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Effect of Kefir on Glucose Concentration and Blood Biochemical Parameters in Alloxan-İnduced Diabetes Mellitus Rabbits

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Abstract

Kefir, a traditionally fermented beverage derived from milk, harbors a diverse microbial community that imparts a wide range of health benefits, including potential anti-diabetic properties. In this study, we investigated the potential positive effects of kefir consumption on blood parameters and blood glucose levels in diabetic rabbits. A total of 48 rabbits with an initial weight of 250 grams were divided into four research treatments (Control, DM+kefir: diabetic rabitts + 3.6 ml kefir/ 200 g rabitts, DM+Gliben: diabetic rabitts + 600 µg Glibenclamide /kg rabitts) and kept under standard conditions for 40 days. Blood parameter measurements were conducted on the 20th and 40th days of the experiment. The results revealed consistent findings for the evaluated indicators across both time points. Significant differences (P<0.05) were observed in the levels of cholesterol, triglycerides, glucose, and LDL between the treated diabetic rabbits and the control group. Furthermore, the diabetic rabbits exhibited significantly lower levels of HDL compared to the control group (P<0.05). Notably, the use of kefir as a treatment led to a significant reduction in these indicators, which were significantly different from the diabetic treatment group (P<0.05). Interestingly, no significant difference was observed between the kefir treatment and the Glibon drug, as indicated by several indicators (P>0.05). These findings highlight the potential beneficial effects of kefir on blood parameters in diabetic rabbits.

Keywords*: Kefir, rabitts, diabetics, Glibenclamide, glucose, lipid*

Introduction

Diabetes was initially documented by the Egyptians and is characterized by weight loss and excessive urination (polyuria). However, it was the Greek physician Aertaeus who coined the term "diabetes mellitus." In Greek, "diabetes" means "to pass through," while "mellitus" is the Latin word for honey, referring to its sweetness. Diabetes is a significant cause of prolonged illness and premature mortality, claiming more lives per year than HIV/AIDS, with nearly one death every ten seconds (Suryasa, Rodríguez-Gámez, & Koldoris, 2021).

With the rise of industrialization worldwide and the alarming increase in obesity rates, diabetes has emerged as a global epidemic. Determining its accurate prevalence is challenging due to variations in data collection standards and methods across different regions. However, recent surveys predict a rise in diabetes prevalence among adults, from 4% in 1995 to 6.4% by 2025 (Nurliyani, Harmayani, & Sunarti, 2015). Additionally, there is an estimated rapid increase of 42%

(from 51 to 72 million) in developed countries and a staggering 170% increase (from 84 to 228 million) in the developing world (Ostadrahimi et al., 2015). The number of adults affected by diabetes worldwide is projected to rise from 194 million in 2003 to nearly 380 million in 2025. India, China, and the USA are expected to be the countries most impacted by this epidemic.

Glucagon and insulin are released into the bloodstream as part of a negative feedback loop that regulates blood glucose levels. High blood glucose levels trigger insulin secretion by the pancreatic islets of Langerhans' beta cells. Two chains, A and B, are connected by disulfide bridges to form insulin, a polypeptide. It is produced from pro-insulin by means of the exo-protease carboxypeptidase and two pro-hormone convertases, PC I and PC 2. The insulin receptor is a tyrosine kinase that is bound to insulin by disulfide bonds between its two intracellular beta subunits and its two extracellular alpha subunits (Figure 1). When insulin binds to the beta subunit of the insulin receptor, which is a tyrosine kinase, the beta subunit is autophosphorylated. After that, insulin tells the liver to store the extra glucose as glycogen and prompts other cells (such those in skeletal muscle and adipose tissue) to translocate the glucose transporter (GLUT4) to their surface so they may take in more glucose. This contributes to the normalisation of blood glucose levels.

