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Effects of Probiotics and Prebiotics Dietary Supplementations on Some Parameters of Diabetic Rats

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Abstract

Currently dietary interventions become very critical widelyso it has been recommended for many diseases. e.g., patients with diabetes mellitus(DM), heart diseases, obesity, stroke and cancers should consume foods rich in flavonoid compounds in order to improve their health states. The current study carried out to investigate the effects of different levels of dietary Arabic Gum (AG)supplemented with Yogurt (Y) on diabetic health states using animal models. Thirty male rats were used and divided into six groups; the 1st is the control healthy group (1) that fed on basal diet while the rest of all the groups were diabetic induced and one of them used as control positive group (2). Additionally, groups 3 &4 were fed basal diets supplemented with 5 % of either Y or AG each separately while group 5 was fed 5 % of mixed Y and AG on their diets. Body weight, blood glucose levels in addition to liver functions and pancreatic histopathology were evaluated after consuming the supplemented diets. Collected data showed that glucose levels were significantly decreased (P<0.05) after AG and Y consumptions. However, the highest effect has been seen with 5% concentration. Also, ALT and AST that were determined as liver functionswere significantly decreased (P<0.05) after AG consumption comparing to the positive diabetic group and the other tested groups. In conclusion, the obtained results suggested that AG is highly recommended with to be used as dietary medication especially at the level 5% for diabetic and hyperlipidemia patients. However, further human studies are needed.

Key words: Arabic Gum, yogurt, hyperlipidemia, cholesterol, blood glucose.

1. Introduction

Diabetes mellitus (DM) is metabolic disorder characterized by a chronic hyperglycemic condition that could be resulted from defects in insulin secretion and/or insulin action. Additionally, the permanent neonatal diabetes has been reported to be resulted from glucokinase deficiency which is an inborn error of the glucose insulin signaling path way (Njolstadet al., 2003). There are mainly two types of DM that both known by high blood glucose levels, hyperglycemia ,(YıldızDinçeret al., 2003). The first one is called types 1 DM (T1DM) and that normally presented with no enough amount of the insulin which could be because of any problems within the pancreas. However, type 2 DM (T2DM) happens when pancreas is effectively working and release amounts of insulin enough but could not beused and that called as insulin resistance, IR (Mahler and Adler et al., 1999 and Rother et al., 2007)). Currently, about two-thirds of diabetic patients live in developing countries and the majority of new cases originated from these areas. Again, increasing in the incidence of diabetes globally is well known to be related to high levels of obesity that possible is associated with different changes from the main traditional diets in addition to decreasing the levels of physical activity and finally increasing urbanization(Cefalu et al., 2014). Reducing the consumptions of sugar and increased useful and healthy foods are the major aims worldwide in order to have nutritional improvement status (ADA et al.,2017).

One of the healthy foods is the Arabic Gum (AG) that has new trends nowadays. It is sticky exudates from the stems and branches of when subjected trees they are stress (Nussinovitch1997). Acacia trees are indeed present in across sub-Saharan Africa, a wide belt of semi-arid land stretching. Sudan being the largest producer of AG which is collected as a white to pale amber colored granular solidandis named after its geographic location (Islam et al.,1997 and Idriset al.,1998). Although AG has many antioxidant properties, but different studies show to have some negative effects especially on the liver function(Abdelkareemet al., 2015). From the positive point of view AG is able to increase feeling satiated with decreasing the caloric intake so it could be used in a dietary approach for controlling the body weight gain(Babikeret al., 2017). Also, AG may cause gas bloating and loose stools but it is still safe for most adultsbut

not for pregnant and breast-feeding women(Calameet al., 2011 and Almohaimeedet al., 2018). Additionally, AG is considered as anti-diabetic, anti-lipidemic, antioxidant and anti-inflammatory as it is well known to slowly ferment by the bacterial flora in the large intestine and that cause producing of short-chain fatty acids. Moreover, AG is able to selectively increase the proportion of lactic acid bacteria and bifidobacterium in healthy subjects (Almohaimeedet al., 2018). Therefore, it could be consider an important source of prebiotics with it is healthy effects which is the main idea of the current research.

