Egypt. J. of Nutrition and Health Vol.17No.2 July (2022)

Effect of Lentil, Chickpeas and Okara with probiotic bacteria on the health status of diabetic induced rats

Heba S Abd Elhaliem[1] and Baseem A. Zwain[2]
[1]Clinical nutrition Dep. NNI,[2]Cairo University Hospitals

Abstract

The effect of different percent of lentil, Chickpeas and okara with probiotics bacteria (lactobacillus acidophilus)as mixtures on some biological and histological parameters of diabetic albino rats were studied. Thirty adult female rats were distributed into five groups, The first one was fed on basal diet and kept as the negative control group. The second group being diabetic rats were fed basal diet as a positive control group while the other three diabetic groups were fed on tested diets without and with probiotic bacteria for 28 days. Results showed that body weight gain was markedly higher especially in the mixture of probiotics (2%) compared to the other diabetic rats. Also, this mixture led to low concentrations of total cholesterol, triglycerides, LDL-c(low-density lipoprotein cholesterol), VLDL-c(very low-density lipoprotein cholesterol) and decreased AST(aspartate aminotransferase) and ALT(alanine transaminase) enzymes nearly to normal ranges. Furthermore, the mixture caused significant increase in HDL-c and total immunoglobulin production (IgG, IgM, IgE, IgA), which were higher than the mixture of 5% lentil, chickpea, okara without probiotics .Histopathological examination showed amelioration of histopathological lesions seen in liver of rats received the mixture of tested materials at different levels with probiotics. So, it could be recommended that intake 5% of lentil , chickpea , okara mixture with 2% from probiotics for diabetic patient after pilot study on human to improve the biochemical analysis and increase the immunity production.
Key words: Female rats, lentil, chickpea, okara, probiotics , histopathological structure.

Introduction

Type 2 diabetes mellitus (T2DM) is one of the most common endocrine and metabolic diseases and is characterized by impaired pancreatic β cell function and insulin resistance in insulin target tissues. Data have shown that the incidence of T2DM has been sharply increasing worldwide and has become one of the most critical public health concerns, Moreover, due to diabetes-related complications such as blindness, kidney failure, heart attacks, stroke, and limb amputation, T2DM is a leading cause of morbidity and mortality worldwide.**Gurung*et al*.,(2020)and Arora *et al.,*(2021).**A probiotic is defined classically as a viable microbial dietary supplement that beneficially affects the host through their effects in the intestinal tract. This definition, however, was initially intended for use with animal feed products. For human nutrition, the following definition has been proposed: A live microbial food ingredient is beneficial to health ***(Morelli ,2014 and Zoke, 2020).***

Legumes furnish food for humans and animals and provide edible oils, fibres, and raw materials for plastics. Many are grown for their edible seeds, which are high in protein and contain many of the essential amino acids. For important members of the legume family as bean; chickpea; cowpea; lentil; peanut; soybean; and tamarind.

lentil, (*Lens culinaris*), small annual legume of the pea family (Fabaceae) and its edible seed. Lentils are widely cultivated throughout Europe, Asia, and North Africa but are little grown in the Western Hemisphere. The seeds are used chiefly in soups and stews, and the herbage is used as fodder in some places. Lentils contains more than 25% protein. They're also a great source of iron and minerals which might be lacking in vegetarian diets. Lentils are high in fibers, which support regular bowel movements and the growth of healthy gut bacteria. Eating lentils can increase your stool weight and improve your overall gut function . Furthermore, lentils contain a broad range of beneficial plant compounds called phytochemicals, many of which protect against chronic diseases such as heart disease and type 2 diabetes ***Kumar and Baojun, (2018) and Lisa et al.,(2018) .***

Chickpea (*Cicer arietinum L*.) is one of the world's most important grain legumes .Chickpea is an excellent source of protein, carbohydrates, and fiber, and provide many essential vitamins and minerals. Their highly nutritional properties have been associated with many beneficial health-promoting properties, such as managing high cholesterol and type-2 diabetes and in the prevention of various forms of cancer. The main antioxidant compounds in legumes are vitamins C and E, phenolic compounds and reduced glutathione (GSH). Different studies have shown that they have a protective antioxidant effect on cancer and cardiovascular ***diseasesTayloret al.,(2016) and Evan and Heather, (2020).***