Diagnostic criteria for diabetes are based on sustained elevation of blood glucose concentration. Diabetic blood glucose levels often rise over the healthy range. Standard blood glucose levels for diabetes include 200 mg/dL (11.1 mmol/L) when measured at random (Association, 2010), 126 mg/dL (7 mmol/L) when measured during fasting (Mehta & Wolfsdorf, 2010), and oral glucose tolerance tests, which measure plasma glucose levels two hours after oral glucose intake and require levels higher than 200 mg/dL (11.1 mmol/L) (Association, 2010). Type 1 diabetes mellitus (T1DM) symptoms include increased thirst, polyphagia, polyuria, weight loss, weakness, weariness, impaired vision, poor wound healing, irritability, tingling in the hands or feet, and frequent bladder, vaginal, and skin infections (Mellitus, 1985).

Methods:

This study aimed to explore the effects of Skimmed Milk-Kefir on diabetic parameters in a rabbit model. A total of forty-eight male rabbits, aged 12-14 weeks and weighing 200-250 grams, were carefully selected to participate in the experiment. The rabbits were housed in the animal facility of the College of Medicine, University of Muthanna, where they received appropriate care and were subjected to optimal environmental conditions. Throughout the study, the rabbits were fed with standard pellets and had unrestricted access to water to ensure their nutritional needs were met. To maintain a consistent and controlled environment, factors such as temperature and lighting were carefully regulated. The rabbits were divided into four groups, each comprising three rabbits. The first group served as the control group and received oral administration of normal saline. The second group was induced with diabetes mellitus through a single intraperitoneal injection of alloxan, and they also received oral administration of normal saline, serving as the diabetic control group. The third group was induced with diabetes mellitus and received Skimmed Milk-Kefir orally at a dose of 3.6 ml/200g. Lastly, the fourth group was induced with diabetes mellitus and administered Glibenclamide, an antidiabetic medication, orally at a dose of 600 µg/kg. On both Day 20 and Day 40 of the experiment, blood samples were collected from six animals in each

group. These samples were collected to assess the impact of Skimmed Milk-Kefir and Glibenclamide on the diabetic parameters being investigated.

Blood Sampling and bichemical analyzing

To obtain the necessary samples for further analysis, blood sampling and plasma preparation procedures were conducted. Direct blood collection from the animals' hearts was performed using 5 mm syringes that were preloaded with heparin anticoagulant. This allowed for the prevention of coagulation during the sampling process. The collected blood samples were subsequently processed to separate the plasma component. To achieve this, the blood samples were subjected to centrifugation at a speed of 3500 g for 10 minutes. This centrifugation process resulted in the separation of the plasma from the cellular components of the blood. The plasma samples, containing a range of relevant indicators, were carefully separated from the cellular fraction and promptly stored at a temperature of -80°C. This storage temperature was maintained to ensure the integrity of the samples and to preserve their biochemical properties. In addition to the plasma samples, further blood samples were taken immediately after collection to be transported to a medical laboratory. To maintain their stability during transportation, these samples were handled under appropriate cooling conditions. Once in the laboratory, various blood parameters were analyzed using standard methods and techniques. These parameters included red and white blood cell counts, hematocrit, hemoglobin levels, and differential counts of white blood cells, specifically lymphocytes, neutrophils, and monocytes. Prior to blood collection, the rabbits participating in the study were weighed using a precise scale to monitor any potential weight variations that could impact the blood parameters being investigated.

Results:

Figure 1 presents the results of the experimental treatments on total cholesterol levels over two time periods of 20 and 40 days. The results indicate a consistent pattern across both the 20-day and 40-day periods. The treatment of diabetic rabbits demonstrated the highest total cholesterol levels, which were significantly different from the other experimental treatments ($P<0.05$). Furthermore, the utilization of kefir showed a significant reduction in cholesterol levels compared to the diabetic treatment ($P<0.05$). However, no significant difference was observed between the kefir treatment and the other experimental treatments (control and gliben drug) ($P>0.05$).

