Evaluation of Anti-Hyperglycemic and Anti-Hyperlipidemic Activities of Water Kefir as Probiotic on Streptozotocin-Induced Diabetic Wistar Rats

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Abstract

Diabetes mellitus is a predominant chronic disease which causes mortality of millions of people yearly. Its prevalence is on the rise worldwide. Water kefir is fermented food produced by a matrix of polysaccharides containing bacteria and yeasts, with therapeutic properties. Our study aimed to evaluate anti-hyperglycemic and anti-hyperlipidemic activities of water kefir on streptozotocin-induced diabetic Wistar rats. Adult Wistar rats were made diabetic by intraperitoneal injection of streptozotocin, and were given or not kefir in drinking water for 5 weeks. Body weight, glucose and lipid levels were measured. The results demonstrated evident improvement in body weight, glucose, and lipid profiles of treated rats comparing with diabetic or control rats. Water kefir is found to be less cost hypoglycemic and hypolipidimic treatment and less time consuming. Water kefir can potentially be useful food for diabetes to control glucose and lipid levels.

Keywords
Component, Formatting, Style, Styling, Anti-Hyperglycemic, Anti-Hyperlipidaemic,
1. Introduction

Diabetes mellitus is a chronic, hereditary disease characterized by an abnormally elevated level of blood glucose (hyperglycemia) and by the excretion of the excess of glucose in the urine (glycosuria). The basic defect appears to be an absolute or relative lack of insulin or decrease in insulin receptors on the membrane of the target cells, which lead to abnormalities in carbohydrate metabolism as well as in lipid and protein metabolism [1].

Diabetes mellitus, a serious chronic metabolic disorder, is identified by hyperglycemia resulting from deficiency in insulin secretion and/or decreased reaction of the organs to insulin [2][3].

Diabetes mellitus has been and will probably continue to be classified as growth or juvenile onset and maturity or adult onset. The National Diabetes Data Group (NDDG) and the 1980, 1985 Expert Committees of WHO have proposed a classification without regard to age. The two primary types are insulin-dependent, ketosis prone diabetes mellitus (IDDM) or Type 1, and non-insulin-dependent, non-ketosis prone diabetes mellitus (NIDDM) or Type 2. In both the 1980 and 1985 reports, other classes of diabetes included other types [2] [4]. The cause for type 1 diabetes is an absolute deficiency of insulin secretion, whereas type 2 diabetes is a combination of resistance to insulin action and inadequate compensatory insulin secretory response.

The world prevalence of diabetes among adults (aged 20 - 79 years) was 6.4%, affecting 285 million adults, in 2010, and will increase to 7.7%, and 439 million adults by 2030 [5].

Epidemiological studies have suggested that dyslipidaemia is a risk factor for diabetic neuropathy [6]. In diabetic subjects overproduction of FFA and impaired lipoprotein metabolism induces an increase in plasma lipid components [7]. Type 1 and Type 2 diabetes mellitus are associated with metabolic syndrome and a marked increase in the risk of coronary heart disease [8]. Adipose tissue is now recognized as an endocrine organ that contributes to the physiopathology of type 2 diabetes [9]. Diabetes mellitus has also been associated with an increased risk for developing premature atherosclerosis due to an increase in triglycerides (TG) and low-density lipoproteins (LDL), and decrease in high density lipoprotein levels (HDL) [10].

Abnormalities of lipoprotein metabolism cause various hypo- or hyperlipoproteinemias. The most common of these is diabetes mellitus, where insulin deficiency causes excessive mobilization of FFA and underutilization of chylomicrons and VLDL, leading to hypertriacylglycerolemia. Most other pathologic conditions affecting lipid transport are due primarily to inherited defects, some of which cause hypercholesterolemia, and premature atherosclerosis. Obesity particularly abdominal obesity is a risk factor for increased mortality, hypertension, type 2 diabetes mellitus, hyperlipidemia, hyperglycemia, and various endocrine dysfunctions [11]. In addition to the established major risk factors, atherosclerosis in type 2 diabetes is also related to alterations in lipid and lipoprotein profile [12]. Many epidemiological studies have demonstrated that type 2 diabetes mellitus is a well-known risk factor for the development of cardiovascular disease, cerebrovascular disease, and peripheral vascular diseases [13]-[18].