All the scientific societies and communities worldwide depending on the country regulation recommending testing any dietary or food supplements before claiming any health benefits especially before using the probiotics in foods. For instance, lactose intolerancerecommended to be reduced by the prevention of microbial infections by stimulating the immune system and immune responses regulation and that have been claimed after doing a research by **Saint-Eve** et al., (2008). Additionally, an important well known probiotics source is the yogurt which is the most consumed within the wider family of fermented milks, many probiotic fermented milks are traded around the world. The probiotic bacteria are defined as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host(Hillet al., 2014).

Yogurtcontains many bacterial species especially lactic acid as Bifidobacteriumbifidum, Bifidobacteriumlactis, Lactobacillus acidophilus, Lactobacillus casei and/or Lactobacillus rhamnosus with many others which are suggested to have multiple positive effects for our health(Bakhshimoghaddamet al., 2018). Again, yogurt has probiotic such as Streptococcus thermophilus and Lactobacillus bulgaricusthat were used to produce conventional vogurt. Furthermore, it has nine essential amino acids so very good source of protein (8.5 g per cup; Barengoltset al., 2016). The most important issue with yogurt, it shows association with overall health improvement with fermented milk which in turn reduced the mortality levels and that may be based on their potential health properties. Many published data indicated such association of the probiotics supplementation that mainly related to their low levels of lactose content as a result of lactic acid production. So patients with T2Dare recommended to consume yogurt because of its probiotics that being able to play a role in the gut

health(Penget al., 2009 and Mazidietal., 2018). Different animal and human studies recommended yogurt for healthy nutrition with diabetic patients. However, most of the probiotic supplements are expensive and not approved yet and that makes content undependable (Penget al., 2009 and Salas-Salvadó et al., 2017). Therefore, the current study investigated the effect of some dietary probiotics (plan low fat yogurt) and prebiotics (Arabic gum) sources in different concentrations on blood glucose levels and different parameter between diabetic animal models; using rats induced diabetes. (Barengoltset al., 2019).

2. Material and methods

2.1. Materials

Source of tested material:

Gum acacia (Arabic Gum; *Acacia Senegal,L*)was obtained from Al-GomhouriaPharmaceuticalCompanyDoki, Egyptand the low fat yogurt (Dina)was purchased from the local market in Shebin El Kom, Menoufia Governorate, Egypt.

The chemical substances and Kits:

Casein, cellulose, choline chloride powder, and DL- Methionine powder, were obtained from Al-GomhouriaPharmaceutical Company, Doki, Egypt. Alloxan obtained from SIGMA Chemical Co., Cairo, Egypt and Chemical Kits which were used in this study were obtained from Bio-diagnosticCompany, Cairo, Egypt.

2.2. Methods

Preparation of Gum acacia (Acacia Senegal, L.):

Gum Arabic were milled by Moulinex miller (France) to be a fine powder.

Induction of diabetic:

Diabetes mellitus (DM) was induced in normal healthy male albino rats via intra-peritoneal injection of Alloxan by 150mg/kg body weight once a day for 3 days according to the method described by **Desai and Bhide** (1985). One week after the injection of alloxan, fasting blood samples were obtained to estimate fasting serum glucose; rats with 150 mg/dl and above were considered diabetic(**NDDG**,1994).

Experimental design:

A total of 30 adult normal male albino rats Sprague Dawley strain weighing 140± 10 were obtained from Vaccine and Immunity Organization, Ministry of Health, Menoufia Farms, Egypt. Basel diet composition was prepared according to **Reeveset al.**, (1993). Vitamin

mixture and salt mixtures were prepared according to The experimental was carried out at the Faculty of Home Economics, Menoufia University, Shebin El-kom, Menoufia Governorate, Egypt. All the thirty albino rats (about 3 months old) were used in this study and weighted as described previously. Rats cages kept in a room at temperature 25° C and under normal healthy conditions. All rats fed on a basal diet for 7days to make adjustment and then divided into 5 rats in 6 groups as following:

Group (1):Healthy rats fed on basal diet (negative control group). Then all the following groups were injected by alloxan for diabetes induction as described early.

Group (2): Diabetic rats fed on basal diet(positive control group).

Group(3): Diabetic rats fed on Yogurt at 5% of the basal diet.

Group(4):Diabetic rats fed on Arabic gum powder at5% of the basal diet.

Group(5): Diabetic rats fed on mixture of Arabic gum powder and Yogurt at 5% of the basal diet.

