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PROTECTIVE EFFECT OF PROBIOTIC SUPPLEMENTATION IN DIABETIC PREGNANT FEMALE MICE ON BLOOD SUGAR AND LIPIDS

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Abstract

The study was conducted in the animal house of the College of Veterinary Medicine / Tikrit University and in the laboratories of the General Company for the Pharmaceutical Industry and Medical Appliances / Samarra. for the period from October/ 2020 until the end of April/ 2021. It included the production of therapeutic yoghurt with the addition of a mixture of probiotic supplements Lactobacillus Casei, Lb. planetarium, Lb. acidophilus and Bifidobacterium. Bifiduem) and a second starter of two types of probiotic (Bif. Bifiduem and Lb. casei) with some fruits (cranberry and strawberry) and therapeutic drugs (Gliclazide and Metformin), to know their effect on some physiological and biochemical parameters such as measuring blood sugar values, total cholesterol (TC), triglycerides (TG), and low-density lipoproteins (LDL). very low-density lipoproteins (VLDL) and high-density lipoproteins (HDL) of female laboratory mice with alloxan-induced diabetes, as well as the effect of female mice with diabetes on their offspring and conducting the same criteria mentioned above.

The results showed a significant increase ($p<0.05$) in blood sugar concentrations (156.4) mg/dL, TC (195.4) mg/dL, TG (159.6) mg/dL, LDL (150) mg/dL, VLDL (31.92) mg/dL, and a significant decrease in HDL (35) mg/dL, while female mice were treated with alloxan.

It was also found that treating mice with therapeutic yogurt containing a mixture of (Bif. Bifiduem and Lb. casei) with some fruits (cranberry and strawberry) and therapeutic drugs (Gliclazide and Metformin), led to a significant decrease ($p<0.05$) in blood sugar concentrations (86.8) mg/dL, TC 162.4) mg/dL, TG (142.2) mg/dL,

LDL (95.4) mg/dL, VLDL (28.44) mg/dL, and a significant increase in HDL (52.60) mg/dL in pregnant female mice.

Key words: Gestational diabetes mellitus, probiotic, yoghurt, lipids, Gliclazide and Metformin, cranberry and strawberry.

Introduction

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance that occurs or is first recognized during pregnancy (Chiefari et al., 2017) and of maternal complications of gestational diabetes, hydramnios, preterm delivery, and caesarean section (Briana et al., 2018) As for the fetus, studies have indicated that fetuses or newborn children of mothers with gestational diabetes suffer from complications, such as congenital malformation, fetal death (Huynh et al., 2014), and congenital dyspnea. In addition, mothers with GDM and their babies They are at increased risk of metabolic dysfunction and type 2 diabetes (Chiefari et al., 2017).

The Food and Agriculture Organization and World Health Organization defines probiotics as “living microorganisms which, when administered in sufficient quantities, confer a health benefit to the host.” A probiotic can rebalance the normal flora of the gut, associated with metabolic diseases such as celiac disease. diabetes mellitus;

Some studies have also indicated that probiotics are given as biological supplements or added to yogurt, making them available for consumption (Asgharian et al., 2019).

Previous studies have shown that probiotics may improve metabolic control, such as blood sugar control, and mitigate some of the adverse effects of type 2 diabetes (Li et al., 2019)

Although the beneficial effects on gestational diabetes and its symptoms are still uncertain. Where Babadi et al (2018) found that probiotic supplementation improved the fasting glucose FBG level in the blood of mothers with gestational diabetes, while Ahmadi et al (2016) compared probiotic supplementation to placebo. There is no difference between them in controlling blood sugar.

As stated by Sanders, (2000) the probiotics added to fermented dairy products such as LAB and Bif. Bifiduem may reduce the level of LDL to the normal limit, in addition to being effective in the metabolism and digestion of fats.

Fruits (cranberries and strawberries) are also good sources of antioxidants, which include phenolic compounds in general, which have strong antioxidant activity in addition to a protective effect on human health (Tyagi et al., 2017).

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Materials and Methods

Animals were purchased from Samarra Pharmaceutical Laboratory / Pharmacology Department. In this study, (65) female and (32) male white laboratory Albino mice of (8) weeks old and weight (80-150) g for both genders were used. Males and females were placed in cages. Separately, they were examined and ensured that they were safe, healthy and free of diseases, and then they were placed in the laboratory animal house Consisting of the yoghurt that was produced according to what was mentioned by Abdullah, (2010) with a modification by adding a mixture of four types of probiotic bacteria (Bif. bifiduem, Lb. casei, Lb. planetarium, Lb. acidophilus) and a second mixture of two types of probiotic bacteria (Bif. Bifiduem and Lb. casei) in addition to cranberries, fresh strawberries and a mixture of drugs (70 mg Metformin + 20 mg gliclazide). Water and food were also given until they start feeding on the previous ration and conduct the required analysisss.

Female mice were divided into 13 groups, which are as follows

The first group (T1): It is the healthy control group, which included 5 healthy mice, and they were fed a regular diet and normal water.

The second group (T2): It is the group with diabetes induced by alloxan and was fed the usual diet and normal water.

The third group (T3): The group with gestational diabetes, fed the usual plain yogurt, and treated with (70 Metformin mg + 20 gliclazide mg.(

Fourth group (T4): It is the group affected by gestational diabetes and fed on a diet of therapeutic yogurt to which four types of probiotic bacteria are added, as (75 mg/ kg body weight) of this mixture of bacteria to yoghurt with cranberries.

The fifth group (T5): The group affected by gestational diabetes and fed on the previously mentioned therapeutic yogurt diet with strawberries.

The sixth group (T6): The group affected by gestational diabetes and fed the previously mentioned therapeutic yogurt diet supplemented with same drug and cranberries.