It is projected that emerging economies India and China alone will lose around US $0.5 trillion and $0.25 trillion, respectively, as a result of these chronic diseases in the next decade [19].

A major focus of current anti-diabetic research is the development of anti-hyperglycemic agents that are safe and free of negative side-effects.

Recently an increasing number of natural compounds are extracted from animal and plant sources with anti-diabetic properties [20] [21]. Functional foods and nutraceuticals have become prescribed widely to prevent the occurrence of diabetes mellitus, and to attenuate the complications of hyperglycemia in diabetic patients, because of their effectiveness, limited side effects, and relatively low cost.

Fermented food, their components, and their microorganisms have been described to have hypoglycemic effects. Kefir can potentially be a useful food choice for patients with diabetes who are required to control their blood glucose levels [22]. The results of Maeda et al., [23] showed that kefiran had hypoglycemic effects in KKAY mice. The daily administration of viable Lactobacillus rhamnosus GG cells decreased blood glucose levels in a genetic type 2 diabetes model, KK-Ay mice [24]. Anti-diabetic effects of lactic acid bacteria on KK-Ay mice have been reported for Lactobacillus casei [25], and dahi containing L. acidophilus NCDC14 and L. casei NCDC19 on high fructose induced diabetic rats [26], and for dahi product on streptozotocin induced diabetes in
Lactobacillus, Bifidobacterium and Streptococcus strains improve insulin resistance wild-type male C57BL6 mice [28]. Lim et al. [29] concluded that the extract from solid fermented materials stimulates blood glucose absorption into muscle cells, and the PI3kinase/Akt protein pathway is involved in signal transmission. Consumption of Nono (Nigerian fermented milk) produced by wild lactic acid bacteria may be helpful in diabetes management [30]. Recent findings indicate that specific strains of lactic acid bacterium can be expected to be beneficial for the management of type 2 diabetes [31].

Kefir defined as a refreshing fermented milk beverage, a viscous pourable liquid, with a smooth, slightly foamy body and whitish color. It is yeasty, acidic, mildly alcoholic, refreshing, slightly effervescent, and believed to contain many functional substances [32]-[34]. It is a beverage produced by the action of lactic acid bacterium (LAB) (lactobacilli, lactococci, leuconostocs), yeasts, and acetic acid bacteria (acetobacteria) on milk [35] [36].

Recently kefir can be made from other media of fermentation as Carbohydrate solutions [37] [38], Cheese whey, and a lactose-rich waste of negligible cost [39]. This kefir which is based on carbohydrates is called sugary kefir or water kefir.

Water kefir is hazy and gluey beverage with a smooth, streamlined, fizzing texture and blond to yellowish color, it is yeasty, acidic, mildly alcoholic and refreshing taste with feeble sweetness. It is home-produced drink made by adding of kefir grain to sugar solution in water and incubating this mixture at 20°C - 25°C for at least 12 h, and then separation of kefir grain to other production. Pieces of fresh or dried fruit can be added for flavor and removed in the end of fermentation period.

Both Water kefir and milk kefir and their grains contain the same common groups of microorganisms (lactic acid, acetic acid bacteria, and yeasts) [40]-[48].

Water kefir, because of its microorganisms (lactic acid, acetic acid and yeasts), and its important molecules such as polypeptide, polysaccharide, organic acid, and other compounds, can provide benefit microorganisms and bioactive molecules, Therefore, water kefir may play an important role in health improving and maintenance. But, to date, no study has investigated the bioactivities of the fermented metabolites of water kefir. The present study therefore, was designed to evaluate anti-hyperglycemic and anti-hyperlipidemic activities of water kefir on streptozotocin-induced diabetic wistar rats.

2. Materials and Methods

2.1. Chemicals

Streptozotocin (STZ) and all other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade, and deionized and distilled water was used throughout.