Body weight was measured before and after running the experimental. Additionally, at the end of the experiment and after 12 hours fasting blood samples were collected using the abdominal aorta the rats which were scarified under ether anesthetized. Blood samples were then centrifuged for 10 minutes at 3000 rpm to separate the serum. Serum was carefully aspirated, transferred into clean cuvette tubes, and stored frozen at-20°C for analysis. All serum samples were analyzed for determination of the following parameters. Enzymatic determination of plasma glucose was carried out colorimetrically according to the method of Tinder (1969). In addition to that, different parameter for liver functions were determined on the collected serumsuchasALTthat was carried out according to the method of Chimica Acta Yuehe Lin et al., (2018). Also, determination of serum AST and ALPwere carried out according to the Method of King .Ret al., (1979). The CBC test included WBC count, HB level, RBC count, Platelet count and lymphocytes. The results of CBC are generated by highly automated electronic and pneumatic analyzers based on aperture-impedance and / or laser beam cell sizing and counting according to Jacobset al. (2001). Finally, the Histopathological changes of the pancreas have been determined between all animal groups as described by (Bancroft &Stevens, 2010).

Statistical analysis

Collected data were analyzed statistically using Statistic Program Sigma Stat (SPSS, Statistical software, SAS Institute, Cary, NC). ANOVA (Analysis of Variance) test have been used to test the effects of different treatments were analyzed. Duncans multiple range test used to indicate the significances between different groups at ($P \ge 0.05$)

3. Results and discussion

In the current study Alloxanhas been used for inducing hyperglycemia which is a useful experimental model for studying antihyperglycemic activities. The main reason for such use is Alloxan can selectively entry into the β cells. Its use because of the structural features of the islets of Langerhans via the low affinity glucose transporter GLUT2 in its plasma membrane. Thus it can cause destruction of β cells, which in turn leads to a reduction in insulin release and that results in a rise in blood glucose concentration. The following collected data is going to show the possible therapeutic effects of using either Arabic gum (AG) and/or yogurt in different measured parameters among diabetic rats.

3.1. Effect of Arabic gum, yogurt and its mixture on feed intake and body weight gain of diabetic rats:

The current study established to measure the effect of Arabic gum (AG) and yogurt in different parameters of diabetic animal models. Rats used in the current study has been effected mostly when consuming both AG and Y together as it can be seen in table (1) that, feed intake (g/d) was increased within all the groups. However, the biggest increased level was seen with diabetic group G5 that was eating mixture of AG and Y at 5% (15.99g/d; Table 1) comparing to the positive diabetic rats (12.42 g/d; Table 1). However, groups G3 (diabetic group consuming 5% Y only) and G4 (diabetic group consuming 5% AG only) have also increased their feed intake by with less amounts; 14.32 and 15.16g/d respectively.

Table (1): Effect of Arabic gum, yogurt and their mixture on feed intake and body weight gain of diabetic rats

Groups	Feed intake (g/d)	Body weight gain/ 28 days	FER
G1; Negative control	$12.42^{\circ} \pm 0.12$	$27.2^{d} \pm 2.11$	$0.08^{a} \pm 0.11$
G2; positive control	$14.56^{b} \pm 0.76$	$23.1^{\circ} \pm 1.77$	$0.057^{\circ} \pm 0.02$
G3; Diabetic rats treated with 5% yogurt.	$14.32^{b} \pm 1.32$	$28.9^{b} \pm 0.95$	0.072 b ± 0.01
G4; Diabetic rats treated with 5% AG.	15.16 a ± 2.21	31.9 ^b ± 1.24	$0.075^{\mathrm{b}} \pm 0.03$
G5; Diabetic rats treated with 5 % mixture.	15.99 a ± 1.55	35.7 ^a ± 0.83	$0.079^{\mathrm{b}} \pm 0.04$

Values are mean \pm SD. Means under the same column bearing different superscript letters are different significantly (p<0.05).

Regarding the body weight gain data collected within the running experimental, it can be noticed in table (1) again that body weight gain at the end of the experimental has been increased between all the animal groups. However, group consumed mixed of AG and Y at 5% showed the wide effect (35.7g) comparing with the positive control and the negative control groups (27.2 and 23.1 g respectively). Indeed, such increase has been reflected by the highest feed intake within the same group (G5).