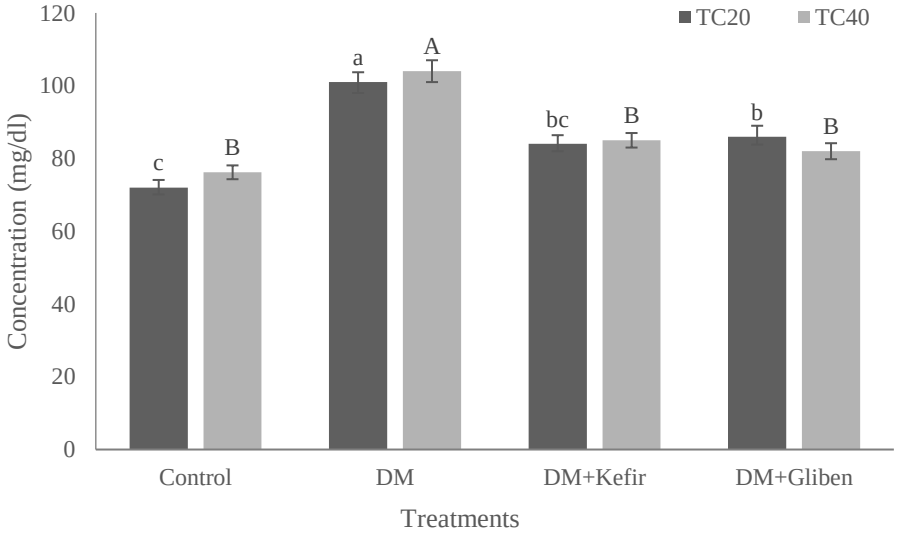
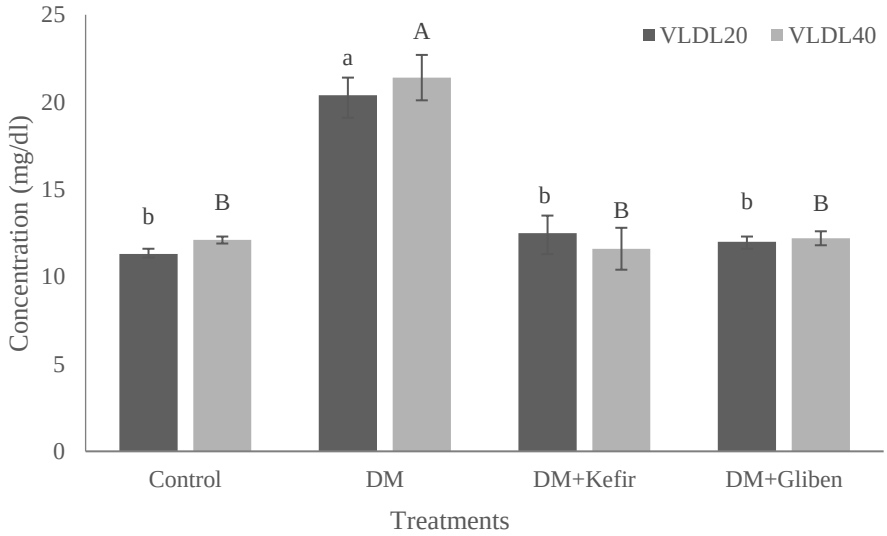
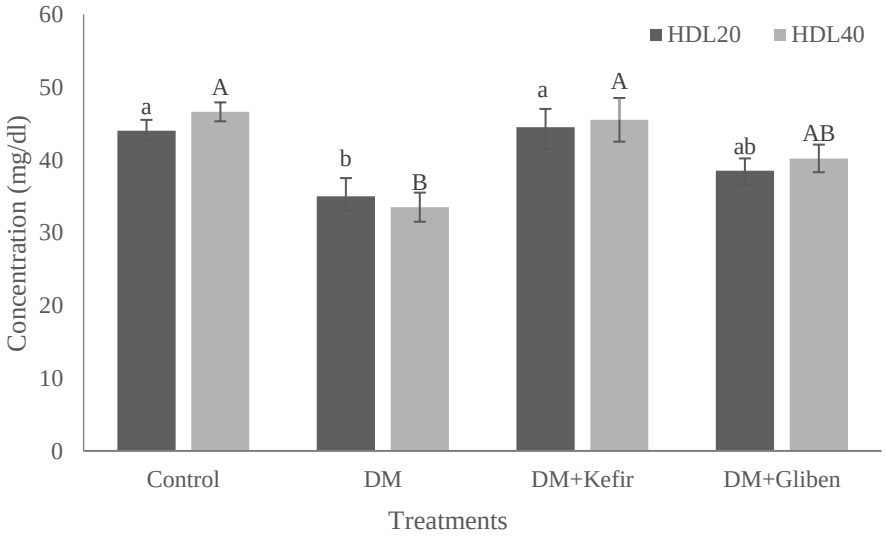