The seventh group (T7): It is the group affected by gestational diabetes and fed on the aforementioned therapeutic yogurt diet added to it same drug and strawberries.

The eighth group (T8): The group affected by gestational diabetes and fed the previously mentioned therapeutic yogurt diet with same drug.

The ninth group (T9): It is the group affected by gestational diabetes and fed on a diet of therapeutic yogurt to which two types of probiotic bacteria are added, which are: (Bif. Bifiduem and Lb. casei) as 75 mg/kg of body weight with cranberries.

The tenth group (T10): It is the group affected by gestational diabetes and fed on the previously mentioned therapeutic yogurt diet with strawberries.

The eleventh group (T11): It is the group affected by gestational diabetes and fed the previously mentioned therapeutic yogurt diet with same drug and cranberries.

The twelfth group (T12): The group affected by gestational diabetes and fed the previously mentioned therapeutic yogurt diet with same drug and strawberry.

Thirteenth group (T13): The group affected by gestational diabetes and fed the previously mentioned therapeutic yogurt diet with same drug.

Carry out measurements and checks

Biochemical tests of blood

Determination of serum glucose, cholesterol, triglycerides and high-density lipoproteins concentration

The concentration of blood glucose, cholesterol, triglycerides and high-density lipoproteins in the blood serum was measured using a special analysis kit (Kit) from the French company (Biolabo) (Fasce, 1982).

$$\text{concentration (mg/dl)} = \frac{\text{(A) Sample}}{\text{(A) Standard}} \times \text{Standard Conc.}$$

Standard Concentration of Glucose, Cholesterol, Triglycerides and HDL-C = (100, 200, 200, 100) mg/dl

Determination of serum low density lipoprotein-cholesterol concentration (LDL-C)

The estimation of LDL-C in serum was based on the following relationship (Burtis and Ashwood, 1999).

LDL-C concentration (mg/dl) = Total cholesterol – (HDL-C) + (VLDL-C)

Determination of serum very low density lipoprotein-cholesterol concentration (VLDL–C)

The concentration of (VLDL-C) in the blood serum was estimated based on the following relationship (Burtis and Ashwood, 1999).

VLDL concentration (mg/dl) = Triglycerides / 5

Statistical Analysis

The statistical program sciences social package Statistical (SPSS) version 16 was used in order to analyze the primary data of the results of the current study, as the analysis of variance (ANOVA) test was used and the level of significant differences between the rates of the criteria included in the current study was extracted using the least significant difference at the statistical level. (0.05, 0.01).

Results and Discussion

Effect of treatments and stage of pregnancy on blood sugar in diabetic rats

Table (1) shows the effect of different treatments and pregnancy stage on blood sugar in laboratory mice.

The results showed a significant increase at the level of $P \leq 0.05$ in the glucose index in rats when they were infected with diabetes, and the results before and after pregnancy were at 89.40 and 147.40 mg/dL, respectively, compared with the healthy control group, which was at 81.80 mg/dL.

The results also showed a significant decrease at $P \leq 0.05$ in sugar values when treated with four mixed bacteria, fruits and drugs used in the treatment of diabetes compared with the group of infected animals.

The best treatment was (T8, T7, T6), with an arithmetic mean of (84.40, 86.80 and 93.00) mg/dL.

Table (1) Effect of treatments and stages of pregnancy on the level of blood sugar mg/dl in female mice with diabetes induced by alloxan

after treatment mL/dL	After pregnancy and gestational diabetes mL/dL	before pregnancy mL/dL	Duration Transactions
82.80 IA	81.80 dA	81.80 aA	T1
150.00 aA	147.40 bcA	89.40 aB	T2
125.40 cB	151.00 abA	85.20 aC	T3
116.00 dB	142.00 cA	87.20 aC	T4
124.20 cB	144.60 bcA	90.00 aC	T5
84.40 hiB	140.00 cA	90.20 aB	T6
86.80 ghiB	148.40 abcA	89.40 aB	T7
93.00 efB	146.80 bcA	86.80 aB	T8
121.80 cB	140.80 cA	89.60 aC	T9
130.60 bB	143.60 bcA	90.80 aC	T10
87.40 ghiB	142.60 bcA	86.40 aB	T11
90.40 fgB	142.60 bcA	87.40 aB	T12
97.00 eB	156.40 aA	89.00 aB	T13

Similar capital letters indicate that there are no horizontal differences between the stages

Similar lowercase letters indicate that there are no vertical differences between the coefficients

T1: healthy control group, T2: gestational diabetes control group, T3: gliclazide 20mg + Metformin 70mg group, T4: group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + cranberry group, T5: group (Bif). Bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + Strawberry, T6: group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + cranberry + gliclazide 20mg + Metformin 70mg, T7: group (Bif). Group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + strawberry + gliclazide 20mg + Metformin 70mg, T8: group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + gliclazide 20mg + Metformin 70mg, T9 group: (Bif. bifiduem Lb. casei,) + cranberry, T10: group (Bif. bifiduem Lb. casei,) + strawberry, T11: group (Bif. bifiduem Lb. casei,) + cranberry + gliclazide 20mg + Metformin 70mg, T12 Group (Bif. bifiduem Lb. casei,) + strawberry + gliclazide 20mg + Metformin 70mg, T13: combination (Bif. bifiduem Lb. casei,) + gliclazide 20mg + Metformin 70mg

The results also showed a significant decrease at the level $P \leq 0.05$ in sugar values when treated with mixed bacteria (Bf. bifiduem Lb. casei,) with same fruits and drugs used in the treatment of diabetes compared with the group of infected animals.

The best treatment was (T13, T12, T11), with an arithmetic mean of (87.40, 90.40 and 97.00) mg/dL.