3.2. Effect of Arabic gum , yogurt and its mixture on blood glucoselevelsof diabetic rats

Our collected data at the end of the experiment and after 12 hours fasting blood samples showed the effects of different Arabic gum and yogurt concentrations on blood glucose of diabetic rats were tabulated in table (2).It could be noticed that the highest mean value was seen in G2; the diabetic group that fed on basal diet as a positive control group and thatshowed blood glucose levels at $257.66 \pm 4.56 (mg/dl)$. However, the negative control group was seen at the lowest levels (about 91 mg/dl).

Table (2): Effect of Arabic gum, yogurt and its mixture on blood glucoseof diabetic rats

Sincopeol aimbette late					
	Rat groups				
Parameters	G1; Negative control	G2; positive control	G3; 5% Y	G4; 5% AG	G5; 5% Y and AG
Blood glucose (mg/dl)	90.87 ^e ±5.96	257.66 ^a ± 4.56	205°± 3.91	235 ^b ± 3.79	185 ^d ± 4.11
Differences from G1		167	114.13	144.13	94.13

Values mean \pm SD. Means under the same raw bearing different superscript letters are different significantly (p<0.05).

Additionally, groups G3 presented blood sugar levels at average of about 205 mg/dl withdifference with the other groups (Table 2). Again, it can be seen from table 2 that the rats group fed on mixture of Arabic gum and yogurt were lower of their blood glucose values than that in the groups fed on 5% of yogurt and 5% Arabic gum. However, all rat groups consumed the different studied material were significantly lowerin glucose than the positive control group (G2; about 257mg/dl) while they were higher significantly than the negative control group (G1; about 90mg/dl). It can be notice that mixture of tested substances had the highest effective reduced blood glucose results on the level of 5% addition (G5). Such results especially the low levels of blood glucose amounts after AG consumptions is in a great agreements with previous recent studies such as Gates. Set al., (2008) and Ahmed. AA and Fedail .JSet al., (2015) which indicated that AG supplementation reduced fasting blood glucose levels significantly in addition to glycosylated hemoglobin (HbAc1). Also, Mohamed, R.E., et al., (2015) showed that AG supplementation in different forms to rat/mice either normal or diabetic or the ones that fed-high fat diet reduced blood glucose levels significantly. Decreasing the blood glucose levels in such conditions may be because of the inhabitation process in the intestine of the AG to the absorption of glucose as it can cause an interaction with membrane abundance of sodium-glucose transporter 1 (SGLT1) as it has been seen in experimental mice.

3.3. Effect of Arabic gum, yogurt and its mixture on liver functions (AST, ALT and ALP levels) of diabetic rats:

Data in table (3) show effect of different concentration of AG and Yon the liver functions which indicated by the changes in serum enzymeALT, AST and ALP) levels among all the used animal models especially the diabetic rats. AST, ALTT and ALP are used in the evaluation of hepatic disorders as any increase in these enzyme activities reflect how deep is the liver damage or the inflammatory hepatocellular disorders. Previous data finds that alloxaninjection has a significant role in the alteration of liver functions since the activities of AST, ALTT and ALP were significantly higher than normal values (Sarwaret al., 2015).

It could be noticed fromtable (3)that AST and ALT values were at their highest levels in the group fed on positive control (G2; about 84 and 81mg/dl respectively) and the lowest mean was in the group fed on basal diet as normal group; the negative control group (G1; both

treatments were about 41mg/dl). Moreover, G3 and G5 show no significant changes between them in both AST and ALT. (**Alubaidyet** *al.*, 2013)

Table (3):Effect of 5% from Arabic gum, yogurt and its mixture on liver enzymes (AST, ALT and ALP) of diabetic rats

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
G1; Negative control	$40.6^{\mathrm{d}} \pm 2.7$	40.3 ^d ±1.13	15.5 °±0.03
G2; positive control	83.4 a ±0.04	81.6 a ±2.15	37.4 a ±1.06
G3; 5% Y	67.9°±1.12	65.7°±1.13	27.3 b ±1.03
G4; 5% AG	72.8 b±2.13	72.9 b±2.34	35.1 a ±0.02
G5; 5% mixture of Y and AG	65.1° ±4.46	63.4°±3.88	$26.4^{\mathrm{b}} \pm 0.76$

Values are mean \pm SD. Means under the same column bearing different superscript letters are different significantly (p<0.05).