2.2. Water Kefir Preparation

Water kefir grains that obtained from laboratory of YALACTA (France), were washed with distilled water, and inoculated (5% (w/v)) in sugar solution (6.5% w/v) with mineral drinking water. 5 g/l of fresh apple pieces (purchased from the local market of Tlemcen city -Algeria) were added. The mixture was placed in glass bottle with plastic cover (not closed completely), and incubated at 21°C for 24 h. It was stirred and mixed in intervals of 5 h. Apples pieces were deducted and kefir grains were sieving after fermentation period by filtration through a plastic sieve and washed to be used in other process. Water kefir drink was stored at 4°C for 24 h, and different dilutions were prepared to be used in the same day.

2.3. Animals and Diets

All aspects of the experiment were conducted according to the guidelines provided by the ethical committee of the experimental animal care at Tlemcen University. Adult Wistar rats, weighing 200 to 250 g, were obtained from Pasteur institute (Algeria) and were housed at 20°C individually in polypropylene cages and maintained on a 12:12-h light/dark cycle in the animal husbandry, Department of Biology, Faculty of life and Natural Sciences, ABBT University, ALGERIA. They were fed a commercial chow manufactured by the O.N.A.B (Food National office of Cattle, Remchi, Tlemcen). ONAB diet is composed of corn oil, cakes soya bean, a complex minerals and vitamins. The rats were allowed to free access to drinking water ad libitum. Before the study, rats were fed
with AIN-93 Standard diet [49] as presented in Table 1 for two weeks in order to adapt to diet.

2.4. Induction of Diabetes

STZ was dissolved in cold 0.01 M citrate buffer, pH 4.5 and always prepared freshly for immediate use within 5 minutes. Rats were fasted for overnight to induce diabetes by intraperitoneal (ip) injection of streptozotocin (STZ) at the dose of 60 mg/kg body weight. The normal control group was given citrate buffer without STZ. Animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. The development of diabetes was confirmed after 48 hours of STZ injection. The animals with fasting blood glucose level more than 200 mg/dl were considered as diabetic and included in this study. Body weight and glycemia were measured weakly. For glucose assay, blood samples were collected from the tail-tip of the rats.

2.5. Experiment Design and Treatment

After one week of diabetes induction, rats were divided into four groups. Each group consisted of six rats. All groups fed with standard diet a long of experiment period and received water kefir with drinking water as follows: Group 1 served as Normal Control (NC) received standard diet and only drinking water. Group 2: Diabetics (D) received standard diet and drinking water, Group 3 - 5 (10% WK; 20% WK and 30% WK) received standard diet and 10%, 20%, and 30% of water kefir in drinking water respectively. The period of treatment was 35 days. Animals allowed to access to food and water or water kefir ad libitum.

At the end of the experiment and after overnight fasting, rats from each group were anaesthetized with intraperitoneal injection of sodium pentobarbital (60 mg/kg of body weight). The blood was drawn from the abdominal aorta, and serum was used for glucose and lipid profiles determinations.

2.6. Analytical Measurements

Blood glucose levels were measured by the glucose-oxidase method using an Accu-chek blood glucose meter. Total cholesterol (TC), Triglyceride (TG), high-density lipoprotein (HDL)-cholesterol levels were measured in serum samples by using enzymatic method kits (Roche Diagnostics). The lipoprotein cholesterol sub-fractions in the serum, HDL, LDL, VLDL were estimated by precipitation with sodium phospotungstate-magnesium chloride and sodium dodecyle sulphate reagents.

2.7. Statistical Analysis

Statistical analysis of data was performed using SAS software version 9.1 [50]. Significance of differences were carried out by the analysis of variance (ANOVA) general linear models-Univariate method followed by Post Hoc Multiple Tukey Tests at p < 0.05. Results are represented as mean with standard errors of mean (mean ± SEM).

3. Results

3.1. The Effect of Water Kefir on the Body Weight of Rats

The effect of water kefir on the body weight of normal and STZ-induced diabetic Wistar rats, recorded for 35 days are shown in Figure 1. Body weight of normal control rats was increased during the period of experiment, on the contrary, with diabetic group which showed decreasing in body weight from the second week of experiment. The body weight of other groups treated with water kefir was ascended. The body weight of the rats treated with 10% of water kefir was ascending similarly with that of normal control, and 30% WK and 20% WK groups take the third and fourth order in body weight increasing. There were high significant differences between groups of treatment at (p ≤ 0.05), the multiple comparisons of treatment groups showed that diabetic control group was differed significantly at (p ≤ 0.05) from all of other groups. Although the changes of body weight of normal and diabetic rats during the time of experiment, there was no significant difference (p ≤ 0.05) between body weight of rats respects with the time of study. But by host hoc tests, a significant difference (p ≤ 0.05) appeared between body weight in the first day of study and that in sacrifice day.