The results agreed with Galletto et al., (2004) in obtaining a rise in the glycemic index, as they showed through a study in which alloxan was used to provoke diabetes in rats. It also agrees with a study conducted on rabbits by Al-kaki, (1999) as alloxan works to break down and cause rapid damage to cells Specifically in Langerhans island in the pancreas, alloxan works to generate reactive oxygen species (ROS), which works to cause damage to pancreatic cells (beta cells) and thus stop insulin production (Al-Moussawi, 2012), which This led to the failure of glucose to enter cells and its high concentration in the blood (Nelson and Cox, 2000), as indicated by Szkudelski, (2001) that alloxan participates in the activity of some compounds containing sulfhydryl groups involved in the formation of the enzyme Glucokinase, which leads to a loss of the activity of this enzyme that has A major role in controlling the balance of glucose concentration in the blood serum because of its very high ability to control the liver in terms of getting rid of glucose and thus raising the level of blood sugar.

The results agreed with Cho et al., (2009); Alsharidah et al., (2018) In the lowering of the glucose index when using the drugs used in the treatment of diabetes, gliclazide and metformin, as they showed in these studies that their use together to treat diabetes led to a decrease in the concentration of glucose in the blood, because these two compounds together improve the control of diabetes. The proportion of glucose in the blood of diabetic patients, and together they are considered more biologically equivalent than using them alone in addition to protection from oxidative stress, while the results differed with Chen et al., (2010) who noted that there were no significant differences in controlling blood sugar whether treatment was used. singly or in combination.

It also agreed with the findings of DaSilva et al., (2021) ; Taghizadeh et al (2020) ; Babadi et al. (2018) where they found that the use of a probiotic with a prebiotic such as cranberry was accompanied by a decrease in the concentration of glucose in the blood compared to the previous treatments. They also confirmed that the probiotic and the prebiotic positively affect the metabolism of the host and can modify the The gene expression of the host thus positively affects the metabolic disorders such as diabetes mellitus and other related disorders.

Gomes et al., (2014) suggest that administration of a probiotic can improve the prognosis of diabetes by modifying the gut microbiota.

The reason may be that the consumption of cranberries and strawberries prevents the occurrence of a number of diseases in humans and animals, as well as the formation of free radicals, because they are rich in vitamins C, E and B-carotene, polyphenols, flavonoids such as anthocyanins, and antioxidants, as anthocyanins reduce inflammation in the body and enhance immunity, The addition of these fruits to biological supplements improves the performance of phenolic compounds by influencing their structures and increasing them after fermentation in addition to marked improvements in antioxidant, antimicrobial and antidiabetic activities, indicating their promising potential as an anti-hyperglycemic agent (Zhong et al., 2021).

These results differed with Ahmadi et al. (2016), where they used in the study a probiotic consisting of (Lb. acidophilus, Lb. casei and Bif. Bifidum) with the prebiotic, and they noticed during this study that there were no significant differences in low blood glucose and unaffected with it during treatment.

Effect of treatments and stage of pregnancy on cholesterol in diabetic rats

Table (2) shows the effect of different treatments and pregnancy stage on cholesterol in laboratory mice.

The results showed a significant increase at the level of P ≤ 0.05 in the cholesterol index in mice with diabetes, and the results before and after pregnancy were at 106.60 and 170.80 mg/dL, respectively, compared with the healthy control group, which was at 97.00 mg/dL.

The results also showed a significant decrease at P ≤ 0.05 in cholesterol values when treated with four mixed bacteria, fruits and drugs used in the treatment of diabetes compared with the group of infected animals.

The best treatment was (T8, T7, T6), as its mean was (160.40, 162.40 and 170.60) mg/dl.

The results also showed a significant decrease at a level P ≤ 0.05 in cholesterol values when treated with mixed bacteria (Bf. bifiduem Lb. casei,) with same fruits and drugs used in the treatment of diabetes compared with the group of infected animals. .

Table (2) Effect of treatments and stages of pregnancy on the level of cholesterol mg/dl in female mice with diabetes induced by alloxan

after treatment mL/dL	After pregnancy and gestational diabetes mL/dL	before pregnancy mL/dL	Duration Transactions
97.80 eA	97.40 eA	97.00 aA	T1
172.00 aA	170.80 cdA	106.60 aB	T2
175.40 aB	182.80 abA	100.80 aC	T3
174.40 aB	193.00 aA	105.80 aC	T4
166.00 bB	178.00 cdA	103.80 aC	T5
160.40 cdB	186.00 abA	100.00 aC	T6
162.40 dB	195.40 aA	104.20 aC	T7
170.60 aB	193.20 aA	102.20 aC	T8
163.20 dB	174.00 dA	104.00 aC	T9
dB 160.40	179.60 bcA	100.60 aC	T10
163.20 dB	187.00 abA	102.80 aC	T11
163.00 dB	190.20 aA	108.00 aC	T12
171.40 aB	192.00 aA	100.80 aC	T13

Similar capital letters indicate that there are no horizontal differences between the stages

Similar lowercase letters indicate that there are no vertical differences between the coefficients

T1: healthy control group, T2: gestational diabetes control group, T3: gliclazide 20mg + Metformin 70mg group, T4: group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + cranberry group, T5: group (Bif). Bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + Strawberry, T6: group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + cranberry + gliclazide 20mg + Metformin 70mg, T7: group (Bif). Group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + strawberry + gliclazide 20mg + Metformin 70mg, T8: group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + gliclazide 20mg + Metformin 70mg, T9 group: (Bif. bifiduem Lb. casei,) + cranberry, T10: group (Bif. bifiduem Lb. casei,) + strawberry, T11: group (Bif. bifiduem Lb. casei,) + cranberry + gliclazide 20mg + Metformin 70mg, T12 Group (Bif. bifiduem Lb. casei,) + strawberry + gliclazide 20mg + Metformin 70mg, T13: combination (Bif. bifiduem Lb. casei,) + gliclazide 20mg + Metformin 70mg

The best treatment was (T13, T12, T11), as its mean was (163.20, 163.00 and 171.40) mg/dL.

