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

By

Fedaa Faysal Abu Safia

Supervised by

Dr. Tarek Elbashiti
Assoc. Prof. of Biotechnology

Dr. Baker M. Zabut
Prof. of Biochemistry

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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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نتيجة الحكم على أطروحة ماجستير

بناءً على موافقة عمادة البحث العلمي والدراسات العليا بالجامعة الإسلامية بغزة على تشكيل لجنة الحكم على أطروحة الباحثة/ فداء فضيلة أ.د. نورة الماجستير في كلية العلوم/ قسم التكنولوجيا الحيوية وموضوعها:

تأثير تناول لبن الكفير على بعض متغيرات الدم الحيوية لدى مرضى السكري الذكور البالغين المستفيدين حديثاً في محافظة غزة

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

وبعد المناقشة التي تمت اليوم الأربعاء 07 جمادي الأولى 1439 هـ الموافق 24/01/2018م الساعة العاشرة صباحاً، اجتمعت لجنة الحكم على الأطروحة والمكونة من:

- أ. د. بكر محمود الزعبي
- د. طارق عبد القادر البشتي
- د. كمال البدolah
- د. أيوب راغب الدلو
- مشرفًا ورئيسًا
- مشرفًا
- مشرفًا
- مشرفًا
- مشرفًا

وبعد المداولات أوصت اللجنة بمنح الباحثة درجة الماجستير في كلية العلوم/ قسم التكنولوجيا الحيوية.

واللجنة إذ تنصح هذه الدورة فإنها توصيها بتفويض الله تعالى وزعم طاعته وأن تسخر علماً في خدمة دينها ووطنها.

والله ولي التوفيق...

عميد البحث العلمي والدراسات العليا

أ. د. مازن إسماعيل هنية
Abstract

Background: Kefir is natural probiotic milk. It is a complex mixture of bacteria, yeasts, many vitamins, minerals, amino acids, and enzymes. Also, it contains many bioactive ingredients that give its unique health effects, for instance, affecting the immune system, metabolism, and improving anti-allergic resistance. It may have an antitumor effect, intestinal immunity, antimicrobial effect, regulate cholesterol, and improve sugars digestion and may have an antioxidant effect.

Aim: The study aimed to evaluate the effect of kefir intake on some blood biochemical parameter among type 2 diabetic adult male patients in Gaza Government.

The study design: it was a case-control study.

Material and method: Kefir starter obtained from El-Zawya market, Gaza. The experiment 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 is a patient who was intake one 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. SPSS V20 system was used to analyze the obtained data.

Results: 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 in the group taking Kefir milk (P< 0.05). After the intervention, HbA1C was reduced significantly (P=0.001). Also, there was a decrease in phosphorus result and no significant difference in Kidney functions, lipid profile, and calcium was observed.

Keywords: Probiotic, Kefir, Diabetic, Biochemical parameters.
ملخص الدراسة

المعلومات الأساسية: الكفير هو غذاء بروبيوتك طبيعي، يتكون من خليط معقد من البكتيريا والخمائر، ويحتوي على العديد من الفيتامينات والمعادن والأحماض الأمينية والإيزيتمات. كما يحتوي على العديد من مواد بيولوجية نشطة تعطي فوائد صحية فريدة من نوعها، على سبيل المثال تحسين الجهاز المناعي والتمثيل الغذائي ويكسب الجسم مقاومة مضادة للحساسية ومضاد للأورام وتحسين مناعة الأمعاء ومضاد للميكروبات وتنظيم الكولسترول وتحسين هضم السكريات ومضاد للأكسدة.

الهدف: تهدف الدراسة إلى التعرف على تأثير تناول لبن الكفير على بعض متغيرات الدم الحيوية لدى مرضى السكري الذكور المشخصين حديثاً في محافظة غزة.

نوع الدراسة: دراسة تجريبية ضابطة.

الطريقة والأدوات: تم الحصول على حبيبات الكفير من أحدى العطارين، سوق الزاوية، غزة، بكميات كبيرة، وتم اختيار مرضى السكري الذكور حديثي الاصابة من عيادات الرعاية الأولية لوزارة الصحة في قطاع غزة، تشتمل عينة الدراسة على 42 مريضاً تتراوح أعمارهم ما بين 37 إلى 65 عاماً، حيث تم تقسيمهم إلى مجموعتين، المجموعة الأولى هي المضابطة التي تتناول علاج الميتفورمين فقط، والمجموعة الثانية هي التجريبية التي تتناول علاج الميتفورمين بالإضافة إلى كوب واحد من لبن الكفير يومياً لمدة عشرة أسابيع، ثم في بداية الدراسة تم ضبط عينات دم لجميع المرضى، وبعد عشرة أسابيع تم السحب مرة أخرى لإجراء الفحوصات البيوكيميائية، تم استخدام نظام (V20, SPSS) لتحليل البيانات التي تم الحصول عليها.

النتائج: أظهرت النتائج الدراسة أنه لا توجد علاقة إحصائية في نسبة السكر في الدم بين المجموعتين في بداية الدراسة (p=0.22) ولكل المرضى الذين احترفت انخفاض في نسبة السكر (p<0.05) وأيضا انخفاض في نسبة مخزون السكر في الدم (p=0.001).

ومن ناحية أخرى أظهرت النتائج أنه لا تأثير للكفير على الدهون، والأحماض الفلوسورية في الدم.

الكلمات المفتاحية: بروبيوتك طبيعي، الكفير، المتغيرات البيوكيميائية، السكري.
Dedication

To soul of my father

To my mother for her endless love

To my beloved husband for his support,

Encouragement and patience

To my son’s Saeed and Faysal

who are the light of my life

To my brother, sisters, family, friends and

Work colleagues.
Acknowledgment

All praise and glory are due to ALLAH the all mighty for all the bounty and support granted to me, peace and blessings are up on the prophet Mohammed.

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I deeply thank the team of medical lab Family, especially Mrs. Monia Majed Hammoda for the kind help given during biochemical analysis of blood samples.

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Finally, needless to say, that without encouragement and support of my family, this work would not come through.

Fedaa Abo Safia
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Abbreviations

ALT  Alanine Aminotransferase
AST  Aspartate Aminotransferase
ATP  Adenosine Triphosphate
BUN  Blood urea nitrogen
FBS  Fasting blood sugar
GPO  Glycerophosphate oxidase
HDL-C High-density lipoprotein cholesterol
IGF  Insulin growth factor
LAB  Lactic acid bacteria
LDL-C Low-density lipoprotein cholesterol
MDA  Malondialdehyde
Mets Metabolic Syndrome
MSG  Monosodium glutamate
NADP Nicotinamide adenine dinucleotide phosphate
TAG  Triacylglycerol
TC   Total cholesterol
VLDL Very-low-density lipoprotein
Chapter 1
Introduction
Chapter 1

Introduction

1.1 Overview

Kefir (pronounced kuhFEER) (alternately kefirs, keefir, kephir, kewra, talai, mudu kekiya, búlgaros), purportedly from either the Turkish "keyif" (joy/pleasure) or "kopur" ((milk) froth, foam) (Gaware, Kotade, Dolas, Dhamak, Somwanshi et al., 2011).

It 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 (Antoniou, Topalidonos, Tsavalia, & Dimitrelio 2008). 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” (Suriasih, Aryanta, Mahardika, & Astawa, 2012).

Inoculating Kefir grains to goat, cow, or sheep's milk produced the Kefir milk. The traditional way to produce Kefir was 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. Many types of dairy are used, like soy milk and coconut milk kefir (Gaware et al., 2011).

Kefir is viscous, acidic and slightly carbonated dairy beverage that consists of mini quantities of alcohol (Farnworth, 2006).

Kefir grains produced Kefir milk traditionally, the characteristic of this grains are small, gelatinous, yellowish in colour, and they are similar to cauliflower in shape and irregularity, they look like a small clamps (Öner, Karahan, & Çakmakçi, 2010) measure 1-3 cm in length, they are lobed, the color is white to yellow-white, look like small calculi flower florets. By transferring Kefir grains into fresh milk daily and making allowance for them to grow for 20 hours approximately grains are still viable (Farnworth, 2006).

The way of producing Kefir relies on the synergistic reaction of the microflora that 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 evidence to be bioactive (Farnworth, 2006).

Kefir has many minerals, vitamins, enzymes and amino acids especially phosphorous, vitamin A, calcium, magnesium, B12 and B2, 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 (Gaware et al., 2011).

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 bacteria culture and the milk matrix. Kefir exemplarity contains both CO$_2$ and ethanol. A sparkling sensation and refreshing of Kefir product refer to the content of CO$_2$ and ethanol. The yeast strains that found in Kefir grains give to the Kefir milk its perfect flavor in particular (Păucean, Rotar, Jimborean, Mudura, & Socaciu, 2009).

Diabetes mellitus is a mixture of metabolic diseases featuring increased blood glucose levels (hyperglycemia) caused by failings in insulin action and or insulin secretion. The beta cells of the pancreas synthesized Insulin as a hormone, which is used to appliance glucose from digested food as an energy source (Loghmani, 2005).

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 (Sartang, Mazloomi, Tanideh, Zadeh, 2015).

Kefir milk has been considered a probiotic because it has anti-inflammatory and antioxidant characteristic (Punaro, Maciel, Rodrigues, Rogero, Bogsan et al., 2014).

1.2 General Objective

The study aimed to evaluate the effect of kefir intake on some blood biochemical parameters among type 2 diabetic adult male patients in the Gaza Governorate.

1.3 Specific objectives

1- To determine the effect of kefir milk on blood glucose level of the diabetic patient.

2- To determine the effect of kefir milk on HbA1c of a diabetic patient.
3- To determine the effect of kefir milk on kidney function test.

4- To examine the effect of Kefir intake on the level of calcium and phosphorous.

5- To determine TC, TAG, HDL, LDL level in serum of diabetic patient after intake kefir milk.

