



Effect of Probiotic Fermented Milk (Kefir) on Some Blood Biochemical Parameters Among Newly Diagnosed Type 2 Diabetic Adult Males in Gaza Governorate

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Abstract

Kefir is natural probiotic milk. It is a complex mixture of bacteria, yeasts, many vitamins, minerals, amino acids, and enzymes. Previous studies reported that Kefir has a hypoglycemic effect, and it may have an antitumor effect, intestinal immunity, antimicrobial effect and regulate cholesterol. The aim of this study was to evaluate the effect of kefir intake on some blood biochemical parameter among newly diagnosed type 2 diabetic adult male patients in Gaza Government. It was a case-control study experiment and was carried out on the 42 newly diagnosed diabetic male patients aged from 37-65 years. They were divided into two groups (control and case). The control group received Metformin only. The case group are patients who intake a cup of Kefir daily with a Metformin. Blood collection sample for biochemical analysis was carried out at the beginning of the study and after 10 weeks. The results of the study showed that there were no statistically significant differences in fasting blood sugar (FBS) at the beginning of the study between the two groups ($P=0.22$). After an intervention with Kefir milk, fasting blood glucose was significantly decreased among patients taking Kefir milk ($P < 0.05$). After the intervention, glycohemoglobin (HbA1c) was reduced significantly ($P=0.001$). Also, there was an increase in calcium concentration correlated with a decrease in phosphorus and no significant differences in Kidney functions except uric acid. There was an improvement in cholesterol and triglyceride (TG) only in lipid profile. On conclusion, kefir has potential effect in the reduction of FBS, HbA1c and phosphorous with increase in calcium among diabetic adult males.



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Introduction

Kefir is a natural probiotic dairy product, produced by the metabolic activity of bacteria and yeasts, reputed to have a variety of health benefits for the consumers.¹ The grains of Kefir consists of a mix of complex microflora like yeast, lactic acid bacteria (LAB) and may be in sometimes acetic acid bacteria found that is lodged by a complex carbohydrate matrix named "kefiran".²

Inoculating Kefir grains to goat, cow, or sheep's milk produced the Kefir milk. The traditional way to produce Kefir was to put the milk and Kefir grains in skin bags that were hung near a doorway; because anyone passing through the doorway could be knocked the bag, this is to keep the kefir grains and milk well mixed.³

Kefir is a viscous, acidic and slightly carbonated dairy beverage that consists of minimal quantities of alcohol.⁴

Kefir grains produced Kefir milk traditionally, the characteristics of these grains are small, gelatinous, yellowish in color, and they are similar to cauliflower in shape and irregularity, they look like small clumps that measure 1-3 cm in length.⁵ By daily transferring Kefir grains into fresh milk and making allowance for them to grow approximately for 20 hours, grains are still viable.⁴

The way of producing Kefir relies on the synergistic reaction of the microflora that are found in Kefir grains. A variety of components made by the yeasts and bacteria in Kefir grains during the fermentation process, these components give the Kefir its texture and unique taste. After the fermentation process, the final product of Kefir consists of many components that are evident to be bioactive.⁴

Kefir has many minerals, vitamins, enzymes and amino acids especially phosphorous, calcium, magnesium, vitamin A, B₂, B₁₂, vitamin K, folic acid and vitamin D. Kefir contain tryptophan which is one of the necessary amino acids, which is well used for resting effect on the central nervous system.³

The products of kefir have a characteristic flavor, which is a result of a complex reaction between compounds that formed through the metabolic

activity of the yeast culture and the applied bacterial culture and the milk matrix. Kefir exemplarity contains both CO₂ and ethanol. A sparkling sensation and refreshing of Kefir product refer to the content of CO₂ and ethanol. The yeast strains that found in Kefir grains give to the Kefir milk its perfect flavor in particular.⁶

Diabetes treatment is based on pharmacological hypoglycemic agents and insulin; however, the efficacy of these therapies is limited due to their many side effects. Therefore, finding natural compounds is essential for overcoming these problems.⁷

Kefir milk has been considered a probiotic because it has anti-inflammatory and antioxidant characteristic.⁸ Kefir supplementation has been identified to potentially reduce hyperglycemia. The underlying mechanism is probably via its bioactive components such as: exopolysaccharide, peptide, antioxidants and through immunomodulatory properties.^{9,10,11} The aim of this study was to evaluate the effect of Kefir intake on some blood biochemical parameters among newly diagnosed type 2 diabetic adult males in Gaza Governorate.