The results agreed with Ahmed et al., (2008) in the occurrence of a rise in the cholesterol index, as they found that dosing laboratory animals with Aloxan leads to an increase in the concentration of cholesterol in the blood, or the cause of the high level of cholesterol in the blood may be due to the fact that the body, while suffering from diabetes, lacks the ability to consume glucose As a source of energy and thus leads to activating the process of lipolysis in the fatty tissues of the body, thus releasing free fatty acids (FFA) that increase the level of cholesterol in the blood (Salmenniemi, 2004).

These results differed with (Rajagopal and Saskala, 2008), where they noticed a decrease in cholesterol concentration after dosing laboratory animals with alloxan, as they indicated the reason is that when the secretion of the hormone insulin is increased, it will lead to a decrease in the process of lipolysis in adipose tissue by inhibiting the action of the enzyme Lipase and thus the process of fatty acid oxidation and production of Acetyl-CoA molecules decrease, and thus the concentration of cholesterol in the blood decreases. This may be due to an increase in the activity of the enzyme Cholesterol acyl transferase, which is responsible for the absorption of cholesterol in the intestine, and that the activity of the mentioned enzyme is stimulated during the presence or absence of the hormone insulin (Hori et al., 2004).

The results also agreed with what was stated by Hassan and Abd-Allah (2015) ; Alsharidah et al., (2018) observed a decrease in the level of total cholesterol while taking the two drugs together. This may be due to the fact that metformin works to reduce fat molecules in the liver, in addition to that it works directly or indirectly in reducing glucose production in the liver and enhances the sensitivity of cells For insulin, clikside acts as an antioxidant and scavenger of free radicals (Alp et al., 2012).
The results agreed with Dehkohneh et al., (2019) ; Tamtaji et al., (2017) indicated that some probiotic strains including Lb. casei and Lb. rhamnosus and Lb. acidophilus, can have beneficial effects on blood lipid profiles such as cholesterol, as they showed that dairy products fermented by types of LAB can cause a reduction in lipid levels to the normal limit, and they also indicated that the reason for the decrease in cholesterol concentration during the use of probiotic supplements is due to Enhancement of cholesterol binding, inhibition of bile acid reabsorption by probiotic bacteria, and cholesterol binding to probiotic cell walls, in addition to Bif. Bifiduem is effective in digesting and metabolizing fats, including cholesterol, and it was stated during this study that the type of probiotics used during treatment and the period during which they take place affect the efficiency of probiotic supplements.
Or it may be that the presence of the probiotic in food leads to the production of a substance known as hydroxy methyl glutamate, which inhibits the gene responsible for the synthesis of the enzyme HMG-CoA-reductase responsible for the synthesis of cholesterol from the Acetyl-CoA compound, and the reason may be due to the consumption of cholesterol probiotics (fats). while growing in the small intestine and making it unusable (Abdullah, 2010).
As for the strawberry and cranberry fruit, studies have proven that they have health benefits for humans, as they contain biologically active compounds such as flavonoids and phenols, including anthocyanins, making them among the functional foods that play an important role against some diseases, including high cholesterol in the blood, in addition to mixing it with The probiotic increases the effectiveness of these compounds (Catinean et al., 2018).
The study data on laboratory animals also indicate that phenolic compounds reduce cholesterol synthesis by reducing the activities of the main cholesterol-regulating enzymes, in addition to the effect of phenols on bile flow, which increases the concentration of bile cholesterol and bile acid and thus its excretion with the feces (Krzeminski et al., 2003).

Effect of treatments and pregnancy stage on triglycerides of diabetic mice

Table (3) shows the effect of different treatments and pregnancy stage on triglycerides in laboratory mice.

The results showed a significant increase at the level of P ≤ 0.05 in the triglyceride index in diabetic rats, and the results before and after pregnancy were at 73.60 and 150.60 mg/dL, respectively, compared with the healthy control group, which was at 69.00 mg/dL.

The results also showed a significant decrease at the level P ≤ 0.05 in the values of triglycerides when treated with four mixed bacteria, fruits and drugs used in the treatment of diabetes compared with the group of infected animals.

The best treatment was (T5, T4), where the mean was (142.20 and 139.20) mg/dL.

Table (3) Effect of treatments and stages of pregnancy on the level of triglycerides mg/dl in female mice with diabetes induced by alloxan.

after treatment mL/dL	After pregnancy and gestational diabetes mL/dL	before pregnancy mL/dL	Duration Transactions
68.40 hA	69.00 dA	68.80 eA	T1
147.50 cdA	150.60 bA	73.60 dB	T2
153.80 abA	151.00 bA	76.40 cB	T3
142.20 efB	159.60 aA	70.60 deC	T4
139.20 fgB	151.60 bA	81.80 bC	T5
139.60 fgB	143.60 bcA	83.60 aC	T6
143.40 eB	147.80 bA	81.40 bC	T7
136.40 gB	144.80 bcA	75.60 cC	T8
144.60 bcA	145.60 deA	83.00 aB	T9
149.20 bB	157.40 aA	77.60 cC	T10
144.40 deA	146.40 bA	82.40 bB	T11
138.00 fgB	152.80 bA	73.00 dC	T12
144.00 deB	150.40 bA	84.60 aC	T13

Similar capital letters indicate that there are no horizontal differences between the stages

Similar lowercase letters indicate that there are no vertical differences between the coefficients

T1: healthy control group, T2: gestational diabetes control group, T3: gliclazide 20mg + Metformin 70mg group, T4: group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + cranberry group, T5: group (Bif). Bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + Strawberry, T6: group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + cranberry + gliclazide 20mg + Metformin 70mg, T7: group (Bif). Group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + strawberry + gliclazide 20mg + Metformin 70mg, T8: group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + gliclazide 20mg + Metformin 70mg, T9 group: (Bif. bifiduem Lb. casei,) + cranberry, T10: group (Bif. bifiduem Lb. casei,) + strawberry, T11: group (Bif. bifiduem Lb. casei,) + cranberry + gliclazide 20mg + Metformin 70mg, T12 Group (Bif. bifiduem Lb. casei,) + strawberry + gliclazide 20mg + Metformin 70mg, T13: combination (Bif. bifiduem Lb. casei,) + gliclazide 20mg + Metformin 70mg

The results also showed a significant decrease at the level $P \leq 0.05$ in the values of triglycerides when treated with mixed bacteria (Bf. bifiduem Lb. casei,) with same fruits and drugs used in the treatment of diabetes compared with the group of infected animals.