1.4 Significance of the study

1- Chemical drugs have many side effects, so natural alternatives are required.

2- Kefir is considered as a probiotic that has a biological value and perhaps treats some diseases.

3- Effects of Kefir on diabetic patients have not been carried out before in the Gaza Strip
Chapter 2

Literature Review
Chapter 2
Literature Review

2.1 Kefir

2.1.1 Origin of Kefir

Kefir is a dairy producer which has been produced for years in Eastern Europe and Mongolia before spreading to Caucasian (Kavas, 2015). The name Kefir is Turkish word "Kef" which refers to pleasure or good feeling (Gaware et al., 2011).

Kefir is a fermented acid–alcoholic dairy product. The starter culture used traditionally to produce kefir is an irregularly shaped, gelatinous, white-yellow structure the kefir grains (Antonioua, Topalidoua, Tsavaliaa, & Dimitrelib, 2008). It has been ascribable to an old world food with exceptive curative characteristic and health-promoting since the beginning of recorded history.

In long-ago day Historically, Kefir has been joined by health, for example, in Soviet countries, to reduce the risk of some diseases, the healthy people have been recommended for consumption Kefir. Many of health benefits have been linked to the drinking of this fermented milk, these health benefits refer to the existence of some metabolic products like organic acids, and also related to its microflora (Prado, Blandón, Vandenberghe, Rodrigues, Castro et al., 2015). Also, in Soviet Union hospitals is used to treat some diseases like cancer, digestive disorders, even atherosclerosis and tuberculosis (Shavit, 2008).

2.1.2 Kefir grains

Kefir grains are key to Kefir production, they measure 1–3 cm in length, its look like small cauliflower florets: are lobed, the color of it is white to yellow-white, irregularly shaped, and have a slimy but beefy texture (Farnworth, 2006). The external surfaces of the kefir grains appear shiny and smooth with the naked eye but, the grain surfaces presented to be very rugged under scanning electron microscopy (Xin Gao et al., 2016).

The microflora of kefir grains is very stable if incubated and preserved under the needful physiological provisions which needed for the continued generation of the probiotic species. Its activity remains for years. Wet kefir grains, if not inoculated into
fresh milk, will keep activity for 8-10 days only and dried grains keep activity for 12-18 months (O’Brien, 2012).

By transferring grains of Kefir every day into fresh milk, Grains are still active and letting them grow for 20 hours approximately; during this time, may be the mass of grains can increase by 25% (Tu, Chen, Tung, Kao, Hu et al., 2015).

Figure (2.1) Kefir grains under a light microscope (Farnworth, 2006)

Because the Kefir grains are reusable, they may be used to culture batch-after-batch of kefir by proper care. To allow culture, the kefir grains are laying in sugar-based liquid or milk after that removed and added to a new sugar liquid or milk. A mini quantity of the kefir made by powdered kefir starter may often be preserved and placed in fresh milk to produce a new batch of kefir. Generally, Kefir grains may be re-cultured many times before the bacteria weaken significantly. Some factors like the freshness of the milk, hygiene, and the quick reculture process of Kefir, affecting the number of times re-cultured of powdered kefir starter

The milk of the most mammals can be successfully fermented by Kefir grains. These Kefir grains will resume the growth in such milk. Idealistic milk used include sheep, cow and goat, each with different in nutritional qualities and organoleptic. The traditional milk has been used is raw milk. Also, kefir grains will ferment milk alternatives like rice milk, coconut milk, and soy milk, in addition to other sugary liquids like coconut water, fruit juice, ginger beer and beer wort (Gaware et al., 2011).

Kefir grains consist of a microbial symbiotic mixture of the LAB, acetic acid bacteria, and yeast that attach to a polysaccharide matrix. At the end of the fermentation process, the kefir grains may develop to new procreating grains, that contain the same properties as the old ones (Prado et al., 2015).
Kefiran is the main polysaccharide of Kefir grains that is a heteropolysaccharide consist of an equal characteristic of galactose and glucose and is essentially generated by *Lactobacillus kefiranofaciens*. Also, Kefiran may be used like a supplement in fermented products, because it develops the viscoelastic and viscosity characteristic of acid milk gels as well as, is capable to form gels that have interesting viscoelastic characteristic at low temperatures. In addition, kefiran can activate the rheological characteristic of chemically acidified skim milk gels which increase the viscosity of Kefir milk (Prado et al., 2015).

### 2.1.3 Microbiota of Kefir grains

The Kefir grains contain a complex of the microbial ecosystem, these microbial types are categorized into four groups: heterofermentative and homofermentative LAB and non-lactose and lactose assimilating yeast (Prado et al., 2015). And mainly composed of *lactococci*, *lactobacilli*, *acetic acid* bacteria and yeasts which are held together by a protein-polysaccharide matrix (Antonioua et al., 2008). Yeasts and *lactococci* are found in the outer layer of the grain. But in the inner layer of the grain, more yeasts cells were found and the number of *lactobacilli* was much higher (Prado et al., 2015). And a symbiotic relationship exists between the microorganisms found in the grains and their population composition may differ depending on their origin and the culturing method used (Antoniou et al., 2008).

![Figure (2.2) Scanning electron microscopy of kefir grain microbiota; outer grain portions (O’Brien, 2012)](image-url)
Figure (2.3) Scanning electron microscopy of kefir grain microbiota; inner grain portions (O’Brien, 2012)

Table (2.1) Average number of colony-forming units (CFUs) of probiotic microorganisms found in 1 gram (g) of traditionally produced Kefir (O’Brien, 2012)

<table>
<thead>
<tr>
<th>Genus</th>
<th>CFU per gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactococcus</td>
<td>1\times10^9</td>
</tr>
<tr>
<td>Leuconostocs</td>
<td>1\times10^8</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>5\times10^6</td>
</tr>
<tr>
<td>Yeasts</td>
<td>1\times10^6</td>
</tr>
<tr>
<td>Acetobacter</td>
<td>1\times10^5</td>
</tr>
</tbody>
</table>

The grains of Kefir shows by a crude analysis which they are a mass of yeasts, bacteria, proteins, and polysaccharides with a chemical component of 890 to 900 g/kg water, 2 g/kg lipids, 30g/kg protein, 60 g/kg sugars, and 7g/kg ashes. Proteins of kefir grains were examined by SDS-PAGE on acrylamide gels method. And the result of this examination shows that the milk proteins have a lower molecular weight than the main proteins of grain, and also mention that they were not proteolysis producers (Farnworth, 2008).

Total yeast counts were ranging from $10^5$ – $10^6$ CFU/ml while, LAB counts were $10^8$ – $10^9$ CFU/ml, no *Escherichia coli*, and coliform was found in any kefir samples. Identification of the yeast and LAB detected that the *Lactobacillus paracasein ssp. paracasein* was the preponderant species appear in the kefir samples, then *Lactobacillus brevis*. (Suriasih et al., 2012). And also *Lactobacillus acidophilus*,
Lactobacillus casei, Lactobacillus Brevis, and Lactobacillus kefir; lactococci including Lactococcus lactis sbsp. lactic, Lactococcus lactis sbsp. Streptococcus salivarius sbsp. cremoris. Thermophiles, Leuconostoc cremoris, Leuconostos mesenteries (Suriasih et al., 2012). And the important yeasts seem to be Saccharomyces kefir and Torula kefir. Also, several other species have been isolated (Lengkey et al., 2013).

Saccharomyces cerevisiae was the most lactose-negative strains of the kefir yeast isolates, which made up 41 of 110 yeast isolates. And Saccharomyces cerevisiae, promote yeastily and an alcoholic aroma, in addition to a refreshing taste, as well as they, participate greatly in the sensory qualities of the kefir beverage. It is also seeing that the founding of non-lactose fermenting yeasts in kefir grains and kefir is relying on the founding of other lactose fermenting species of yeasts and bacteria able to hydrolyze lactose. Also, another type of non-lactose fermenting species isolated included Lachancea meyersii (15 isolates) and Kazachstania aerobia (23 isolates) these two types had been previously unreported in kefir grain and kefir studies yeasts. (O’Brien, 2012).

Table (2.2) Microorganisms in kefir grains, mother culture, and kefir drink.

<table>
<thead>
<tr>
<th></th>
<th>Lactococci</th>
<th>Lactobacilli</th>
<th>Yeasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kefir grains</td>
<td>7.37</td>
<td>8.94</td>
<td>8.30</td>
</tr>
<tr>
<td>Mother culture</td>
<td>8.43</td>
<td>7.65</td>
<td>5.58</td>
</tr>
<tr>
<td>(wash of grains)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kefir drink</td>
<td>8.54</td>
<td>7.45</td>
<td>5.24</td>
</tr>
</tbody>
</table>

2.1.4 Chemical compositions of the Kefir

Table 3 shows the chemical composition of the Kefir milk drink, as analyzed by Ministry of National Economy Labs. The kefir milk drink contains 2.98%, 3.00%, 3.61% proteins, fats, and carbohydrates, respectively.
Table (2.3) Chemical compositions of the kefir (El-bashiti et al., 2017)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>89.81</td>
</tr>
<tr>
<td>Protein</td>
<td>2.98</td>
</tr>
<tr>
<td>Fat</td>
<td>3.00</td>
</tr>
<tr>
<td>Lactose</td>
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<tr>
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</tr>
<tr>
<td>Calcium mg/100gm</td>
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</tr>
</tbody>
</table>

2.1.5 Health Benefits of Kefir

Kefir composition may contain a bioactive component which gives it unique health benefits, and this means that Kefir has a very important probiotic producer (Farnworth, 2006).

Kefir is milk drink produced by the fermentation process. It is a complex (Prado et al., 2015) symbiosis of more than 30 microflora which forms cauliflower-like structures or grains in the milk. Also, it is wealthy in, enzymes, minerals, amino acids, and vitamins. essentially magnesium, phosphorus, calcium, B12 and B2, vitamin D, vitamin K and vitamin A. In addition Kefir has numerous antioxidant and therapeutic properties and beneficial bacteria and yeast (Gaware et al., 2011).