Additionally, ALP results in table 3 revealed that the highest mean value was in positive control group (G2; about 37mg/dl) while the negative control group (G1; about 15mg/dl)was the lowest mean. There is no significant change between groups G2, G4 (about 35and 35mg/dl in both treatments respectively). However, groups fed on 5% mixture of tested material (G5) had the lower mean values than the other groups with no significant changes between this group and group (3) (about 26mg/dl).Indeed previous collected data indicated that AG treatments caused significant regulation in the activities of these enzymes, showing the protective effect of the extract(Ali et al., 2008). This may be because of AG main components; substantial amount of fiber which even giving fiber supplement and of that a prebiotic product. It is non-digestible food ingredients which benefit the host by stimulating selectively the growth or activity of one or more of the colonicbacteria(AL Mosawlet al., 2009; Ali etal., 2010 and NiamaMirghaniet al., 2017). Moreover, yogurt is considering very good source for the probiotic; helpful living organisms (Pace F et al., 2015). Again early collected data of a research that was studying the probiotic yogurt consumption for 8 weeks revealed significant reductions in serum ALT and AST because they can generate a health benefit on the hostsuch as improving the liver enzymes (Fryaral., 2016). Additionally, yogurt such as many probiotics supplementations, it is generally preferred over food sources because of the high potassium, phosphorus, sodium, and sugar content (Atlanta et al., 2017 and Barengelts etal., 2019).).

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Improves the absorption of minerals, especially calcium and help. improves the absorption of minerals, especially calcium. and helps to maintain a healthy balance of bacteria in the GI tract.

3.4. Effects of Arabic gum, yogurt and its mixtureonhemoglobin, WBC and RBClevelsof diabetic rats:

Data showed in table (4)illustrate the effect of feeding different used substrates (AG, Y and its mixture) onblood components between used animal models. It can be notice that there is no significant difference among G4, G5 and normal rats (G1) for haemoglobin values. However, the red blood cells (RBC)×10⁶ within the negative control group (healthy; G1) showed a significantly level which was higher than all other groups (diabetic animals; G2-5)and the differences among all tested groups werenon-significant. The WBC of the normal control group showed significantly changes as compared the other groups and the positive group was the highest value. The tested groups didn't significantly differ between each other.

Table (4):Effects of Arabic gum , yogurt and its mixtureonhaemoglobin. WBC and RBCof diabetic rats

Groups	Hemoglobin(g/dl)	WBC×10 ³	RBC × 10 ⁶
G1; Negative control	12.5 a ±1.04	4.8 °±2.70	5.4 ^a ±0.06
G2; positive control	$7.3^{\circ} \pm 0.19$	8.6 a ±3.0	3.1 °±0.92
G3; 5% Y	9.9 ^b ±1.05	$4.01^{\text{ b}} \pm 2.7$	4.1 b ±0.43
G4; 5% AG	12.2 a ±0.91	$5.79^{b} \pm 1.9$	4.1 ^b ±1.04
G5; 5% mixture of Y and AG	11.3 ^a ±0.64	$5.3^{\text{b}} \pm 2.5$	$4.5^{\text{ b}} \pm 1.05$

Values are mean \pm SD.Means under the same column bearing different superscript letters are different significantly (p<0.05).

3.5. Effects of Arabic gum, yogurt and its mixture onhistopathological changes of the pancreas between diabetic rats:

Pancreas of rats from group 1 fed on basal diet as a negative control group showed no histopathological changes (Photo 1) meanwhile, pancreas of rats from group 2 which injected with alloxan and fed on basal diet as positive control group revealed vacuolation of acinar epithelium, vacuolation of cells of islets of Langerhans's and congestion of pancreatic blood vessel (Photo . 2). Moreover, sections from group 3which injected with alloxan and fed on basal diet and 5% Y revealed vacuolation of some acinar epithelium, congestion of pancreatic blood vessel (Photo . 3) .Some examined sections from group 4which injected with alloxan and fed on basal diet with 5% AG revealed vacuolation of acinar epithelium and vacuolation of cells of islets of Langerhans's (Photo 4), whereas pancreas from group 5 which injected with alloxan and fed on basal diet with mixture of Y and AG revealed no histopathological changes except vacuolation of epithelial lining some acini in some sections (Photo 5). The pancreas is the organ that produces insulin, and it plays a major role in regulating blood glucose levels, with an insufficient amount of insulin in the body, diabetes develops. Over time, the beta cells become damaged and may stop producing insulin altogether. Results from epidemiological studies generally support that consumption of milk and dairy products is associated with a lower incidence of T2D or improvements in glucose homeostasis indices, and studies of animal and cell models support a positive effect of dairy-rich diets or components on metabolic and inflammation factors relevant to T2D and insulin resistance. Emerging evidence indicates that dairy components that alter mitochondrial function (e.g., leucine actions on silent information regulator transcript 1 (SIRT1)-associated pathways), promote gut microbial population