The best treatment was (T12, T10), with an arithmetic mean of (149.20 and 138.00) mg/dL.

The results agreed with Shehata and Moussa, (2014) in the occurrence of an increase in the triglyceride index during diabetes, while these results did not agree with the findings of Al-Fleih,(2005), as it was found that there is a non-significant increase in the Triglyceride concentration in diabetic laboratory animals (mice).

The reason for the high level of triglycerides in people with diabetes may be due to a decrease or loss of the hormone insulin due to the breakdown of pancreatic cells (beta) and this led to the inhibition of the enzyme lipoprotein lipase. Fatty acids and glycerol (Nelson and Cox, 2005), or the cause may be the activation of the lipolysis process in adipose tissue cells due to diabetes and insulin deficiency, thus releasing free fatty acids (FFA) to be used as an energy source and also participating in the process of building triglycerides in addition In addition, the liver absorbs large amounts of free fatty acids, and thus VLDL-C increases, leading to an increase in the level of triglycerides in the blood (Salmenniemi et al., 2004; Zhang, 2010).

The results also agree with what was stated by Al-Naeaimy (2011) reported that there was no decrease in the triglyceride index by using the two drugs together, and they differed with (Hassan and Abd-Allah, 2015; Alsharidah et al., 2018).

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The results are in agreement with Costabile et al., (2017) who showed that some probiotic strains, including lactic acid bacteria, can have beneficial effects on blood lipid profiles such as triglycerides. Also, recent findings show that the reason for this may be that the biostimulant can attenuate lipid metabolism by altering the expression of genes involved in the lipid biosynthesis pathway (Tamtaji et al., 2017).

Metformin also works to reduce fat molecules in the liver and thus insulin works better to reduce the level of glucose in the blood and may work directly or indirectly in reducing the production of glucose in the liver hepatic gluconeogenesis. Glucose levels in the blood by increasing insulin secretion from pancreatic beta cells and thus lowering levels of body lipids (Al-Naeaimy, 2011).

As for berries and strawberries, their content of phenolic compounds, which led to a change in the concentrations of epochs, may be due to the fact that they stimulate osteoblasts. It was hypothesized that since osteoblasts and adipocytes are derived from the same mesenchymal stem cells, this may explain this effect on lipid profiles (Filip et al. , 2015).

The study also agreed with what was done by Luo et al., (2019) In a study on functional foods rich in phenolic compounds and dosed to experimental animals following a high-fat diet for two months with probiotics, the animals during 28 days observed a decrease in triglycerides (TG) and low-density lipoprotein (LDL) cholesterol (LDL) in the blood serum.

The reason for the lack of a decrease in the level of triglycerides in both treatments (T11, T9) may be due to the fact that the lipid disturbance and its increase in diabetic patients depends on the concentration of glucose in the blood because of its effective role in controlling lipid disorders in patients with diabetes, especially triglycerides or may be due to the mothers' exposure to increased oxidative stress due to the high concentrations of free radicals formed in the body and thus the decrease in the activity of the enzyme Lipoprotein lipase present in the body tissues, and this decrease leads to a high concentration of (TG) in the blood and as a result, the proportion of VLDL-C that enters TG in their synthesis and thus contribute to its high concentration in blood serum (Flora ; et al., 2012).

The reason may also be due to the period used in the treatment or the amount of enhancers used for treatment is insufficient, in addition to the type of biostimulant used (Dehkohneh et al., 2019).

Effect of treatments and stage of pregnancy on high-density lipoprotein cholesterol (HDL-C) of diabetic rats

Table (4) shows the effect of different treatments and pregnancy stage on HDL in laboratory mice.

The results showed a significant decrease at the level of $P \leq 0.05$ in the HDL index in rats when infected with diabetes, and the results before and after pregnancy were at 55.60 and 47.50 mg/dL, respectively, compared with the healthy control group, which was at 43.20 mg/dL.

The results also showed a significant increase at $P \leq 0.05$ in HDL values when treated with mixed bacteria, fruits and drugs used in the treatment of diabetes compared with the group of infected animals.

The best treatment was (T8, T7, T4), with an arithmetic mean of (47.80, 46.80 and 52.60) mg/dL.