Kefir used as the first weaning food for babies because it is easily digested (Farnworth, 2006).

Kefir is capable to stop enterocolitis and diarrhea caused by Clostridium difficile. Also, Kefir attack the pathogenic bacteria such as Helicobacter, Salmonella, Escherichia coli, Shigella, Staphylococcus, Proteus vulgaris, Enterobacter aerogenes, Micrococcus luteus, Bacillus subtilis, Streptococcus pyrogens, and Listeria monocytogenes (Prado et al., 2015). And Kefir makes the body strong and active to destroy harmful pathogens, like harmful viruses and bacteria. Also, the tumor cells can destroy by Kefir friendly bacteria (O’Brien, 2012).
The most important health benefits of Kefir, it is can promote the functioning of the brain. It looks like a brain-food also helps to fight the exertion. In addition, the memory-retention power, focus, and reflexes of the brain by Kefir get better. And Kefir also makes the heart healthy and fit, because it regulates the blood pressure and also clears the vessels of the body. And so by drinking milk Kefir, you will maintain the health of the heart (Gaware et al., 2011).

Kefiran has distinguished benefits like antibacterial, antifungal, antitumor characteristic, immunomodulation or epithelium protection, anti-inflammatory, antioxidant activity and healing (Prado et al., 2015).

There are many studies shown that kefir has conservative effects against cancer, this is by inhibiting the growth of bacteria which change procarcinogens into carcinogens in the digestive system, Kefir has shown anti-mutagenic effects (by decreasing 2-glucuronidase) the risk of colon cancer may be decreased because of the anti-mutagenic effects (by decreasing 2-glucuronidase) of Kefir. That also plays an important role in the stop of the disease. Kefir Probiotics have activating effects on the immune system by increasing the numbers of T-lymphocytes and NK cells and by improving phagocytosis. Kefir forming bioactive peptides in the fermentation process which have an indirect effect on the immune system. On account of these findings, now many foods are had many probiotics, as well as now animal feed provider by this probiotic food (Shavit, 2008).

Consuming probiotic foods and Kefir reducing blood sugar levels, blood lipid levels, high blood pressure and lowering the symptoms of food allergies effectively. Animal studies Proved the importance of Kefir in decreasing effect on blood pressure, and on levels of LDL cholesterol (Shavit, 2008).

Kefir lactobacilli are able to produce a wide range of antimicrobial components, like carbon dioxide, organic acids (acetic acid and lactic), ethanol, hydrogen peroxide, peptides and diacetyl (bacteriocins) that not only beneficial in the decreasing of spoilage bacteria and foodborne pathogens through food storage and production but also may be in the prohibition and treatment of vaginal infections and gastrointestinal deficiency (Vahdatpour & Babazadeh, 2016).
2.2 Probiotic food

The Food and Agriculture Organization of the United Nations (FAO), and the World Health Organisation (WHO) published that Probiotics are components of microbial cells or microbial cell preparation which have a useful effect on the health of the host (Prado et al., 2015). It is used to recover gastrointestinal deficiency like irritable bowel syndrome such as Cohn's disease, constipation, diarrhea, and to prevent the too much proliferation of pathogenic intestinal bacteria. Probiotic may promote regularity, decrease lactose intolerance, recover serum cholesterol levels, lower the risk of certain cancers, modify gut immune response and enhance its barrier function, control or decrease the development of certain allergies (Lye, Kuan, Ewe, Fung, & Liong, 2009).

Probiotics are vital microorganisms that give the host health benefits when consumed in suitable amounts, this is by elevating the reproduction of beneficial gastrointestinal natural microflora. Several microorganisms have been present to possess as characteristic, and the more common probiotic bacteria Lactobacillus and Bifidobacterium are used as food adjuvant (Lye et al., 2009).

Probiotics are resistance to pancreatic secretions and stomach acids such as digestive enzymes and bile would be important in the small intestine for probiotics needing to survive in high numbers. The beneficial effect, nontoxic, nonpathogenic, and free of significant adverse side effects keep the stabilizing through the intended shelf life of the product, contain a sufficient number of vital cells to award the health benefit (Sanders, Gibsonill, & Guarner, 2007).

Probiotics usually are isolated from animal and human intestinal tracts. Probiotic products are Yogurt that perhaps the most common probiotic carrying food. Earliest types of probiotic food were Cheese, milk made by the LAB and fungal fermentation, unfermented and fermented milk Kefir, smoothies, juices, cereals, nutrition and infant/toddler. As well as being sold like foods, probiotics are sold as a dietary complement, drugs, and medical foods. Predominately, these products consist of dried microbes, concentrated packaged into tablets, sachets or capsules. This format is suitable for the distribution of major numbers of microbes (Awaisheh, 2012).
The products of many probiotic have been shaped to consist of small numbers of various bacteria. Kefir is a much more complex probiotic because it has a special chemical composition and microbiological. Also, this complexity refers to a large number of different yeast and bacteria, which distinguishes Kefir from other probiotic producers. Since the bacteria and yeasts found in Kefir grains have undergone a long association, the resulting microbial population inhibits many analogous properties, performing identification and isolation of individual types complicated (Farnworth, 2006).

2.3 Diabetes mellitus

Diabetes mellitus is a largely occurring endocrine disorder in many countries. In diabetes, due to defects in the production of insulin or its action, blood glucose levels become elevated. Also impaired is the functioning of the macronutrient metabolism (Sartang et al., 2015).

Multiple metabolic disorders including a defect in lipoprotein and lipid metabolism, oxidative stress (overproduction of free radicals and disorder in endogenous antioxidant defense system), hypertension, vascular endothelial disorder, and sub-clinical inflammation are usually linked to type 2 diabetes. These metabolic deficiencies lead to long-term pathogenic situations like micro- and macrovascular complexities including nephropathy, retinopathy, neuropathy, and increased the rate of mortality and a reduced quality of life (Mirmiran, Bahadoran, & Azizi, 2014).

Diabetes is a combination of metabolic deficiency distinguished by a chronic hyperglycemic condition which results from a disorder in insulin action and insulin secretion or both. Many groups of genetic disorders close to weakly insulin resistance, insulin secretion and environmental factors like overeating, obesity, stress, and lack of exercise, as well as aging, are caused type 2 diabetes. While type 1 diabetes is caused by an autoimmune interaction to proteins of the islet cells of the pancreas. The pathogenesis of selective β-cell annihilation within the islet in type 1 diabetes mellitus is hard to follow because of remarkable heterogeneity of the pancreatic lesions. A mixture of pseudoatrophy islets with cells producing the pancreatic polypeptide, somatostatin and glycogen, normal islets and islets containing both β-cells and infiltrating monocytes and lymphocytes may be seen at the onset of overt hyperglycemia. A deficiency of insulin secretion which leads to the metabolic
disarrangement related to type 1 diabetes caused by the autoimmune destruction of pancreatic β cells. Impaired insulin secretion and increased insulin resistance are the major pathophysiological features of type 2 diabetes. The fragility of pancreatic β cell function in a special way shows advancement over time in type 2 diabetes although obesity, aging, alcohol drinking, smoking, consumption energy insufficiently, etc are unattached risk factors of the pathogenesis of type 2 diabetes mellitus (Ozougwu, Obimba, Belonwu, & Unakalamba, 2013).

Non-insulin dependent diabetes mellitus (NIDDM) is the name of Type 2 diabetes, which caused by lowered sensitivity of target tissues to insulin (Ozougwu et al., 2013).

Type 2 Diabetes caused by insulin resistance in the liver and skeletal muscle characterized by the production of glucose in the liver increased, overproduction of free fatty acids by fat cells and relative insulin disorder, and gradual beta cell malfunction leading to decrease in Insulin Secretion. Decreasing in blood glucose levels pre dominantly may be effected with changes in physical activity patterns and food intake. Insulin injections and/or oral medication are required finally (Loghmani, 2005).

Diabetes increases the risk of affecting mortality and atherosclerosis due to cardiovascular coronary disease. In diabetic patients, the major cause of death is cardiovascular diseases. In diabetic people, the relative risk of these diseases is 2-4 times more than other people. Dyslipidemia is one of the known risk factors lead to diabetes’ complications such as cardiovascular diseases, that has a high incidence in diabetic patients. In people with hypercholesterolemia, the risk of heart attack is times more than people with a normal level of serum lipid profile. Most of type 2 diabetic patients have high serum cholesterol and triglyceride, since a deficiency in glucose and fat metabolism. In type 2 diabetic patients high triglyceride, and low HDL cholesterol and high LDL cholesterol are the properties of dyslipidemia (Toghyani, Kazem Mosavi, Modaresi, & Landy, 2015).

The prevalence of diabetes is rapidly increasing worldwide and the World Health Organization (2003) has prospected that by 2030 the number of adults with diabetes would have almost doubled worldwide, from 177 million in 2000 to 370 million. Experts project that the incidence of diabetes is set to climb by 64% by 2025,
meaning that a surprising 53.1 million citizens will be affected by the disease. The evaluated worldwide prevalence of diabetes among adults in 2010 was 285 million (6.4%) and this value is prospected to rise to about 439 million (7.7%) by 2030 (Ozougwu et al., 2013). However with the increased insulin resistance and incidence obesity of childhood, the number of children diagnosed with type 2 diabetes has also raised worldwide (Loghmani, 2005).

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 (Sartang et al., 2015).

Probiotics are live microorganisms that can exert antidiabetic effects, improve glucose homeostasis and delay the progression of diabetes in different studies. Dietary recommendations for both high-risk and healthy individuals such as pre-diabetic patients can be an effective planning to prevent diabetes or its complexity. Studies demonstrate that probiotic bacteria can improve glycemia and dyslipidemia (Mahboobi, Iraj, Maghsoudi, Feizi, Ghiasvand et al., 2014).