Figure 2- the results of the effects of experimental treatments on totla cholestrol in day 20 and 40. The data are presented as the mean ± SD (n = 3 per treatment). In the figures, DM group refer to diabetic rabbits while DM+Kefir group represents diabetic rabbits + 3.6 ml kefir /g per body weight of rabbits, and DM+Gliben clamide group represents diabetic rabbits + 600 µg Gliben clamide /kg per body weight of rabbit.

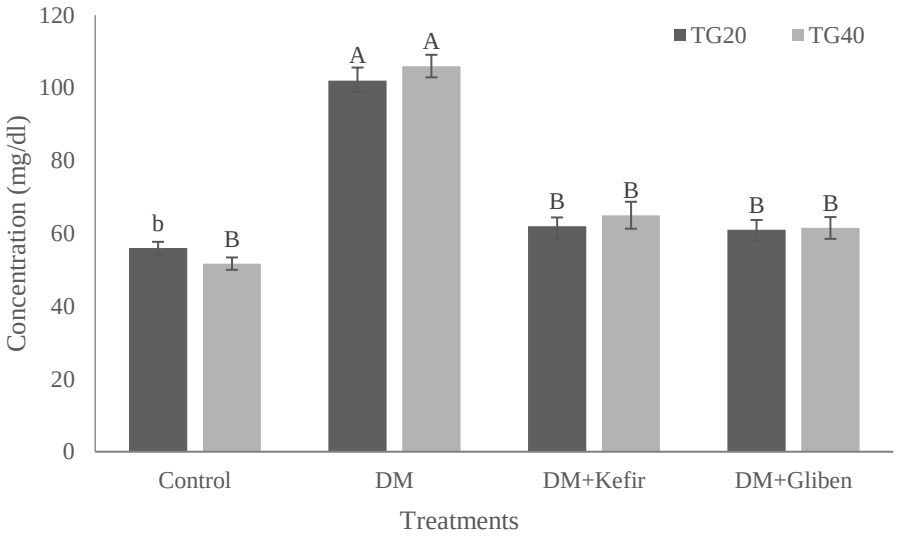
The figure below illustrates the results of experimental treatments on triglyceride concentration on days 20 and 40. The results demonstrate a consistent pattern throughout both the 20-day and 40-day periods of the study. The findings reveal that the treatment of diabetic rabbits exhibited the highest triglyceride levels, which were significantly different from the other treatments ($P<0.05$). Additionally, no significant difference was observed in the total triglyceride concentration among the other experimental treatments ($P>0.05$).



The results depicting the changes of HDL concentration induced by experimental treatments are displayed in Figure 12, representing the 20th and 40th days of the experiment. The results demonstrate a consistent pattern throughout both the 20-day and 40-day periods of the study. According to these findings, the treatment of diabetic rabbits exhibited the lowest rate of HDL, which significantly differed from the control treatment ($P<0.05$). Furthermore, the results indicate no significant difference between the kefir treatment and the control treatment ($P>0.05$) in term of HDL, with the utilization of kefir significantly elevating the observed levels compared to the diabetic treatment ($P<0.05$).