Table (4) Effect of treatments and stages of pregnancy on the level of (HDL-C) mg/dL in female mice with diabetes induced by alloxan.

after treatment mL/dL	After pregnancy and gestational diabetes mL/dL	before pregnancy mL/dL	Duration Transactions
35.60 gA	35.40 eA	35.20 cdA	T1
78.50 aA	76.20 aA	45.20 aC	T2
63.40 bB	73.80 abA	44.00 aC	T3
58.00 cdeB	70.40 abcA	44.00 aC	T4
63.20 bA	68.80 bcA	40.40 abcB	T5
52.60 fB	59.40 dA	32.20 dC	T6
55.20 eB	64.60 cdA	38.20 bcC	T7
57.40 deB	64.00 cdA	35.60 cdC	T8
60.40 bcdB	69.20 abcA	39.40 abcC	T9
63.40 bB	73.00 abA	40.60 abcC	T10
56.60 defB	67.00 bcA	39.00 abcC	T11
60.00 bcdB	69.20 abcA	42.00 abC	T12
56.20 defB	66.40 bcdA	45.20 aC	T13

Similar capital letters indicate that there are no horizontal differences between the stages

Similar lowercase letters indicate that there are no vertical differences between the coefficients

T1: healthy control group, T2: gestational diabetes control group, T3: gliclazide 20mg + Metformin 70mg group, T4: group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + cranberry group, T5: group (Bif). Bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + Strawberry, T6: group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + cranberry + gliclazide 20mg + Metformin 70mg, T7: group (Bif). Group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + strawberry + gliclazide 20mg + Metformin 70mg, T8: group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + gliclazide 20mg + Metformin 70mg, T9 group: (Bif. bifiduem Lb. casei,) + cranberry, T10: group (Bif. bifiduem Lb. casei,) + strawberry, T11: group (Bif. bifiduem Lb. casei,) + cranberry + gliclazide 20mg + Metformin 70mg, T12 Group (Bif. bifiduem Lb. casei,) + strawberry + gliclazide 20mg + Metformin 70mg, T13: combination (Bif. bifiduem Lb. casei,) + gliclazide 20mg + Metformin 70mg

The results also showed a significant increase at a level $P \le 0.05$ in sugar values when treated with mixed bacteria (Bf. bifiduem Lb. casei,) with same fruits and drugs used in the treatment of diabetes compared with the group of infected animals. .

The best treatment was (T12, T11, T10), as its mean was (43.00, 48.00 and 47.00) mg/dL.

The results are in agreement with Shehata & Moussa, (2014) showed a decrease in the HDL-C index in diabetic rats, and the reason for the decrease in HDL-C during diabetes may be due to the increased activity of the enzyme cholesterol ester transferase, which works to transfer cholesterol esters from HDL-C to VLDL-C leaving HDL-C rich in triglycerides and less affinity for Apo-A, remaining free and easily filtered through the kidneys (Al-Jubouri, 2008), or perhaps the reason is that the development of diabetes in laboratory mice resulted in the formation of free radicals and decreased The activity of the enzyme lipoprotein lipase and the increase of the activity of the hepatic lipase enzyme. Also, HDL-C contains many triglycerides, and this is a goal to increase the activity of the hepatic lipase enzyme, leading to a decrease in the concentration of HDL-C in the blood (Betteridge, 2000).

The results also agreed with what was stated by Hassan and Abd-Allah (2015) ; Alsharidah et al., (2018) noticed an increase in the level of HDL during treatment with both drugs, as it was found that they have an effect on body fat profiles, and differed with what was stated by Tessier et al., (1999), where they indicated that there were no significant differences in HDL levels.

The results agreed with Tamtaji et al., (2017) ; Karamali et al., (2018) who indicated during their study that probiotics affect lipid profiles, including HDL, where they observed an increase in HDL level in laboratory animals during treatment with probiotic supplementation. This may be due to the fact that probiotics can attenuate lipid metabolism by altering the expression of genes involved in the lipid biosynthesis pathway, while this study did not agree with what Samimi et al., (2018) Dehkohneh et al., (2019), indicated that the probiotic did not have a significant effect on the whole blood lipid profile.

As for the biological precursor, a study conducted by Lockyer et al., (2017) indicated that the use of fruits such as cranberries and strawberries has a beneficial health effect on the levels of lipid profiles in the blood, including (HDL) because they contain phenolic compounds.

Effect of treatments and stage of pregnancy on low-density lipoprotein cholesterol (LDL-C) of diabetic mice

Table (5) shows the effect of different treatments and pregnancy stage on LDL in laboratory mice.

The results showed a significant increase at the level of $P \leq 0.05$ in the LDL index in diabetic rats, and the results before and after pregnancy were at 44.80 and 99.00 mg/dL, respectively, compared with the healthy control group, which was at 36.40 mg/dL.

The results also showed a significant decrease at $P \leq 0.05$ in LDL values when treated with four mixed bacteria, fruits and drugs used in the treatment of diabetes compared with the group of infected animals.

The best treatment was (T7, T4), with an arithmetic mean of (100.80 and 95.40) mg/dL.

Table (5) Effect of treatments and stages of pregnancy on the level of LDL-C cholesterol mg/dL in female mice with diabetes induced by alloxan.

after treatment mg/dL	After pregnancy and gestational diabetes mg/dL	before pregnancy mg/dL	Duration Transactions
37.80 hA	36.80 fA	36.40 bA	T1
109.80 cdA	99.00 deA	44.80 abC	T2
120.00 abB	150.00 aA	44.80 abC	T3
100.80 efB	127.00 bcA	41.20 bC	T4
115.00 bcA	120.40 cdA	50.20 aB	T5
93.60 gB	104.00 eA	39.00 bC	T6
95.40 fgB	129.00 bcA	35.40 bC	T7
114.00 bcB	139.80 abA	38.00 bC	T8
103.00 deA	102.60 eA	43.40 abB	T9
108.60 cdA	109.80 deA	50.20 aB	T10
101.40 efB	108.00 deA	39.80 bC	T11
97.20 eB	105.40 deA	50.20 aC	T12
124.20 aB	131.80 bA	50.20 aC	T13

Similar capital letters indicate that there are no horizontal differences between the stages

Similar lowercase letters indicate that there are no vertical differences between the coefficients

T1: healthy control group, T2: gestational diabetes control group, T3: gliclazide 20mg + Metformin 70mg group, T4: group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + cranberry group, T5: group (Bif). Bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + Strawberry, T6: group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + cranberry + gliclazide 20mg + Metformin 70mg, T7: group (Bif). Group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + strawberry + gliclazide 20mg + Metformin 70mg, T8: group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + gliclazide 20mg + Metformin 70mg, T9 group: (Bif. bifiduem Lb. casei,) + cranberry, T10: group (Bif. bifiduem Lb. casei,) + strawberry, T11: group (Bif. bifiduem Lb. casei,) + cranberry + gliclazide 20mg + Metformin 70mg, T12 Group (Bif. bifiduem Lb. casei,) + strawberry + gliclazide 20mg + Metformin 70mg, T13: combination (Bif. bifiduem Lb. casei,) + gliclazide 20mg + Metformin 70mg

The results also showed a significant decrease at a level $P \leq 0.05$ in LDL values when treated with mixed bacteria (Bf. bifiduem Lb. casei,) with same fruits and drugs used in the treatment of diabetes compared with the group of infected animals. .