2.3.1 Biochemical parameter

Hemoglobin A1c is a fraction of hemoglobin composed mainly of glycol hemoglobin. It measures the percentage of hemoglobin that is glycated, bound by glucose. The value of HbA1c is highly correlated with the concentration of blood glucose. Erythrocytes have a lifespan of approximately 120 days. Glycation occurs over the entire lifespan of erythrocytes. In general, HbA1c reflects the average concentration of blood glucose over the preceding 3 months. In addition to the concentration of blood glucose, disease states that alter the lifespan of erythrocytes, such as renal failure and anemia, could affect the value of HbA1c, resulting in under- or over-estimation of glycemic control.

Human Hemoglobin inside erythrocytes undergoes a non-enzymatic chemical interaction with glucose. Though the extent and rate of this interaction are depended on the rate concentration of blood glucose through the lifetime of the erythrocytes there are many interaction steps, Glycated hemoglobin, that collectively HbA1. In diabetic patients, the most profuse of these is HbA1c the ratio of HbA1 or HbA1c to
the total HbA concentration has been proposed as a trustable measure of the rate of metabolic control.

Urea is the major end product of protein catabolism. It is a synthesis in the liver and is excreted in the urine. The urea concentration in plasma is same as that in the glomerular filtrate. Urea represents about 80-90% of the total urinary nitrogen excretion and 45-50% of the non-protein nitrogen of blood. Blood urea nitrogen inversely varies with the rate of excretion of urea and directly with protein intake (Jagarati, 2004).

The waste product of muscle is creatinine which produced by creatine metabolism. Creatine is formed in the liver that then passes into circulation where it is taken up by skeletal muscle then converted to creatine phosphate that in skeletal muscles used as a storage form of energy. Creatine phosphate and Creatine are readily converted to creatinine at an average of about 2% the total per day. This is related to muscle body weight and mass (Jagarati, 2004).

The end product of purine metabolism in man is Uric Acid which formed by oxidation of Purine bases (Jagarati, 2004).

Now serum lipid profile has become an approximate routine test and measured for cardiovascular risk evaluation. The test includes four basic parameters, triglycerides, LDL cholesterol, HDL cholesterol and total cholesterol. The blood specimen of it is must be done by fasting patients. Fasting about 12-14 h overnight prevent complete dietary except water and medication (Nigam, 2011).

Triglycerides are fats that are present in foods like dairy products, cooking oils, and meats. The human body stores the fat in tissues, this fat comprised of triglycerides. This triglyceride eaten by foods are absorbed in intestines and transported by the bloodstream to tissues where they may be used to supply energy or stored as fat. In addition, triglycerides are made in the liver. For example, when more calories are produced than requires of the body, the liver forms triglycerides that are then stored as fat (Albert, 1998). The hyperlipidemias may be inherited trait or they may be secondary to many disorders of diseases like biliary obstruction diabetes mellitus, nephrosis, and metabolic deficiency associated with endocrine deficiency. Also, high levels of plasma triglycerides have been considered as risk factors attached to atherosclerotic diseases (Jagarati, 2004).
Cholesterol is a fat-like matter that is present in all cells of the body. All the cholesterol that body needs it to make certain hormones and to compose cell membranes produced by the liver. One of the important tools in the classification and diagnosis of lipemia is the determination of serum cholesterol. Also, one of the main risk factors for heart disease is high blood cholesterol (Jagarati, 2004).

The HDL particles increase the removal of cholesterol from cells, especially those in atherosclerotic plaques, and transport them to the liver, but the mechanisms by which HDL award protection from atherosclerosis contain more than just reverse cholesterol transport. The HDL can decrease the risk of thrombosis by preventing platelet aggregation and activation. HDL particles also appear to have antioxidant properties and anti-inflammatory, prohibiting the oxidation of LDL cholesterol and the expression of cellular cohesion molecules and monocyte induction. (Bitzur, Cohen, Kamari, Shaish, & Harats, 2009).

Aggressive LDL cholesterol (LDL-C) decrease strategies are recommended for secondary and primary inhibition of cardiovascular events (Meeusen, Lueke, Jaffe, & Saenger, 2014).

Calcium is the most micronutrient in the body (2% of total body weight). It has two key roles. A very little rate of body calcium has a vital part in regulating definitive functions like muscle contractions, nerve impulses, and the activities of enzymes. Is located in the bones in percent more than 99%, where it plays an important role in their strength and structure (Angelis-Pereira, Barcelos, Sousa, & Pereira, 2013). Calcium is found in serum in three forms – ionized calcium, calcium bound to proteins, and calcium bound to other organic substances, e.g. citrate. The ionized fraction is the most of the physiological functions of calcium depend upon, but, for routine work of serum evaluation only total calcium is used (Jagarati., 2004).

Phosphorus is found in the blood such as inorganic phosphate and in combination with various organic compounds including lipids, nucleotides, and carbohydrates. The main phosphorus found in serum is Inorganic phosphorus (Jagarati, 2004).

2.4 Previous studies

St-Onge, Farnworth, Savard, Chabot, Mafu et al., (2002) reported that after 4 weeks of Kefir supplementation. There is no effect on triglyceride
concentrations, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol or total cholesterol nor on cholesterol fractional synthesis degrees. With diet, in plasma fatty acid levels no significant change appeared. Both milk and Kefir increased (p < 0.05) fecal isovaleric, propionic and isobutyric acids, in addition, the total amount of fecal little chain fatty acids. Also, the result of supplement with Kefir increased the content of fecal bacterial in the majority of the subjects.

**Yaman, Ulukanli, Elmali, & Unal, (2006)** studied the effects of Kefir as a probiotic for 5 weeks on the microbiology of Gosling feces in comparison with a control diet. It is found that *Lactobacilli spp* increased in group 2 and total aerobic bacteria populations in group 1 and 2, Kefir has significantly affected the fecal bacteria population. In addition to that, the number of *Enterobacteriaceae* lowered in the group2, and coliforms in the group 1 and 2. The yeast, *Staphylococci* and the *Enterococci* populations were very slightly but not significantly, minimized in feces of birds supplementation. The results provided initiatory information that *Lactobacilli spp* and yeast of Kefir can be a helpful candidate for competitive exclusion preparation to develop intestinal microflora of poultry and lead to best carcass hygiene.

**Liu, Wang, Chen, Chen, Yueh et al., (2006)** evaluated the hypocholesterolemic characteristic of milk-Kefir and soymilk-Kefir. The soya milk, milk-kefir, and soya milk-Kefir diets all trended towards a reducing of total cholesterol concentrations and triacylglycerol in serum, and a lowering the accumulation of cholesterol in the liver. The lower cholesterol concentration in serum being essentially in the non-HDL fraction. They reported that soymilk-kefir diet gives a significant increase in the fecal excretion of bile acids and neutral sterols contrast with the other two diets. They also reported that the soymilk-kefir diet extracts a significant reduce the serum rate of HDL-cholesterol to non-HDL-cholesterol, compared with the control, that was the case for the other diets. These result finding in hypocholesterolemic action, soymilk-Kefir can be considered to be the most promising food composition.

**Urdaneta et al., (2007)** reported information about the effects of Kefir on proteins and enzymes found in the intestine. Daily recorded body weight and food intake. And the glucose, cholesterol, triacylglycerols, alkaline phosphatase, and uric acid activity were examined in the serum. When measured the weight of the organs
found that no significant differences. The intestinal enzymatic analysis was achieved, and shown in the results an increase in this activity as well as the uptake of D-galactose by brush border membrane vesicles. These findings showed that Kefir, might decrease glycemic index and benefit protein digestion, in conditions studied.

Cenesiz et al., (2008) investigated the effect of varying amounts of Kefir applied in drinking water in relation to changes in total lipid, total cholesterol serum, (AST) and (ALT) activities in broiler chicks. At the end of the experiment, live weights of the groups were increased significantly contrast to that of the control group. In response to Kefir treatment, total serum lipid and cholesterol levels were significantly reduced contrast to that of in control group. Moreover, did not result in any changes in serum AST and ALT activity by Kefir treatment in the groups. The obtained results demonstrated that use of Kefir as a probiotic in drinking water lowers total cholesterol and total lipid and increased live weight, thus suggesting that it may have beneficial effects when using in human diets.

Huey et al., (2009) discussed the antihypertensive functions of probiotics via the modification of insulin resistance and sensitivity, enhancement and/or treatment of lipid profiles, the modification of renin levels and also the conversion of bioactive phytoestrogens like an alternative replacement of sex hormones like progesterone and estrogen.

Gaware et al., (2011) reported that Kefir was beneficial for diabetics as it lowers the glucose level in the blood and maintains the normal blood sugar level, In addition to Kefir is beneficial for prohibition the occurrence of many cardiovascular diseases as stroke and heart attack by it helps to lower high cholesterol levels. Kefir can stop the growth of cancerous cells and can inhibit a certain type of cancers like breast cancer, colon cancer etc. also lower the size of tumors. The Journal of Medicinal Foods in 2007 published that many women who have been diagnosed with breast cancer are adopting kefir as an alternative to milk because the extracts of kefir have an ingredient that specifically targets and prevent the growth of cells of human breast cancer.

Panesar (2011) indicated that fermented milk producers have a hypocholesterolemic effect. It is proposed that ingestion of large quantities of fermented milk provide factors that fail cholesterol synthesis. It has been published
that *L. acidophilus* has expressed the ability to reduce the levels of cholesterol in serum. This enhances the potential healthful aspects of dairy products which fermented with *L. acidophilus* (or other lactic acid bacteria) since hypercholesterolemia is considered to be one of the main factors leading to cardiovascular disease. However, it is possible that some strains can demonstrate this characteristic while others do not.

**Hadisaputro (2011)** investigated the effect of oral Clear Kefir probiotic on lipid peroxidation, glycemic status and antioxidative characteristic of Streptozotocin elicited hyperglycemia Wistar Rats. The indiscriminate pretest: posttest control group study design was performed in 84 male hyperglycemia. Rats were randomized into four groups. The result of this study showed that clear Kefir supplementation 3.6 ccs/day for 30 days administration, influenced by blood glucose, and increased antioxidant capacity. The result of the statistical analysis showed that there were lowered of glucose, antioxidant capacity was increased.