The figure below depicts the outcomes of experimental treatments on triglyceride concentration on days 20 and 40. The results consistently demonstrate a pattern across both the 20-day and 40-day periods of the study. It is evident that the treatment of diabetic rabbits exhibited the highest levels of VLDL, which were significantly different from the other treatments ($P<0.05$). Conversely, no significant difference was observed in the VLDL level among the remaining experimental treatments ($P>0.05$).



Discussion:

The findings of this experiment revealed that administering kefir at a concentration of 3.6 ml per 200 grams of rabbit weight has beneficial effects on plasma indices in rabbits with type 2 diabetes. These results indicate that the induction of type 2 diabetes leads to elevated levels of blood cholesterol, triglycerides, glucose, and LDL, while simultaneously reducing plasma HDL levels. However, the use of kefir effectively counteracts these detrimental effects and improves these indicators in rabbits with type 2 diabetes. In this section, we will delve into the mechanisms behind the observed positive effects of kefir on plasma indices.

High blood glucose levels, or hyperglycemia, can be caused by insufficient insulin production or action, or both. This disease is complicated and long-lasting, and it puts patients at risk for microvascular and macrovascular consequences down the road (Chaudhury et al., 2017). Worldwide, 463 million people, or almost 1 in 11 adults aged 20–79, have diabetes, making it a massive epidemic according to the International Diabetes Federation (IDF). Insulin production can be impaired over time due to glucose toxicity, which can occur in the absence of therapy for prolonged hyperglycemia. High blood glucose levels are harmful to the pancreas and require costly insulin treatment to prevent these effects (Chaudhury et al., 2017). Kefir, on the other hand, has been the subject of growing evidence of anti-diabetic benefits in studies conducted in the last decade, making it an attractive low-cost treatment alternative.

Research has shown that kefir may have anti-diabetic properties. An example of this is the work of Teruya et al. (2002), who found that the insulin signalling pathway molecules that were activated by the water and methanol-soluble fractions of kefram-kefir successfully controlled Type II diabetes. The absorption of glucose was enhanced as a result of this activation. A follow-up study by Maeda et al. (2004) found that after 30 days of kefiran feeding, genetically diabetic mice (KKAy) exhibited a marked trend towards decreased blood glucose levels in comparison to the control group, whose blood glucose concentrations remained consistently elevated. In addition, research by Kwon et al. shown that α-glucosidases and pancreatic α-amylase are hydrolytic

enzymes that, when inhibited, may considerably lessen the rise in blood glucose levels following a mixed carbohydrate meal. The treatment of type II diabetes relies heavily on this inhibitory method. In their 2006 study, Kwan et al. showed that fermented soymilk with Rhodiola extracts added to it through kefir culture had better anti-diabetic efficacy, as measured by a slight increase in α-glucosidase inhibitory activity. A new study showed that fermented soy milk (FSM) made via kefir might decrease the activity of α-amylase, with an IC50 value of 52.71 µg/mL. The intestinal and pancreatic α-amylase activities of HFFD rats that were given FSM were 26% and 31% lower, respectively, than those of untreated HFFD rats. According to Tiss et al. (2020), a 36% reduction in blood glucose levels was the result. In addition, Hadisaputro et al. (2012) studied the impact of oral supplementation with plain kefir on streptozotocin-induced hyperglycemia in Wistar rats. Consumption of kefir significantly reduced plasma glucose levels in comparison to the control group, according to the study. Similarly, Alsayadi et al. (2014) discovered that streptozotocin-induced diabetic Wistar rats that were fed water kefir instead of water for 35 days had lower blood glucose levels. The probiotic qualities of kefir have also been shown to help in the control of type 2 diabetes. Possible mechanisms for its beneficial effects on the colon and immune system include the synthesis of short-chain fatty acids (SCFAs) such butyrate, acetate, and propionate (Brasil et al., 2018). There is mounting evidence that kefir may have a therapeutic effect because to the inclusion of short-chain fatty acids (SCFAs), which have recently become key therapeutic targets in the management of type 2 diabetes.

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