis of proinsulin and its conversion to insulin by increased cell mass and insulin sensitivity via reduced the inflammatory therapy on beta cells of pancreas. The onset of glucose intolerance, hyperglycemia, hyperinsulinemia, dyslipidemia, and oxidative stress were significantly delayed in rats with induced diabetes mellitus when fed with the probiotic dahi-supplemented diet Hariom(2007) indicating reduced risk of diabetes and its complications. Skimmed milk Kefir consumption modulates significantly blood glucose, SOD, Catalase and GPx, immune response, peroxidation lipids (MDA), and function of β-cell in pancreas.

Conclusions
In this study and depend on the results it is possible to observe that skimmed milk kefir products has antidiabetic and antihyperlipidemic effect in induced diabetic rats which showed elevation in serum glucose, hyperlipidemia and decrease in HDL. Consumption of skimmed milk kefir products in 20 and 40 days during the period of study was very useful. The beneficial effects of Kefir products were found to be decrease.
serum glucose and other lipid picture but there is increase in HDL. Therefore skimmed milk kefir products are considered to be a useful bioactive natural food which have a hypolipidemic and hypoglycemic effect, in addition to the usefulness of its microorganism in gastrointestinal tract, it is also benefit for patients whether human or animals like pet animals (dog and cats) suffering diseases or the patients with diabetes who need safe and useful food products to fight the dangerous effects associated with the disease.

REFERENCES