**Honda, Moto, Uchida, He & Hashizume (2012)** concluded that the persistent prohibition of the postprandial blood glucose during the repression of absorption of glucose from the intestine may refer to antidiabetic activity. The resultant point that for the management of type 2 diabetes the specified strains of the lactic acid bacterium can be prospective to be useful.

**Kizak & Çelik, (2012)** examined the effects of different doses of Kefir on the oxidant-antioxidant status and growth performance in liver tissues and the blood of *Salmo coruhensis*, Coruh trout, in different periods. In condition factor among fish fed diets with Kefir, there were no significant differences in specified growth rate, feed protection rate. Although in control groups compared to all groups, there was no statistical difference among groups and was observed an increase in the glutathione peroxidase enzyme activity. The statistical data gained from this experiment mentioned that the same doses of Kefir were less effective at the end of 2-month treatment than 3-month treatment, whereas catalase activity increased in control group compared to all groups. Finally, it was concluded that kefir could play an antioxidant role and its efficacy relied on time and dosage of application in *S. coruhensis*, Coruh trout.
Jascolka et al., (2013) evaluated the effects of brown sugar-fermented Kefir solution on the associated risk factor and promoting of atherosclerosis in mice. The results showed that in Kefir group, triacylglycerols lowered and HDL increased significantly, as compared to the control group. Catalase activity and lipid peroxidation were also lowered in the liver of Kefir supplemented mice. Kefir supplementation, despite increasing HDL-c, was not associated with a reduction of oxidized lipoproteins or atherosclerosis development. Kefir supplementation improved oxidative stress and lipid profile but did not decrease atherosclerotic lesion.

Angelis-Pereira et al., (2013) investigated the effect of Kefir and skin flours and banana pulp on the levels of triacylglycerols, LDL-c, HDL-c and total cholesterol in serum in rats fed cholesterol-rich diet. They found that the fermented Kefir significantly lowered the levels of triacylglycerols, VLDL and LDL-c, as well as having increased HDL-c.

Punaro et al., (2014) investigated the effects of Kefir on the synthesizing of renal damage and oxidative stress and nitric oxide in STZ-induced diabetic rats. This study indicated that Kefir therapy may share to best control of oxidative stress and glycemia, that is participating with the amelioration of renal function, proposing its use as a non-pharmacological adjuvant to retarding the improvement of diabetic complexities.

Judiono et al., (2014) investigated the effects of clear kefir on the glycemic status of Type 2 Diabetes Mellitus in Bandung. They concluded that lowering of glucose levels in serum (FBG, HbA1c, PBG) and e-peptide increased refer to supplementation of clear kefir. They reported also chemical properties and biomolecular mechanisms of clear Kefir’s is a challenge for future studies.

Mohamadshahi et al., (2014) studied the effect of probiotic and conventional yogurt on lipid profile in type 2 diabetes mellitus patients. And to progress dyslipidemia in patients with type 2 diabetes proposed that probiotic yogurt consumption may be used as a treatment method and an alternative prevention approach.

Alsayadi, Al Jawfi, Belarbi, Soualem-Mami, Merzouk, et al., (2014) evaluated the anti-hyperglycemic and antihyperlipidemic efficacy of water kefir on streptozotocin-induced diabetic Wistar rats. By intraperitoneal injection of
streptozotocin adult diabetic Wistar rats were made, and then given Kefir in drinking water for 5 weeks. Water Kefir is present to be hypoglycemic and hypolipidemic treatment in less cost and less time-consuming. To control lipid levels and glucose for diabetes Water Kefir can be useful food.

Maria R. Prado et al., (2015) mentioned that the reason for the increased interest in probiotic strains from Kefir is its capacity to reduce cholesterol levels. Bacteria can change serum cholesterol in different ways, during the absorption and binding into the cell before it can be absorbed into the body producing deconjugating and free bile acids preventing the HMG-CoA reductase enzyme.

Mikelsaar, Sepp, Štšepetova, Hütt, Zilmeret al., (2015) tested the possibility of kefir which includes the antioxidative probiotic strain, Lactobacillus fermentum ME-3 to regulate the plasma lipid profile. The experiment was making in clinically healthy adults by high serum triglyceride (TG) and/or borderline-high serum low-density lipoprotein-cholesterol (LDL-C) levels based on guidelines from the European Atherosclerosis Society and the European Cardiology Society. They reported that, after eight weeks of consuming kefir with the antioxidative probiotic L. fermentum ME-3, lowered serum TG, LDL-C, and LDL values in the clinical healthy volunteers with borderline-high lipid profile indices. Therefore, L. fermentum ME-3 has the prospect to reduce the risk of CVD which is tightly associated with protecting of plasma lipid profile.

Carl et al., (2015) reported that fasting blood glucose concentrations and glycosylated hemoglobin (HbA1c) levels were reduced in type 2 diabetic patients by consumption of probiotic yogurt, but whether or not probiotics can also prohibit diet-induced insulin resistance in otherwise healthy individuals is not yet known. Therefore, provided novel evidence that probiotics protect glycemic control and inhibit insulin resistance through a dietary challenge consisting of severe lipid overload, and also proposing that probiotics might be beneficial in the resistance against the human metabolic disease.

Ostadrahini, Taghizadeh, Mobasseri, Farrin, Payahoo et al., (2015) study aimed to evaluate the effect of probiotic fermented milk (kefir) on glucose and lipid profile control in patients with type 2 diabetes mellitus. they reported that in the treatment of diabetes, Probiotic fermented milk can be useful as a complementary or
adjuvant therapy. The reduction of fasting blood glucose and HbA1C in comparison with conventional fermented milk caused by the consumption of probiotic fermented milk.

Nurliyani (2015) aimed to investigate the effect of Kefir combination from soy milk and goat milk on plasma glucose, lipid profile, glutathione peroxidase activity and the enhancement of pancreatic β-cells in diabetic rats. Results of this study mention that diabetic rats fed with goat milk or soy milk Kefir had lower triglyceride than the rates fed Kefir combination. Lowering of plasma glucose in diabetic rats fed goat milk Kefir was lower than rats fed Kefir combination.

Damiana, Lukasz, Celia, Ana, Sandra et. al (2016) evaluated the effect of supplemented kefir in the diet of Spontaneously Hypertensive Rats (SHR) in which metabolic syndrome (Mets) were stimulated with monosodium glutamate (MSG) and determined its effect on inflammatory, metabolic parameters, and glycemic index control and oxidation marker expression. In the experiment, thirty animals were used. Feeding was completed by gavage for 10 weeks and the animals received standard water and food *ad libitum*. The main variables were evaluated are pro- and anti-inflammatory markers, insulin resistance, obesity and the histology of adipose tissues and pancreatic adipose tissues. The supplementation of Kefir decrease liver lipids, plasma triglycerides, liver triglycerides, fasting glucose, insulin resistance, thoracic circumference, fasting insulin, abdominal circumference, products of lipid oxidation, increased anti-inflammatory cytokine expression (IL-10 and pro-inflammatory cytokine expression (IL-1β) Finally mentioned that kefir has the practicability to interest the management of MetS

Tahere et al., (2016) determined the effect of probiotics on lipid profile level of serum of patients with type II diabetes mellitus. And the result of this study mentioned that probiotic supplementation via serum lipid profile lowering, in type 2 diabetes. may effective in enhancing risk factors for cardiovascular diseases

El-bashiti, Zabut, Al- Krenawie (2017) evaluated the effects of kefir intake on some biochemical profiles and growth performance among domestic rabbits. They reported, there was a significant reduction in kidneys, spleen, skin weight, lungs, internal body fat, and liver. In contrast, there were significant increases in head, Caracas and viscera weights by increased Kefir concentration to 10% of water. But
when increased to 20% of water there were significant reductions in internal body fats, viscera weights, and liver. In addition to, there were significant reductions in insulin growth factor1 (IGF-1), fasting blood sugar (FBS), low-density lipoprotein (LDL), uric acid and free thyroxine as kefir percentage increased to 20%. In contrast to these results, there were significant increases in alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total cholesterol among groups.
Chapter 3

Materials and Methods
3.1 Materials

3.1.1 Chemicals

Table (3.1): Chemicals that used in the study.

<table>
<thead>
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<th>Country</th>
</tr>
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<tbody>
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</tr>
<tr>
<td>Urea Kit</td>
<td>Quimica Clinical Aplicada S.A</td>
<td>Spain</td>
</tr>
<tr>
<td>Creatinine Kit</td>
<td>Quimica Clinical Aplicada S.A</td>
<td>Spain</td>
</tr>
<tr>
<td>Uric acid Kit</td>
<td>Quimica Clinical Aplicada S.A</td>
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</tr>
<tr>
<td>Phosphorous Kit</td>
<td>Quimica Clinical Aplicada S.A</td>
<td>Spain</td>
</tr>
</tbody>
</table>

3.1.2 Equipment

EDTA blood collection tube. Test tubes and plain tube. 5cc syringes and needles were used to collect the blood sample. Rayto Spectrophotometer and cuvettes were used in this work for analysis the sample. Clover system used to tested HbA1c. The centrifuge was used to separate the samples. Micropipettes with disposable plastic tips. Vortex mixer. Water bath at 37 °C.

3.2 Method

3.2.1 Study design

The present study was a case-control and before after.
3.2.2 Study population

Study populations consist of 21 newly diagnosed diabetic male patients that were given one cup kefir milk daily with Metformin (Metformin, marketed under the trade name Glucophage among others, is the first-line medication for the treatment of type 2 diabetes) for ten weeks and compared with 21 patients who were given Metformin only.