Materials and Method

Materials

Chemicals

All chemicals and kits that were used in the determination of blood biochemical parameters of this study were purchased from Quimica Clinical Aplicada S.A, Spain. The kits for glucose, urea, creatinine, uric acid, cholesterol, TG, high density lipoprotein (HDL), calcium and phosphorus were used.

Equipment

EDTA blood collection tube, test tubes and plain tube, 5ml syringes and needles were used to collect the blood samples. Rayto Spectrophotometer (RT 9200) and cuvettes were used in this work for analysis the samples. Clover system used to measure HbA1c. The bench top centrifuge was used to separate the samples. Micropipettes with disposable plastic tips, vortex mixer and water bath at 37 °C were used.

Kefir Grains

Kefir grains were obtained from Mr. Abo Mustafa Zain Edeen from Ezawya market, in Gaza city.

Methods

Study Design

The present study was a case-control study.

Study Samples

The study sample included 42 newly diagnosed diabetic male patients who are between the ages of 37-65 years. They are divided into two groups, the first group has 21 patients was a control who were given Metformin only (Metformin, marketed under the trade name Glucophage used as the first-line medication for the treatment of type 2 diabetes). The case group has 21 patients were given daily one cup kefir milk (250 ml) with Metformin for ten weeks. The patients were taught how to produce kefir milk as mentioned in the next section and regularly followed by the research team.

Blood Sample Collection

The blood samples were collected from the patients for biochemical analysis of FBS, HbA1c, TG, HDL, low density lipoprotein (LDL), urea, creatinine, uric acid, Calcium and phosphorus at the beginning of the study and after 10 weeks. All these biochemical tests were carried out using ready kits as mentioned above.

Kefir Production

Kefir production was achieved by adding kefir grains directly to warm pasteurized milk. After a period of fermentation, 18-24 hours at room temperature, the grains filtered with a sieve separated from the milk, then these grains are reused in the next inoculation.¹²

Ethical Consideration

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Local Ethics Research Committee. All patients (volunteers) provided written informed consent prior to the study.

Data Analysis

All obtained data were analyzed by paired and independent sample T-test sample using SPSS (Version 20) system. Difference between variables will be considered statistically significant if p-value ≤ 0.05 .

Results

Biochemical Parameters of Case Group Before and After Kefir Intake

The present study is a case control included 42 males (41 cases patients and 21 controls). Both

Table 1: Statistical analysis of blood biochemical parameters of case group before and after Kefir milk intake

Parameters	Cases (n=21)		P-value
	Before (Mean \pm SD)	After (Mean \pm SD)	
FBS (mg/dl)	112.5 (\pm 45.05)	91.7 (\pm 12.79)	0.045*
HbA1c (%)	8.54 (\pm 1.56)	7.20 (\pm 1.12)	0.000*
Urea (mg/dl)	30.85 (\pm 6.46)	33.89 (\pm 6.03)	0.092
Creatinine (mg/ml)	0.75 (\pm 0.16)	0.77 (\pm 0.11)	0.489
Uric acid (mg/dl)	4.02 (\pm 1.31)	3.50 (\pm 1.07)	0.038*
Cholesterol (mg/dl)	160.66 (\pm 21.98)	150.66 (\pm 28.43)	0.107
TG (mg/dl)	92.76 (\pm 41.90)	88.23 (\pm 28.18)	0.367
HDL (mg/dl)	67.07 (\pm 11.87)	66.38 (\pm 12.48)	0.706
LDL (mg/dl)	75.55 (\pm 19.09)	66.58 (\pm 25.47)	0.114
Phosphorus (mg/dl)	3.44 (\pm 0.44)	3.36 (\pm 0.44)	0.410
Calcium (mg/dl)	8.15(\pm 0.73)	8.45 (\pm 0.58)	0.000*

* Significantly different (P ≤ 0.05).

case and control were newly diagnosed T2DM and matched for baseline characteristics such as gender, age, BMI (body mass index), Diet, duration and family history of T2DM.

FBG and HbA1c

The Table (1) shows that for FBS the p-value was 0.045. This means a significant difference resulted between before and after taking Kefir. The Table also showed that there was a highly significant decrease in HbA1c ($p=0.000$) after the intake of Kefir.