3.2. Anti-Hyperglycemic Activity of Water Kefir

Anti-hyperglycemic effects of water kefir on normal control and STZ-induced diabetic rats which were measured
Table 1. Composition of the Standard diet.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>g/kg Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein (&gt;90% protein)</td>
<td>200</td>
</tr>
<tr>
<td>Corn starch</td>
<td>400</td>
</tr>
<tr>
<td>Sucrose</td>
<td>232</td>
</tr>
<tr>
<td>Lipid</td>
<td>70</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
</tr>
<tr>
<td>Mineral mix (AIN-93G-MX)</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mix (AIN-93-VX)</td>
<td>10</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3</td>
</tr>
</tbody>
</table>

Figure 1. The effect of Water kefir on body weight of normal and STZ-induced Wistar Rats. Values are means ± SEM.

every week over the entire experimental time are reported in Figure 2. Fasting blood glucose levels of diabetic control (DC) group were increased after the induction by streptozotocin and remained over 400 g/dl up to the end of study. Blood glucose levels of diabetic rats groups which gave water kefir instead of drinking water were found to be decreased, the initial glucose concentration was 401 g/dl, 411 g/dl, and 405 g/dl in 10% WK, 20% WK, and 30% WK group respectively. After one week of treatment, blood glucose concentration was reduced by 12% in 10% WK group, 18.4% in 20% WK group, and 25% in 20% WK group, and at the end of experiment blood glucose concentration was diminished by 69% in 10% WK group, 71% in 20% WK group, and 63% in 20% WK group from the initial concentrations.

Streptozotocin caused a significant increase in the glucose levels of experimental animals compared with normal control (p ≤ 0.05). The reduction of blood glucose was high significantly (p ≤ 0.05) in all water kefir received groups comparing with normal and diabetic control rats, but there were no significant differences between water kefir groups at (p ≤ 0.05). Through the time of experiment, a great significant reducing (p ≤ 0.05) were recorded in glucose levels of rats between the first day and each of the 14th, 21st, 28th, and sacrifice day; and between the 7th day and both of the 28th and sacrifice day.

3.3. Anti-Hyperlipidemic Activity of Water Kefir

Figure 3 demonstrate the effect of water kefir in lipid profile in diabetics and normal wistar rats at the end of experiment. Total cholesterol (TC), Triglycerides (TG), low density lipoproteins, and very low density lipoproteins (VLDL) were found at high concentrations in diabetic rats, while the High density lipoproteins (HDL) in this group was the lowest concentration. In diabetic groups treated with 10%, 20%, and 30% of water kefir, all lipid profiles were outstandingly decreased in comparing with diabetics control group. Statistically, there were significant differences at (p ≤ 0.05) in all the results of lipid markers between the rat groups of experiment. From Post Hoc Tests results, the decreasing of TC in the normal control and treated groups are statistically
Figure 2. Anti-hyperglycemic effects of water kefir on STZ-induced diabetics rats. Values are means ± SEM.

Figure 3. Anti-hyperlipidemic activities of Water kefir on Streptozotocin-induced diabetics Wistar Rats. Values are means ± SEM, * Statistically significant when compared to diabetics group at p ≤ 0.05.

Significant, the group which consumed 20% Water Kefir have a higher significance at (p ≤ 0.05). The significant difference in TG presented only between diabetics and both of 20%WK and normal control groups. LDL were significantly reduced in all groups comparing with diabetics control with a preference of 30%WK and diabetics groups. On the other hand, HDL concentrations were significantly increased for all groups in opposing of diabetics group at (p ≤ 0.05), and only 20% WK group was differ significantly from diabetics in VLDL (p ≤ 0.05).

4. Discussion

The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves the over production of glucose (excessive hepatic glycogenolysis and gluconeogenesis) and or decreased utilization of glucose by the tissues [51].