3.2.3 Sampling and sample size

3.2.3.1 Study samples

The study sample included 42 newly diagnosed diabetic male patients who aged 37-65 years. They divided into two groups, the first group has 21 patients was a control. The case group has 21 patients.

3.2.3.2 Blood sample collection

The samples will be collected from all the patients for biochemical analysis (FBS, HbA1C, TG, Cholesterol, HDL, LDL, urea, creatinine, uric acid, Calcium, phosphorus), at the beginning of the study and after 10 weeks.

3.2.4 Kefir Grains and samples

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

The method of producing kefir achieved by adding kefir grains directly to 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 using in the next inoculation (Semih, 2003).

3.2.5 Glucose Measurement

Glucose concentration is determined by a hexokinase-mediated reaction. In this enzymatic method, in the presence of a phosphate donor, ATP, glucose is converted to glucose-6-phosphate (G-6-P) by hexokinase, Glucose-6-phosphate dehydrogenase after that the G-6-P converts to gluconate-6-P in the existing of NADP+. Also, during this reaction, the NADP+ is reduced to NADPH, in absorbance at 340 nm. This is an endpoint interaction that is specific for glucose (NHANES, 2007).
The method was used is GOD_POD METHOD

3.2.6 HbA1c test
The clover instrument was used to test the sample. The clover A1c system used to determine the average of Hemoglobin A1c (HbA1c %) in whole blood and is a fully automated boronate affinity assay. The clover A1cTest Cartridge includes a Reagent Pack and a Cartridge.

The Reagent Pack is pre-filled with reagent solution and rinsing solution. The reagent solution consists of agents that lyse erythrocytes and boronate bead that links cis-diols of glycated hemoglobin. A blood sample size of 4µL is obtained with the sampling area of the reagent pack.

By admitting the Reagent Pack into the Cartridge the blood is immediately lysed releasing the boronate bead binding the glycated hemoglobin and the Hemoglobin. The mixture of the blood sample is rotated to the measurement zone of the cartridge, by the reflectance of the photosensor, then measured the amount of total hemoglobin in the blood sample. This photosensor consists of PD (Photo Diode) and LED (Light Emitting Diode).

The cartridge is then rotated so that the rinsing solution washes out non-glycated Hemoglobin from the blood sample, also measured the amount of glycated hemoglobin photometrically. Then calculate the ratio of glycated hemoglobin with respect to total Hemoglobin in the blood sample.

3.2.7 Lipid profile Test

3.2.7.1 Cholesterol Measurement

Cholesterol is enzymatically examined in plasma or serum in a chain of connected interactions that oxidize the 3-OH group of cholesterol and hydrolyze cholesteryl esters. One of the interaction byproducts, H$_2$O$_2$ is quantitatively determined in a peroxidase-catalyzed interaction that produces a color. Absorbance is carried out at 500 nm. The intensity of the color is proportional to cholesterol level (NHANES 2003-2004).

Reagent contents.

(i) Ether and ethyl alcohol (95%) and are mixed in a ratio of 1:3.
(ii) Chloroform- must be a high clarity and completely anhydrous.

(iii) Conc. sulphuric acid and acetic anhydride-sulphuric acid mixture-Acetic anhydride are mixed in a ratio of 1:20 just before use.

(iv) standard Stock cholesterol solution 200 mg of chemically clear cholesterol is dissolved in and diluted to 100 ml with chloroform(Jagarati 2004).

**3.2.7.2 Triglyceride Measurement**

GPO METHOD was used. The principle is Triglyceride present in the sample are enzymatically hydrolyzed by the action of lipases leading to glycerol and fatty acid. In the presence of glycerol Kinase, the ATP phosphorylates glycerol to give glycerol-3-phosphate and the corresponding ADP. By GPO, glycerol-3-phosphate is oxidized to hydrogen peroxide and dihydroxyacetone phosphate. In the last stage, with the peroxidase as a catalyst, hydrogen peroxide reacts with 4-amino antipyrine and 4-chlorophenol to give quinonimine. Absorbance is measured at 500 nm. The intensity of the red color is proportional to a number of triglycerides found in the sample. The kit purchase from Abnaa Ghanem company.

Reference Values

Women 40 -160 mg/dl

Men 35-135 mg/dl

**3.2.7.3 HDL cholesterol Measurement**

DEXTRAN SULFATE - Mg METHOD, the principle is VLDL and LDL are precipitated from serum by the action of a sulfated polysaccharide, in the presence of divalent cations. Then the cholesterol bound to high-density lipoprotein present in the supernatant is determined. The kit was purchased from Abnaa Ghanem Company.

R1 reagent (6 x 54 mL). CHES pH 7.4, HEPES buffer, peroxidase, dextran sulfate, ascorbate oxidase, magnesium nitrate hexahydrate, HSDA, preservative.

R2 reagent (6 x 20 mL). PEG-cholesterol esterase, HEPES buffer, pH 7.0, PEG-cholesterol oxidase, 4-amino-antipyrine, peroxidase, preservative. See insert for concentrations. (NHANES 2008).
3.2.7.4 LDH Cholesterol Measurement

LDL cholesterol (LDL-C) are recommended for secondary and primary forbidding of cardiovascular events. It is not measured directly and primarily calculated measure via the Friedewald equation. triglycerides (TG), HDL cholesterol (HDL-C), and total cholesterol (TC) are used by the Friedewald equation to calculate LDL-C [LDL-C= TC-HDL-C-(TG/5)] (Meeusen et al., 2014).

3.2.8 Creatinine Measurement

Jaffe's Alkaline Picrate Method: A red tautomer of creatinine picrate color result from the interaction between Creatinine and picric acid in alkaline medium, the intensity of red tautomer color is measured at 520nm. And have Sodium hydroxide – 0.75N. Picric acid – 0.04M (9.16g/L). Sodium tungstate – 10%. 2/3 N H2SO4. Creatinine standard stock – 100mg%. Working standard – 3mg% (Jagarati 2004).

3.2.9 Urea test

Diacetyl Monoxime Method was used:

1) Reagent A: Transfer to a graduated cylinder 5g of ferric chloride dissolved in 20ml of water and slowly with stirring add 100ml of orthophosphoric acid (85%). Complete the volume to 250ml with water. Keep in the brown bottle at 4°C.

2) Reagent B: To 800 ml water in 2L flask add 200 ml conc, H2SO4 slowly with cooling and stirring.

3) Acid Reagent: To 1 L of reagent B Add 0.5 ml of reagent A. keep in the brown bottle at 4°C.

4) Reagent C: Diacetyl monoxime 20g/L of water. Keep and filter in the brown bottle at 4°C.

5) Reagent D: Thiosemicarbazide 5g/L of water.

6) Color Reagent: Make a mixture of 67 ml of C with 67 ml of D and complete the volume to 1000 ml with d.H2O put in the brown bottle at 4°C to keep it.

7) Stock urea standard: 100mg/100 ml water.

8) Working standard urea: Dilute 1 ml stock to 100ml with dH2O so conc. is 1 mg/100ml (Jagarati 2004).
3.2.10 Blood Uric Acid Measurement

Caraway's Method of Estimation: Reagents: Sodium tungstate 10%. 2/3 N Sulphuric acid. Tungstic acid: Add a drop of phosphoric acid, 50ml 2/3 N H$_2$SO$_4$ and 50ml of 10% sodium tungstate with mixing to 800ml water. When cloudy reject. Store in a brown bottle. Phosphotungstic acid: In about 400ml of water Stock-Dissolve 50g sodium tungstate. Add 40ml 85% phosphoric acid and softly reflux for 2 hours, cool, make volume to 500m. Store in a brown bottle. For use dilute 1to 1. Na$_3$CO$_3$ 10%. Standard uric acid solution stock-100mg%. Working uric acid solution-1mg% (Jagarati 2004).

3.2.11 Calcium test

Colorimetric Method at pH 8.5 arsenazo dibenzenearsonic acid reacts with calcium ion to form a complex colored. The intensity of color progressing is proportional to the calcium concentration in the sample. The Kit is purchased from Abnaa Ghanem Company.

Normal range in serum is 8.8 – 10.5 mg/dl.

3.2.12 phosphorous test

FISKE _ SUBBAROW Method was used. To produce phosho-molybdate, the phosphate ion reacts with molybdate. phosho-molybdate is later reduced to a molybdenum blue, which is measured photometrically in the UV range.

Normal values

Serum: 2.5 – 5.0 mg/dl

Urine: 0.3 – 1.0 g/24 hours.

3.2.13 Result analysis

All obtained data were analyzed by T-test paired sample using SPSS (Version 20) system. Difference between variables will be considered statistically significant if p-value < 0.05.
Chapter 4

Results
Chapter 4
Results

4.1 The effect of Kefir on the different Biochemical parameters.

4.1.1 Blood glucose and HbA1c of case and control at the beginning of the study

Table 4.1 showed that the p-value for FBS equal 0.723 which was greater than 0.05 that means there was no significant difference between control group and case group. And the p-value for HbA1c equal 0.848, this means there was no significant difference between control group and case group.