Kidney Function Test

As shown in Table (1) the p-value for urea and creatinine were 0.092 and 0.489, respectively which are greater than 0.05. That means there was no significant difference before and after taking Kefir. It also shows that the p-value for uric acid equal 0.038 which means there was a significant difference in uric acid concentration.

Lipid Profile

As shown in Table (1) there was an improvement in lipid profile parameters except LDL but there was no significant difference among these parameters.

Phosphorous and Calcium

The result in Table (1) showed that there was a decrease in phosphorus concentration but there

was no significant difference, but there was a highly significant difference before and after taking Kefir for calcium concentration.

Biochemical Parameters of Case and Control Groups at the End of the Study

FBG and HbA1c

Table (2) shows that the p-value for both FBS and HbA1c equal 0.000 which means there was a highly significant difference between the case group and control group for both FBS and HbA1c.

Kidney Function Test

As shown in Table (2) there was no significant difference between the case group and control group in urea, creatinine and uric acid, although there was a clear decrease in uric acid concentration and little decrease in urea concentration in case group.

Lipid Profile

Although there was an improvement in cholesterol and TG concentration in case group, there was no significance difference in any of lipid parameters between case and control groups (Table 2).

Phosphorous and Calcium

As shown in Table (2) there was an increase in calcium concentration in case group compared with control group but not significant difference. For

Table 2: Statistical analysis of blood biochemical parameters of control and case groups at the end of the study

Parameters	At the end		P-value
	Control (n=21) Mean(\pm SD)	Case (n=21) Mean(\pm SD)	
FBS (mg/dl)	145.47 (\pm 45.63)	91.7 (\pm 12.79)	0.000*
HbA1c (%)	9.14 (\pm 1.71)	7.20 (\pm 1.12)	0.000*
Urea (mg/dl)	35.47 (\pm 9.95)	33.89 (\pm 6.03)	0.529
Creatinine (mg/ml)	0.766 (\pm 0.187)	0.776 (\pm 0.11)	0.815
Uric acid (mg/dl)	4.17 (\pm 1.43)	3.50 (\pm 1.07)	0.164
Cholesterol (mg/dl)	165.04 (\pm 26.79)	150.66 (\pm 28.43)	0.109
TG (mg/dl)	111.52 (\pm 52.73)	88.23 (\pm 28.18)	0.104
HDL (mg/dl)	66.51 (\pm 12.76)	66.38 (\pm 12.48)	0.969
LDL (mg/dl)	66.47 (\pm 32.45)	66.58 (\pm 25.47)	0.969
Phosphorus (mg/dl)	3.86 (\pm 0.751)	3.36 (\pm 0.44)	0.014*
Calcium (mg/dl)	7.66 (\pm 1.769)	8.45 (\pm 0.58)	0.073

* Significantly different from control ($P \leq 0.05$).

phosphorus concentration, there was a significant decrease in case group compared with control.

Discussion

Management of diabetes without any side effects by natural food is a challenge for medical nutrition therapy of diabetes. The present study is a case-control investigation, comprised of 42 newly diagnosed diabetic patients males aged 37–65 years. The 42 patients divided into two groups, 21 patients in each group. The control group received Metformin only and the case group were given one cup of kefir milk daily with Metformin for ten weeks.

Biochemical Measurement

FBG and HbA1c

In this study, the probiotic fermented milk consumption causes significant decline of fasting blood glucose and HbA1C in comparison with the control group. Antidiabetic effect of *Lactobacillus* and *Bifidobacteria* has been investigated in several animal and human studies.^{7,13,14,15} Many possible mechanisms of this effect are expressed. A possible confirmation of hypoglycemic effect is that probiotics affected gut bacteria to introduce insulinotropic polypeptides and glucagon-like peptide to induce uptake of glucose by muscle. In addition in the form of glycogen, liver induces the absorption of more blood glucose.¹⁶ As well as, clear kefir also proved to affect pancreatic cell regeneration. This underlying mechanism caused by its bioactive ingredients, like amino acids and peptides. This peptide stimulated digestibility values and a high biological protein, and it continued to regenerate and maintain cells. Kefir enhances the production of amino acids like arginine, glutamine, and nucleotides as well as the usability of fats and biological proteins by the hydrolysis of enzymes and bacteria hydrolysis. The nucleotide is mainly needed for establishing and working arrangements of proteins in the small intestine, lymph nodes, and liver, as well as for genetic mechanisms. Where the result showed clear kefir supplementation this result correlated with a former finding in the vivo study. During the intervention process, it was systemically regenerating and repairing cells in the number of normal pancreatic β cells of Langerhans Island.¹⁷

Hulston *et al.*,¹⁸ reported that FBS concentrations and HbA1c levels were reduced in type 2 diabetic patients by consumption of probiotic yogurt.