The best treatment was (T12, T11), with an arithmetic mean of (101.40 and 97.20) mg/dL.

The results agreed with Shehata and Moussa, (2014) on the occurrence of an increase in the LDL index, where they indicated with Ai-juka, (2007), that the infection of mice and male rats with diabetes led to an increase in the concentration of LDL in the blood serum for laboratory animals with diabetes.

The reason for the high concentration of LDL can be attributed to the increase in the level of free radicals in the body that leads to a defect in the pancreatic beta cells (beta) and their damage, in addition to the decrease in the concentration of the hormone insulin and the disintegration of fat cells in the fatty tissue, thus releasing free fatty acids. Fatty acids that are frequently used by the liver to form VLDL and thus increase the level of VLDL until it later turns into LDL and thus its level in the body increases, causing harmful side effects such as atherosclerosis and heart diseases (Zhang, 2010). The cause may also be the free radicals formed as a result of the use of alloxan and damage to the pancreatic beta cells, which leads to an increase in the level of oxidative stress, which causes an imbalance in the LDL receptors in the liver (Brewer, 2004).

The results also agree with Erem et al., (2014) in the decrease in the LDL index during the use of the two drugs together, and the reason may be due to the fact that the two compounds are more equivalent when mixed together. Significant differences during the use of the two drugs together on the level of LDL in the blood serum.

The results agreed with Tamtaji et al., (2017) ; Karamali et al., (2018) During their study, they indicated that probiotics affect lipid profiles, including LDL, as they observed an increase in LDL level in laboratory animals during treatment with probiotic supplementation. The reason may be that probiotics can reduce lipid metabolism by altering the expression of genes involved in The lipid biosynthesis pathway, and the results differed with that of Samimi et al., (2018) ; Dehkohneh et al., (2019), indicated that the probiotic had no significant effect on the whole blood lipid profile except for VLDL and TG.

The study also agreed with what Luo et al. (2019) had done in a study on functional foods rich in phenolic compounds and dosed to experimental animals following a high-fat diet for two months with probiotics in animals. During 28 days, triglycerides (TG) and low-density lipoprotein cholesterol were decreased (LDL) in the blood serum.

Effect of treatments and pregnancy stage on very low-density lipoprotein cholesterol (VLDL-C) of diabetic rats.

Table (6) shows the effect of different treatments and pregnancy stage on VLDL in laboratory mice.

The results showed a significant increase at $P \leq 0.05$ in the VLDL index in diabetic rats, and the results before and after pregnancy were 14.72 and 30.840 mg/dL, respectively, compared with the healthy control group, which was at 13.76 mg/dL.

The results also showed a significant decrease at $P \leq 0.05$ in VLDL values when treated with four mixed bacteria, fruits and drugs used in the treatment of diabetes compared with the group of infected animals.

The best treatment was (T4), with an arithmetic mean of (28.440) mg/dL.

The results also showed a significant decrease at a level $P \leq 0.05$ in sugar values when treated with mixed bacteria (Bf. bifiduem Lb. casei,) with same fruits and drugs used in the treatment of diabetes compared with the group of infected animals. .

The best treatment was (T12), with an arithmetic mean of (27.600) mg/dL.

The results agreed with what was stated by Al-Naeaimy (2011) where they noticed that there were no significant differences when using the two drugs Metformin and gliclazide in the level of VLDL, and the results differed with Hassan and Abd-Allah,

(2015) ; Alsharidah et al., (2018), as they showed during their study that the use of the two drugs together leads to a reduction in the level of VLDL lipoprotein.

The results of the treatments (T11, T9, T8, T7, T6) also agreed with what was reported by previous studies by Dehkohneh et al., (2019), as they indicated during their studies that the use of probiotics with the probiotic did not affect VLDL levels, and the reason may be due to the period The reason for the lack of VLDL level in the above-mentioned treatments is that the lipid disturbance and its increase in diabetic patients depends on the concentration of glucose in the blood because of its effective role in controlling On lipid disorders in diabetic patients.

Table (6) Effect of treatments and stages of pregnancy on the level of VLDL mg/dl in female mice with diabetes induced by alloxan

after treatment mg/dL	After pregnancy and gestational diabetes mg/dL	before pregnancy mg/dL	Duration Transactions
14.220eA	14.020 eA	13.76 dA	T1
29.500 cA	30.840 bA	14.72 dB	T2
30.760 abA	30.200 bA	15.28 abB	T3
28.440 cdB	31.920 aA	14.12 dC	T4
27.840 dB	30.320 bA	16.36 cdC	T5
27.920 cdA	28.720 cdA	16.72 cdB	T6
28.680 deA	29.560 bcA	16.28 bcB	T7
27.280 cdA	28.960 cdA	15.12 abB	T8
28.920 cdA	29.120 cdA	16.60 cdB	T9
29.840 bcB	31.480 aA	15.52 abC	T10
28.880 cdA	29.520 bcA	16.48 cdB	T11
27.600 cdB	30.560 bcA	14.06 abC	T12
28.800 cdB	30.080 bcA	16.92 bcC	T13

Similar capital letters indicate that there are no horizontal differences between the stages

Similar lowercase letters indicate that there are no vertical differences between the coefficients

T1: healthy control group, T2: gestational diabetes control group, T3: gliclazide 20mg + Metformin 70mg group, T4: group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + cranberry group, T5: group (Bif). Bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + Strawberry, T6: group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + cranberry + gliclazide 20mg + Metformin 70mg, T7: group (Bif).