Table (4.1): Measurement of blood Glucose parameters of diabetic patients before intake the Kefir milk (case-control)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FBS (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>112.5</td>
<td>45.05</td>
<td>0.723</td>
</tr>
<tr>
<td>Control</td>
<td>117.76</td>
<td>49.90</td>
<td></td>
</tr>
<tr>
<td><strong>HbA1c</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>8.54</td>
<td>1.56</td>
<td>0.848</td>
</tr>
<tr>
<td>Control</td>
<td>8.63</td>
<td>2.03</td>
<td></td>
</tr>
</tbody>
</table>

*critical value of t at df “20” and significance level 0.05 equal

4.1.2 Kidney function test of case and control at the beginning of the study

Table 4.2 showed that the p-value for urea was 0.107 which is greater than 0.05 that means there was no significant difference between case group and control group. The p-value of creatinine equal 0.862, this means there was no significant difference between case group and control group. Also, table 4.2 showed that the p-value for uric Acid equal 0.702, this means there was no significant difference between case group and control group.
Table (4.2): Measurement of kidney function parameters of diabetic patients before intake the Kefir milk (case-control)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urea (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>30.85</td>
<td>6.46</td>
<td>0.107</td>
</tr>
<tr>
<td>Control</td>
<td>35.85</td>
<td>11.8</td>
<td></td>
</tr>
<tr>
<td><strong>Creatinine (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>0.752</td>
<td>16619.</td>
<td>0.862</td>
</tr>
<tr>
<td>Control</td>
<td>0.759</td>
<td>18413.</td>
<td></td>
</tr>
<tr>
<td><strong>Uric Acid (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>4.023</td>
<td>1.31</td>
<td>0.702</td>
</tr>
<tr>
<td>Control</td>
<td>4.20</td>
<td>1.47</td>
<td></td>
</tr>
</tbody>
</table>

4.1.3 lipid profile parameters of case and control at the beginning of the study

The table 4.3 shows that the p-value for cholesterol equal 0.170 which was greater than 0.05, this means there was no significant difference between case group and control group. The p-value for triglyceride equal 0.066, this means there was no significant difference between case group and control group. Also, shows that the p-value for HDL equal 0.942, this means there was no significant difference between case group and control group. And the p-value for LDL equal 0.086, that means there was no significant difference between case group and control group.
Table (4.3): measurement of lipid profile parameters of diabetic patients before intake the Kefir milk (case-control)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cholesterol (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>160.66</td>
<td>21.98</td>
<td>0.170</td>
</tr>
<tr>
<td>Control</td>
<td>179.62</td>
<td>55.08</td>
<td></td>
</tr>
<tr>
<td><strong>Triglyceride(mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>92.76</td>
<td>41.90</td>
<td>0.066</td>
</tr>
<tr>
<td>Control</td>
<td>128.47</td>
<td>66.10</td>
<td></td>
</tr>
<tr>
<td><strong>HDL(mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>67.07</td>
<td>11.87</td>
<td>0.942</td>
</tr>
<tr>
<td>Control</td>
<td>67.40</td>
<td>14.99</td>
<td></td>
</tr>
<tr>
<td><strong>LDL(mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>75.55</td>
<td>19.09</td>
<td>0.086</td>
</tr>
<tr>
<td>Control</td>
<td>90.77</td>
<td>34.26</td>
<td></td>
</tr>
</tbody>
</table>

*critical value of t at df "20" and significance level 0.05 equal

4.1.4 Phosphorous and Calcium of case and control at the beginning of the study

The result of Table 4.4 shows that the p-value for phosphorous equal 0.712 which is greater than 0.05, this means there was no significant difference between case group and control group. And the p-value for calcium equal 0.573 which is greater than 0.05, this means there was no significant difference between case group and control group.
Table (4.4): measurement of Minerals parameters of diabetic patients before intake the Kefir milk (case-control)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorous (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>3.44</td>
<td>0.436</td>
<td>0.712</td>
</tr>
<tr>
<td>Control</td>
<td>3.49</td>
<td>0.690</td>
<td></td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>8.15</td>
<td>0.727</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.04</td>
<td>0.709</td>
<td>0.573</td>
</tr>
</tbody>
</table>

*critical value of t at df "20" and significance level 0.05 equal

4.1.5 Blood glucose and HbA1c of case group before and after intake Kefir

The table 4.5 shows that for FBS the p-value was 0.045 this means a significant difference 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.

Table (4.5): Statistically analysis of blood Glucose parameters of case group before and after intake of Kefir milk.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>112.5</td>
<td>45.05</td>
<td></td>
</tr>
<tr>
<td>After</td>
<td>91.7</td>
<td>12.79</td>
<td>0.045*</td>
</tr>
<tr>
<td>HbA1c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>8.54</td>
<td>1.56</td>
<td></td>
</tr>
<tr>
<td>After</td>
<td>7.20</td>
<td>1.12</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

*critical value of t at df "20" and significance level 0.05 equal

4.1.6 Kidney function test of case group before and after intake Kefir

The result of Table 4.6 shows that the p-value for urea was 0.092 which is greater than 0.05, that means there was no significant difference between before and after taking Kefir. The table 4.6 showed that the p-value for creatinine equal 0.489, this means there was no significant difference between before and after taking Kefir. It shows that the p-value for uric Acid equal 0.038 which is less than
0.05, this means there was a significant difference between before and after taking Kefir.

Table (4.6): Statistically analysis of kidney function parameters of case group before and after intake of Kefir milk.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urea (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>30.85</td>
<td>6.46</td>
<td>0.092</td>
</tr>
<tr>
<td>After</td>
<td>33.89</td>
<td>6.03</td>
<td></td>
</tr>
<tr>
<td><strong>Creatinine (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0.75</td>
<td>0.16</td>
<td>0.489</td>
</tr>
<tr>
<td>After</td>
<td>0.77</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td><strong>Uric Acid (mg/dl)</strong></td>
<td></td>
<td></td>
<td>*0.038</td>
</tr>
<tr>
<td>Before</td>
<td>4.02</td>
<td>1.31</td>
<td></td>
</tr>
<tr>
<td>After</td>
<td>3.50</td>
<td>1.07</td>
<td></td>
</tr>
</tbody>
</table>

*critical value of t at df "20" and significance level 0.05 equal

4.1.7 Lipid profile parameters of case group before and after intake Kefir

Table 4.7 shows that the p-value for cholesterol equal 0.107 which is greater than 0.05, this means there was no significant difference between before and after measurement. And table showed that the p-value for triglyceride equal 0.367 this means there is no significant difference between before and after taking Kefir. The table also, shows that the p-value for HDL equal 0.706 which is greater than 0.05, this means there is no significant difference between before and after taking Kefir. The result shows that the p-value for LDL equal 0.114, this means there was no significant difference between before and after taking Kefir.
Table (4.7): Statistically analysis of lipid profile parameters of case group before and after intake of Kefir milk.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cholesterol (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>160.66</td>
<td>21.98</td>
<td>0.107</td>
</tr>
<tr>
<td>After</td>
<td>150.66</td>
<td>28.43</td>
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</tr>
<tr>
<td><strong>Triglyceride (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>92.76</td>
<td>41.90</td>
<td>0.367</td>
</tr>
<tr>
<td>After</td>
<td>88.23</td>
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<tr>
<td><strong>HDL (mg/dl)</strong></td>
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<td></td>
</tr>
<tr>
<td>Before</td>
<td>67.07</td>
<td>11.87</td>
<td>0.706</td>
</tr>
<tr>
<td>After</td>
<td>66.38</td>
<td>12.48</td>
<td></td>
</tr>
<tr>
<td><strong>LDL (mg/dl)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>75.55</td>
<td>19.09</td>
<td>0.114</td>
</tr>
<tr>
<td>After</td>
<td>66.58</td>
<td>25.47</td>
<td></td>
</tr>
</tbody>
</table>

*critical value of $t$ at df “20” and significance level 0.05 equal

4.1.8 Phosphorous and Calcium of case group before and after intake Kefir

The result in table 4.8 showed that the p-value for phosphorous equal 0.410 which is greater than 0.05, this means there was no significant difference between before and after taking Kefir. Also, table 4.8 showed that the p-value for calcium equal 0.000, that means there was a highly significant difference between before and after taking Kefir.
Table (4.8): Statistically analysis of Minerals parameters of case group before and after intake Kefir milk.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phosphorous (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>3.44</td>
<td>0.44</td>
<td>0.410</td>
</tr>
<tr>
<td>After</td>
<td>3.36</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td><strong>Calcium (mg/dl)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>8.15</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>After</td>
<td>8.45</td>
<td>0.58</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

*critical value of t at df "20" and significance level 0.05 equal

4.1.9 Blood glucose and HbA1c of case and control at the end of the study

Table 4.9 shows that the p-value for FBS equal 0.000 which is less than 0.05, this means there was a highly significant difference between case group and control group. Also the p-value for HbA1c equal 0.000 which is less than 0.05, this means there was highly significant difference between case group and control group.

Table (4.9): Measurement of blood Sugar parameters of diabetic patients after intake the Kefir milk (case-control)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FBS (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>91.71</td>
<td>12.78</td>
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</tr>
<tr>
<td>Control</td>
<td>145.47</td>
<td>45.63</td>
<td></td>
</tr>
<tr>
<td><strong>HbA1c</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>7.20</td>
<td>1.123</td>
<td>0.000</td>
</tr>
<tr>
<td>Control</td>
<td>9.14</td>
<td>1.71</td>
<td></td>
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</tbody>
</table>

*critical value of t at df "20" and significance level 0.05 equal

4.1.10 Kidney function test of case and control at the end of the study

Table 4.10 shows that the p-value for urea equal 0.529 which is greater than 0.05, that means there is no significant difference between case group and control group. And also, showed that the p-value for creatinine equal 0.815, that means there was no
significant difference between case group and control group. And the p-value for uric Acid equal 0.164, this means there was no significant difference between case group and control group.