Soybean, as well as its by-product okara represents a rich source of dietary fibers, protein and oil. The use of okara proteins as meat extenders has spread significantly due to the interesting nutritional and functional properties that are present in soybean proteins. Together with these properties, health and economical reasons are the major causes for the addition of soybean proteins to meat products. Nevertheless, despite the good properties associated to soybean proteins, there are many countries in which the addition of these proteins is forbidden or in which the addition of soybean proteins is allowed up to a certain extent ***(Belloque et al., 2002); Fei et al., 2013) and Ostermann et al., (2017).***This research aimed to study the effect of tested mixtures with probiotic on some biological and histopathological parameters of diabetic rats. There are few studies in the literature about the relationship between the use of Okara supplements and thyroid functions ***(yen, 2001).***

Materials and methods

Lentil, chickpea and okara were obtained from the local market and probiotics lactobacillus acidophilus were obtained and scientifically identified at Horticultural Research Institute, Agriculture Research Center, Egypt. All chemicals and diagnostic kits were purchased from El-Gomhoria Co., Cairo, Egypt.

This study was carried out on thirty adult mail or female Sprague Dawley albino rats weighing 150±5 g body weight. The rats were obtained from Laboratory Animal Colony, Helwan, Egypt. Before their use in the experiment, the rats were kept for one week for acclimatization to the laboratory conditions. They were fed on basal diet and provided with water and food ad libitum.

The basal diet consisted of casein (10%), cellulose (5%) salt mixture (4%), vitamin mixture (1%), corn oil (10%) and corn starch (70%) according to***Reeves et al. (1993).***

Diabetes mellitus (DM) was induced in normal healthy male or female 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 200 mg/dl and above were considered diabetic ***NDDG,(1994)***.

Experimental procedure: Rats were divided into five groups consisting of six rats each. The first group (G1) wasnormal rats, which fed on the basal diet and kept as a negative control group, while the other diabetic groups were fed on lentil,chickpea and okara with probiotics for 4 weeks as the following : Group 2 (G2) diabetic rats were fed on basal diet as positive control group , group3 (G3): Diabetic rats were fed on 5% mixture of lentil , chickpea and okara without probiotics. Group 4 (G4) on5% mixture of lentil, chickpea with 1% probiotics. Group 5 (G5) 5% mixture of lentil, okara and 2% probiotics.

During the experimental period, the feed intake and body weight were recorded weekly. Body Weight Gain (BWG) and Feed Efficiency Ratio (FER) were calculated at the end of the experimental period according to the following equations: BWG (g) = final body weight (g) – initial body weight (g); FER = weight gain (g/ day) /feed intake (g / day).

Collection of blood samples and organs following 12 h fast:
At the end of the experimental period, rats were lightly anaesthetized by ether and about 7 ml of blood was withdrawn from hepatic portal vein into dry centrifuge plastic tubes. After centrifuge serum was separated and kept in tubes at -20 ºC till biochemical analysis ***Schermer, (1967).***

Biochemical analysis: Serum total cholesterol was calorimetrically determined according to ***Allain et al. (1974)*** and triglyceride was determined calorimetrically as stated by ***Fassati and Prencipe (1982). High Density Lipoprotein cholesterol (HDL-c) was determined calorimetrically reported by Lopez (1977).*** Low Density Lipoprotein cholesterol (LDL-c) and Very Low Density Lipoprotein cholesterol (VLDL-c) were determined in line with**Friedewald*et al*. (1972).**

The activity of Aspartate Aminotransferases (AST) and Alanine Aminotransferases (ALT) enzymes were determined by the methods of ***Yound, (1975) and Tietz, (1976),*** total immunoglobulin (IgG, IgM, IgE and IgA) were determined by Radioimmunoassay as described by the method of ***Engvall and Perlman (1971).***The determination of leptin hormone was carried out according to***Cosidine et al. (1996).*** The determination of thyroid stimulating hormone (TSH) was carried out according to ***Uotila et al. (1981).*** Thyroid hormones (free T4 and free T3) were estimated in serum using Radioimmunoassay (RIA) as described by ***Patrono and Peskar (1987).*** The determination of Insulin Hormone was carried out according to***Defronzo et al. (1979).***

Histopathological study: Livers of the sacarified rats were dissected, taken, washed with normal saline and put in 10% formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol, Then were cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness, stained with Hematoxylen and Eosin (H and E) and then studied under an electronic microscope according to ***Bancroft et al.,(1996).***

Statistical analysis: Results are expressed as mean values with their standard deviation of the mean. Statistical differences between groups were evaluated using one-way ANOVA followed by

Duncan post hoc test using SPSS version 11.0 for Windows SPSS(2000). Differences were considered significant at ($p<0.05$) according to SAS 2002.