Judiono *et al.*,¹⁷ found that clear kefir decreases oxidative stress conditions and hyperglycemia. The mechanism underlying it may cause the lowering of oxidative stress. It played a climacteric role in lowering the blood lipid peroxidation levels that are measured by malondialdehyde (MDA). El-bashiti *et al.*,¹⁹ also, found significant reductions in insulin growth factor and FBS. These results were compatible with our results, after intake Kefir milk for ten weeks, there was a high reduction in HbA1c and FBS.

Kidney Function Test

Diabetic complications, such as retinopathy, neuropathy, and nephropathy strongly linked with Hyperglycemia and oxidative stress.⁸ Probiotic Kefir has been proposed to contribute to reduce the progressing of renal injury in diabetes.²⁰

In diabetic rats, Kefir treatment resulted in better glycemic control by decreased polydipsia, polyuria, and polyphagia, a partial progression in renal function but the mechanisms not fully understood by which probiotic bacteria change hyperglycemia. In our study urea, creatinine and the uric acid levels were not affected by Kefir diet. There was no significant variance between the two groups which in good agreement with Urdaneta *et al.*,²¹

El-bashiti *et al.*,¹⁹ reported that effect of Kefir on kidney function was not clear and required further investigations, because of increasing level of creatinine. Although creatinine concentration increased, urea concentration was not affected. Uric acid decreased slightly with increased kefir diet compared to control, which lowers the probability of causing gout.

Kanbank *et al.*,²² found that by intake Kefir, creatinine clearance was higher. Kefir playing as an Angiotensin-converting enzyme inhibitor so Kefir reduced renal function damage. It was concluded that kefir protects renal function and renal damage stimulated by high salt diet in rats.

Lipid Profile

We found in our study that no significant decrease in TG, cholesterol and LDL. Some previous findings are in agreement with our findings, St-Onge *et al.*,²³ reported that Kefir had no effect on TG

concentrations, HDL-c, LDL-c and total cholesterol after 4 weeks of Kefir supplementation. Ostadrahimi *et al.*,¹⁶ also reported that TG, total cholesterol and LDL-c in probiotic fermented milk (Kefir) reduced to conventional fermented milk but these changes were not statistically significant.

Other studies reported that Kefir diets tended towards a lowering of serum total cholesterol concentrations and TG, and a decreasing of cholesterol accumulation in the liver, causes the reduction in serum cholesterol concentration being essentially in the non-HDL fraction.^{24,25}

Tu *et al.*,²⁶ reported that different strains of lactic acid bacteria may have different effects on serum cholesterol concentration. Animal studies proved the importance of Kefir in decreasing effect on blood pressure, and on levels of LDL cholesterol.²⁷

So, lately, it implored to apply other beneficial productions like probiotics in lowering blood cholesterol.²⁸

Calcium and Phosphorus

Tu *et al.*,²⁶ indicated that calcium of serum was slightly increased in patients who consume Kefir treatment for six months. This is due to Kefir containing proteins or bioactive peptides with the potential that improve calcium absorption and bone mineral density. Chen *et al.*,²⁹ reported that a 12-week treatment with kefir increased the level of serum Calcium. Our study in consistent with the previous studies in which the group receiving Kefir for ten weeks, there was a significant increase on serum calcium. Also, our

finding showed that intake of Kefir milk for ten weeks decreased the serum phosphorous which is opposite to calcium concentration.

Conclusion and Recommendations

Conclusion

Kefir has potential effect in the reduction of FBS, HbA1c and phosphorous with increase in calcium among diabetic adult males. No significant effect on Kidney function test by Kefir except of uric acid which significantly increased. Also, there was no significant effect on TG, LDL, HDL, and cholesterol level. Probiotic fermented milk can be useful as a complementary or adjuvant therapy in the treatment of diabetes.

Recommendations

- Further studies with long-term duration and larger scale studies are needed to clarify the effect of Kefir on human health.
- Further work on the effect of Kefir on other biochemical parameters also recommended.

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Conflict of Interest

The authors declare no conflict of interest.

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