Table (4.10): Measurement of kidney function parameters of diabetic patients after intake the Kefir milk (case-control)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urea (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>33.80</td>
<td>6.03</td>
<td>0.529</td>
</tr>
<tr>
<td>Control</td>
<td>35.47</td>
<td>9.95</td>
<td></td>
</tr>
<tr>
<td><strong>Creatinine (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>0.776</td>
<td>0.109</td>
<td>0.815</td>
</tr>
<tr>
<td>Control</td>
<td>0.766</td>
<td>0.187</td>
<td></td>
</tr>
<tr>
<td><strong>Uric Acid (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>3.50</td>
<td>1.07</td>
<td>0.164</td>
</tr>
<tr>
<td>Control</td>
<td>4.17</td>
<td>1.43</td>
<td></td>
</tr>
</tbody>
</table>

*critical value of t at df "20" and significance level 0.05 equal

4.1.11 Lipid profile parameters of case and control at the end of the study

Table 4.11 shows that the p-value for cholesterol equal 0.109 which is greater than 0.05, this means there was no significant difference between case group and control group. And the p-value for triglyceride equal 0.104, this means there was no significant difference between case group and control group. Also, table 4.11 showed the p-value for HDL equal 0.969, that means there was no significant difference between case group and control group. In addition to the p-value for LDL equal 0.989, this means there was no significant difference between case group and control group.
Table(4.11): Measurement of lipid profile parameters of diabetic patients after intake the Kefir milk (case-control)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cholesterol (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>150.66</td>
<td>28.43</td>
<td>0.109</td>
</tr>
<tr>
<td>Control</td>
<td>165.04</td>
<td>26.79</td>
<td></td>
</tr>
<tr>
<td><strong>Triglyceride (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>88.23</td>
<td>28.17</td>
<td>0.104</td>
</tr>
<tr>
<td>Control</td>
<td>111.52</td>
<td>52.73</td>
<td></td>
</tr>
<tr>
<td><strong>HDL (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>66.38</td>
<td>12.48</td>
<td>0.969</td>
</tr>
<tr>
<td>Control</td>
<td>66.51</td>
<td>12.76</td>
<td></td>
</tr>
<tr>
<td><strong>LDL (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>66.58</td>
<td>25.47</td>
<td>0.969</td>
</tr>
<tr>
<td>Control</td>
<td>66.47</td>
<td>32.45</td>
<td></td>
</tr>
</tbody>
</table>

*critical value of t at df "20" and significance level 0.05 equal*
4.1.12 Phosphorous and Calcium of case and control at the end of the study

Table 4.12 shows that for phosphorous the p-value was 0.014. This means there is a significant difference between case group and control group. And the p-value for calcium was 0.073 this means there is no significant difference between case group and control group.

Table (4.12): Measurement of Minerals parameters of diabetic patients after intake the Kefir milk (case-control)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phosphorus (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>3.36</td>
<td>0.438</td>
<td>0.014*</td>
</tr>
<tr>
<td>Control</td>
<td>3.86</td>
<td>0.751</td>
<td></td>
</tr>
<tr>
<td><strong>Calcium (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>8.45</td>
<td>0.575</td>
<td>0.073</td>
</tr>
<tr>
<td>Control</td>
<td>7.66</td>
<td>1.769</td>
<td></td>
</tr>
</tbody>
</table>

*critical value of t at df "20" and significance level 0.05 equal
Chapter 5

Discussion
Chapter 5
Discussion

5.1 Characteristic of the study population

The present study is a case-control investigation, comprised of case group and control group. The experiments carried out from September to December of the year 2016. 42 newly diagnosed male diabetic patients who aged 37–65 years were involved in the study. The 42 patients divided into the two groups, 21 patients in each group. The control received Metformin only and the cases were intake one cup of kefir milk daily with Metformin for ten weeks.

5.2 Biochemical Measurement

Eleven biochemical measurements were obtained from each patient who includes the following:

5.2.1 Fasting Blood Glucose and HbA1c

In insulin resistance status or diabetes, failure of insulin encouraged glucose uptake by muscle and fat leads high concentrations of glucose in the blood, also increase the glucose uptake by insulin-independent tissues. Consequently, in diabetes mellitus this condition the multiple interacting pathways causes the elevated of the damage of antioxidant defenses and oxidant products (Ostadrahimi et al., 2015).

This study demonstrated that the supplementation of one cup of clear kefir drink for 10 weeks, significantly affects HbA1c and blood glucose. Kefir milk reduced hyperglycemia. In several animal and human studies, some studies have been investigated the antidiabetic effect of Lactobacillus and Bifidobacteria. Also, other studies have expressed treatment by probiotic can lower blood glucose levels in diabetic status. Many possible mechanisms of this effect are expressed. A possible confirmation of hypoglycemic effect is that probiotics affected gut bacteria to introduce insulinitropic polypeptides and glucagon-like peptide so induce uptake of glucose by muscle. In addition in the form of glycogen, liver induces the absorption of more blood glucose (Ostadrahimi et al., 2015).

As well as, clear kefir also proved to affect pancreatic β cell regeneration. This mechanism underlying 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 (Judiono et al., 2014).

Carl et al., 2015 reported that fasting blood glucose concentrations and glycosylated hemoglobin (HbA1c) levels were reduced in type 2 diabetic patients by consumption of probiotic yogurt.

Judiono et al., 2014 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). In Ostadrahimi et al., 2015 studied, the consumption of probiotic fermented milk causes the reduction of HbA1C and fasting blood glucose in comparison with conventional fermented milk. El-bashiti et al., 2017 also, found significant reductions in insulin growth factor and fasting blood sugar. And these result was compatible with our results, after intake Kefir milk for ten weeks, there was highly reduction in HbA1c and FBS.

5.2.2 Kidney function test

Diabetic complications, such as retinopathy, neuropathy, and nephropathy strongly linked with Hyperglycemia and oxidative stress (Punaro et al., 2014). Probiotic Kefir has been proposed to contribute to reducing the progressing of renal injury in diabetes (Badawi, 2016).

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. Previous studies have reported that the onset of, hyperinsulinemia, hyperglycemia, oxidative stress and glucose intolerance in rats with type 2 diabetes significantly delayed by supplementation with a strain of Lactobacillus and in
addition to decreasing the risk of the evolution of associated complications (Punaro et al., 2014).

In our study urea, creatinine and the uric acid levels were not affected by Kefir diet. There was no significant variance between two groups. Urdaneta et al., (2007) reported the same result. That is mean Kefir milk does not affect the kidney function.

El-bashiti et al., 2017 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.

Gungor et al., 2014 found that by intake Kefir, creatinine clearance was higher. Kefir playing as an Angiotensin-converting enzyme inhibitor so Kefir reduced renal function damage. And concluded that kefir protects renal function and renal damage stimulated by high salt diet in rats.

5.2.3 Lipid profile

We found in our study that, no significant decreases in triglyceride, cholesterol, LDL, and HDL. Some previous findings are in agreement with that obtained by St-Onge et al. (2002) Who reported that Kefir had no effect on triglyceride concentrations, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol or total cholesterol nor on cholesterol fractional synthesis rates after 4 weeks of Kefir supplementation. Ostadrahimi et al., (2015) also reported that triglyceride, total cholesterol, and LDL cholesterol in probiotic fermented milk (Kefir) reduced toward to conventional fermented milk but these changes were not statistically significant.

Other studies reported that Kefir led towards a reducing of total cholesterol concentrations and serum triglyceride (Je-Ruei Liu et al., 2006) reported Kefir diets tended towards a lowering of serum total cholesterol concentrations and triacylglycerol, and a decreasing of cholesterol accumulation in the liver, the reduction in serum cholesterol concentration being essentially in the non-HDL fraction.
In response to Kefir treatment total cholesterol serum and total lipid levels were significantly lowered compared to that of in control group (Cenesiz et al., 2008) reported triacylglycerols, total cholesterol, LDL-c and HDL-c in rats fed cholesterol-rich diet, fermented Kefir decrease significantly the levels of LDL-c, VLDL, and triacylglycerols, also, having increased HDL-c.

The mechanistic approach for hypocholesterolemic characteristics implies that probiotic strains use cholesterol for their own metabolism. Probiotics bind to cholesterol and convert the binding cholesterol to its catabolic products. Therefore, the cholesterol levels lowered by the deconjugating cholesterol to the bile acids. This mechanism causes the lowering of total body pool of cholesterol. *L.acidophilus* exhibits the 3-hydroxy 3-methyl glutamyl CoA reductase, that is a rate-limiting enzyme responsible for the endogenous cholesterol biosynthesis in the body and this enzyme can deconjugate bile acids in the gut and, eventually, this process causes the lowering cholesterol concentration(Ostadrahimi et al., 2015). As previously mentioned, these significant changes were not shown in this study; the possible reasons, which might be noted, are differences in probiotic strains and genetic differences in our patients. And also the conflicting results may be due to the difference in kefir grains used in the experimental study.

Tu, et al., 2006 reported that different strains of lactic acid bacteria may have different effects on serum cholesterol concentration. The microbial composing of kefir grains has been reported to show variations in a population depending on the manufacturing method, the origin of the grains, and the cultivation method.

Consuming probiotic foods and Kefir reducing blood sugar levels, blood lipid levels, and high blood pressure and lowering the symptoms of food allergies effectively. Animal studies Proved the importance of Kefir in decreasing effect on blood pressure, and on levels of LDL cholesterol (Shavit, 2008).

One of the hypercholesterolemia treatments is to apply cholesterol- reducing drugs but these drugs have known many side effects. So, lately, it implored to apply other beneficial productions like probiotics in lowering blood cholesterol (Tahere et al., 2016).
5.2.4 Calcium

Min-Yu Tu et al., (2015) indicated that calcium of serum was slightly increased in patients who consume Kefir treatment for six months this due to Kefir contain proteins or bioactive peptides with the potential that improve calcium absorption and bone mineral density. Chen et al., (2015) reported that a 12-week treatment with kefir increased the level of serum Calcium. But in our study receiving Kefir for ten weeks had no significant effect on serum calcium.

5.2.5 Phosphorous

Our finding showed that intake Kefir milk for ten weeks had an effect on serum phosphorous. There is no previous study reported about this.
Chapter 6

Conclusion and Recommendation
Chapter 6
Conclusion and Recommendation

6.1 Conclusion

- There were significant decreases in blood glucose and HbA1c in patients who intake Kefir milk
- There was no significant effect of Kefir on serum Calcium level after intake for 10 weeks.
- In contrast, there was a significant decrease in serum phosphorus level
- No significant effect on Kidney function test by Kefir was observed
- Also, there was no significant effect on triglyceride, low-density lipoprotein, High-density lipoprotein, and cholesterol were observed.
- Probiotic fermented milk can be useful as a complementary or adjuvant therapy in the treatment of diabetes.

6.2 Recommendations

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