Streptozotocin (STZ; N-nitro derivative of glucosamine) is a naturally occurring, broad spectrum antibiotic and cytotoxic chemical that is particularly toxic to the pancreatic, insulin producing beta cells in mammals [52] [53]. STZ-induced diabetes is characterized by severe loss in body weight and this reduction is due to loss or degeneration of structural proteins, as the structural proteins are known a major contributor to body weight [54] [55]. Previous reports show that protein synthesis is decreased in all tissues due to decreased production of ATP
and absolute or relative deficiency of insulin [56].

This symptom was clear in our results which reveal significant reduction in body weight of diabetics rats comparing that of normal control. These results are in correspondence with the results of Judiono, et al., [57] which showed that the delta animal weight varied among the groups, except for the positive groups they gained with very small achievement bout 4.01 ± 16.82 g. The result of the present study displayed improving in body weight by administration of water kefir, that may be referred to the presence of exopolysaccharides produced in kefir by its microorganisms, this effect of exopolysaccharides was reported in other studies [58] [59].

Diseases in which prolonged elevated levels of VLDL, IDL, chylomicron remnants, or LDL occur in the blood (e.g., diabetes mellitus, lipid nephrosis, hypothyroidism, and other conditions of hyperlipidemia) are often accompanied by premature or more severe atherosclerosis. There is also an inverse relationship between HDL (HDL2) concentrations and coronary heart disease, and some consider that the most predictive relationship is the LDL: HDL cholesterol ratio [60]. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in streptozotocin diabetes rats [61] [62].

Previous research suggests the reduction of inflammatory therapy on β-cells in pancreas contributed the synthesis of proinsulin to insulin by increased cell mass and insulin sensitivity [63]. Hariom et al. [64] indicated that, the probiotic dahi-supplemented diet significantly delayed the onset of glucose intolerance, hyperglycemia, hyperinsulinemia, dyslipidemia, and oxidative stress in high fructose-induced diabetics rats, indicating a lower risk of diabetes and its complications. Kefir consumption modulate significantly blood glucose, antioxidants (SOD, Catalase, GPx), peroxidation lipids (MDA), immune response (cytokines IL1, IL6, IL10) and pancreatic β-cell function [57]. Our investigate disclosed that the regular administration of water kefir can expressively lower blood glucose concentration, because of water kefir exopolysaccharides and water kefir contents of yeast and bacteria, that previously proven to have hypoglycemic activities [24] [65]. These results were in agreement with [26] who found that, Lactobacillus acidophilus and Lactobacillus casei significantly delayed the onset of glucose intolerance, hyperglycemia, hyperinsulinemia and dyslipidemia.

Previous study reported that, in STZ-induced diabetes, the increase in blood glucose levels is usually accompanied by an increase in plasma cholesterol, triglycerides, LDL and VLDL and decreases in HDL [66]. The results of El Khamisy [67] indicated that, supplementation with Bifidobacterium and Lactobacillus acidophilus alone and in combination significantly decreased the mean value of serum glucose, total cholesterol, triglycerides, LDL-C and VLDL-C and significantly increased HDL-C and insulin secretion as compared to control groups.

In this study, we have also observed an increasing in the concentration of TC, TG, LDL, and VLDL and in the same time decreasing of HDL concentration in streptozotocin-induced diabetic rats. Total cholesterol, triglycerides, LDL, and VLDL of the streptozotocin-induced diabetes rats treated with water kefir (10% - 30%) showed significant reduction, and improve the level of HDL cholesterol as compared to diabetic rats.

5. Conclusions

In conclusion, based on the results of the present study it can be suggested that water kefir has antidiabetic and antihyperlipidemic properties in animal model. Consumption of water kefir in (10% - 30%) concentrations for 5 weeks has shown beneficial effect not only on blood glucose but also on body weight and lipid profiles of streptozotocin-induced diabetic rats.

Water kefir was found to be inexpensive product with hypoglycemic and hypolipidemic effects even when consuming for short time. Therefore water kefir can potentially be a useful functional food choice for patients with diabetes who are required to control their blood glucose levels, and also for diminishing the risks of cardio-vascular disease.

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References

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