shifts(Lee et al., 2010). Studies supported that Guar gum lowers the postprandial glucose response when mixed into a variety of test meals. The supplementation of high carbohydrate diets with Guar gum has been demonstrated to effectively enhance insulin sensitivity(Calameet al.,

2011and Mohammed *et al.*, **2018**)

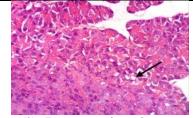
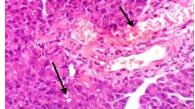


Photo (1): Pancreas of rat from group 1 fed on basal diet as a control group(H & which diabetic and fed on basal diet as E X 400).

Photo (2): Pancreas of rat from group 2 positive control group (H & E X 400).



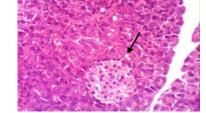


Photo (3): Pancreas of rat from group 3 Photo (4): Pancreas of rat from group 4 fed on basal diet and 5% yogurt (H & E fed on basal diet and 5% AG(H & E X X 400). 400).

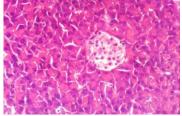


Photo (5): Pancreas of rat from group 5 fed on basal diet and 5% mixture of AG and Y (H & E X 400).

In conclusion, the obtained results noticed that natural fibres of Arabic gum act as prebiotics especially when it has been compared with the yogurt that was the main probiotic source in our study. Both of them have improved the healthy stats of the diabetic animal models used in the current study in different shapes. For instance, it has decreased the blood glucose levels, indicated healthy liver functions. However, much more human studied are needed.

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التأثيرات الناتجة لبعض مصادر البروبيوتيكوالبريببوتك الغذائية على بعض الخواص لدى الفئران المصابة بالسكرى

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الملخص العربي:

توصى العديد من الدراسات بالتنخل الغذائي لكثير من الامراض ,وعلى سبيل المثال: تقلل الاطعمة الغنية بالبكتريا المفيدة من خطر الاصابة من الامراض مثل السكرى وامراض القلب والقلب والتوحد والسرطانات وامراض الكلى والاصابة بالسمنة...الخ لذلك يهدف هذا البحث الى دراسة تأثير مصادر مختلفة من البروبيوتكوالبريبيوتك على مستوى سكر الدم و معدل الشهية او تناول الطعام بالإضافة الى وظائف الكبد و فحص البنكرياس هستولوجيا باستخدام فئران التجارب. تم استخدام الزبادى المخفض الدهون كمصدر للبروبيوتك بتركيز (5%) و الصمغ العربي بتركيز (5%) مع عمل مخلوط منهما بتركيز (5%) من الوجبة الاساسية للفئران وذلك بجانب مجموعتين ضابطتين احدهما سالبة والاخرى موجبة . تم حساب مقدار الزيادة في وزن الفئران , كما تم تجميع عينات دم لتقدير مستوى السكر ووظائف الكبد (AST,ALT،ALP). قد اوضحت النتائج ان جميع المصادر قامت بتحسين مستوى السكر فىالدم لجميع الفئران لوحظ تحسين وظائف الكبد و خلايا البنكرياس مقارنه بالمجموعات المصابة. بالنهاية و طبقا لوحظ تحسين وظائف الكبد و خلايا البنكرياس مقارنه بالمجموعات المصابة. بالنهاية و طبقا للمتائج يوصى البحث باستخدام مخلوط الزبادي والصمغ العربي بنسبة (5%) كعلاج غذائي المرضى السكر.

الكلمات المفتاحية: الزبادي منخفض الدهون, الصمغ العربي, مرض السكري, وظائف والكلي, البنكرياس.