Group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + strawberry + gliclazide 20mg + Metformin 70mg, T8: group (Bif. bifiduem Lb. casei, Lb. planetarium, Lb. acidophilus) + gliclazide 20mg + Metformin 70mg, T9 group: (Bif. bifiduem Lb. casei,) + cranberry, T10: group (Bif. bifiduem Lb. casei,) + strawberry, T11: group (Bif. bifiduem Lb. casei,) + cranberry + gliclazide 20mg + Metformin 70mg, T12 Group (Bif. bifiduem Lb. casei,) + strawberry + gliclazide 20mg + Metformin 70mg, T13: combination (Bif. bifiduem Lb. casei,) + gliclazide 20mg + Metformin 70mg

The reason may be due to the mothers being exposed to increased oxidative stress due to the high concentrations of free radicals formed in the body and thus the decrease in the activity of the enzyme Lipoprotein lipase present in the body tissues and this decrease leads to a high concentration of (TG) which is the main component in the formation of VLDL-C and thus its rise in blood serum (Salmenniemi et al., 2004 ; Flora et al., 2012).

The increase in the level of VLDL-C may be due to an increase in the concentration of free radicals in the body in people with diabetes, which leads to the destruction of pancreatic beta cells (beta) and fatty tissue, and thus increases the chance of releasing fatty acids that are used by the liver to form VLDL-C (Zhang, 2010).

The results of the treatments (T13, T12, T10, T5, T4) agreed with what was reported by previous studies Samimi et al., (2018), observed that the consumption of probiotic supplements leads to a decrease in VLDL concentration, and the reason for this is that the probiotic can moderate lipid metabolism by altering the genes involved in the lipid biosynthesis pathway (Tamtaji et al., 2017).

As for the biological precursor, the results are in agreement with what was indicated by a study conducted by Lockyer et al., (2017) that the use of fruits such as cranberries and strawberries has a beneficial health effect on blood lipid levels, including (VLDL) because they contain phenolic compounds.

Conclusion

The addition of supplementation of probiotic bacteria as a preventive treatment to the therapeutic yoghurt of diabetic pregnant female mice had positive effects on blood sugar and lipids functions compared to the control group, and the addition of medicinal drugs that included gliclazide with Metformin and the fruits used in the trial enhanced the benefits of the health probiotic supplements.

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الخلاصة

أجريت الدراسة في البيت الحيواني التابع الى كلية الطب البيطري/ جامعة تكريت وفي مختبرات الشركة العامة لصناعة الادوية والمستلزمات الطبية/ سامراء، أجريت هذه الدراسة للمدة من بداية شهر تشرين الأول لسنة 2020 ولغاية نهاية شهر نيسان لسنة 2021). تضمنت انتاج البان علاجية مع اضافة خليط من مكملات المعززات الحيوية/ *Lactobacillus. Casei, Lactobacillus. planetarium, Lactobacillus. acidophilus, Bifidobacterium. Bifiduem* وخليط ثاني من نوعين من بكتريا المعزز الحيوي (*Bifidobacterium. Bifiduem*) *Lactobacillus. Casei,*) مع بعض الفواكه (التوت البري والفراولة) وعقاقير علاجية (Gliclazide وMetformin)، لمعرفة تأثيرها على بعض المعايير الفسلجية والكيموحيوية كإجراء قياس قيم سكر الدم، الكولسترول الكلي (TC)، الكليسريدات الثلاثية (TG)، البروتينات الدهنية واطئة الكثافة (LDL) ، البروتينات الدهنية واطئة الكثافة جداً (VLDL) والبروتينات الدهنية عالية الكثافة (HDL) لإناث الفئران المختبرية المصابة بالسكري المستحدث بالآلوكسان , وكذلك بيان تأثير اصابة اناث الفئران بالسكري على مواليدها واجراء نفس المعايير المذكورة أعلاه.
بيَنت النتائج وجود ارتفاع معنوي ($p<0.05$) في تراكيز في كل من سكر الدم (156.4، ملغم/ديسيلتر) TC (195.4، ملغم/ديسيلتر) TG 159.6) ملغم/ديسيلتر، (150) LDL ملغم/ديسيلتر، (31.92) VLDL ملغم/ديسيلتر، وحصول انخفاض معنوي في HDL 35) ملغم/ديسيلتر، اثناء معاملة اناث الفئران بالالوكسان.
كما تبينت ان علاج الفئران باللبن العلاجي الذي يحتوي على خليط من مكملات المعززات الحيوية (*Bif. bifiduem* , *Lb. casei*) وعقاقير علاجية (Gliclazide و Metformin) مع بعض الفواكه (التوت البري والفراولة)، ادى الى حصول انخفاض معنوي ($p<0.05$) في تراكيز في كل من سكر الدم (86.8، ملغم/ديسيلتر) TC (162.4 ملغم/ديسيلتر، TG 142.2) ملغم/ديسيلتر، (95.4) LDL-C ملغم/ديسيلتر، (28.44) VLDL ملغم/ديسيلتر، وحصول ارتفاع معنوي في HDL-C 52.60) ملغم/ديسيلتر في اناث الفئران الحوامل.
الكلمات المفتاحية: سكر الحمل، المعززات الحيوية، اللبن، الدهون، الكليكسايد والمتفورمين، التوت البري والفراولة.

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