Results and Discussions

Probiotics are live microorganisms when administered in adequate amounts, confer a health benefit on the host. The healthy human body contains such microbes physiologically; and they can be obtained in forms of over the counter food supplements as well. Over the last few years, probiotics, especially the lactobacillus species were shown to be effective in the therapy of type 2 diabetes. In type 2 diabetes, gut microbiome is found to be different from that in the healthy population. In a human study, the amount of Firmicutes bacteria was lower, whereas the number of Bacteroides and Proteobacteria is higher in the gastrointestinal tract of patients with type 2 diabetes compared to non-diabetic persons .

The study showed that the ratio of Bacteriodes and Firmicutes species had positive correlation with decreased insulin resistance, however, causality has not been proven yet. Following innovative dietary strategies, it seems possible to maintain euglycemia by normalizing the altered microbiome, and to prevent long term micro- and macrovascular complications of type 2 diabetes. Although, there have been numerous bacterial species investigated in the therapy of type 2 diabetes, no consensus has been obtained regarding the effectivity and the most effective species. For instance, an earlier meta-analysis suggested, that the intake of certain Lactobacillus species, such as L. fermentum, L. ingluviei and L. acidophilus can lead to weight gain, while the ingestion of L. gasseri and L. plantarum might end up in weight loss both in animal and human studies. Previous meta-analysis in this field were not conducted with assessment of the evidence quality levels and the number of identified trials that met their inclusion criteria was relatively low. Two meta-analysis found no significant effects of probiotics on lipid profile and two meta-analysis found decreased indexes of lipid profiles ***He et al., 2017; Abbasi et al., 2018 and Sun, (2019).***

Effect of mixture of lentil, chickpea, okara and probiotic on feed intake (FI), body weight gain (BWG) and feed efficiency ratio (FER) in diabetic rats (table 1) showed that feeding the mixture in group (5) (5% mixture of lentil , chickpea , okara) and 2% probiotics led to increase the feed intake which was near to the normal group. The feed intake of the other tested groups were lower than group (5). There is no significant change between group 2 and 3. Group (3) fed on 5% mixture of lentil ,chickpea and okara without probiotics was the lowest in feed intake . There were significant differences between positive control group and the groups 4 and 5. In case of feed efficiency ratio, the fifth group was the best group and there is no significant change as compared with the negative control group. The lowest group for FER was the positive control group. These results were matched with ***Salminenet al.(1998)*** who found that probiotic improved the feed intake and body weight of rats. Probiotic as functional foods enhanced the gastrointestinal functions, and motility as well as those that modulate epithelial cell proliferation ***Clydesdale, (1997).***

Table (1):
Effect of mixture of lentil,chickpea, okara and probiotic on feed intake (FI), body weight gain (BWG) and feed efficiency ratio (FER) in diabetic rats.

Groups / Parameters	G1	G2	G3	G4	G5
Feed intake g/day	14.08[a] ±0.75	11.58[c] ±0.38	12.53[b] ±1.01	13.5[a] ± 0.18	13.98[a] ±0.75
BWG g / 4 weeks	43.08[a] ±2.32	30.41[c] ±0.56	35.17[b] ±4.42	39.27[a] ±3.01	41.58[a] ±2.32
FER	0.110[a] ±0.01	0.094[d] ± 0.01	0.100[c] ± 0.06	0.104[b] ± 0.01	0.106[b] ±0.01

Values are mean±SD. Values in the same row sharing the same superscript letters are not statistically significantly different at ($p \leq 0.05$)

Effect of mixture of lentil, chickpea ,okara and probiotic mixtures on blood glucose in diabetic rats(Table 2). It could be noticed that the highest mean value of serum glucose was that in group fed on basal diet as a positive control group(G2).The negative control group(G1)hasthe lowest value . The mean values of blood glucose recorded in group (5)was lower than the other tested groups . Both of the groups fed on tested material significantly lower than the positive control group but they significantly were higher than negative control group. Alloxan is a urea derivative, which causes selective necrosis of the β cells of pancreas. In addition, it has been widely used to produce experimental diabetes in animals such as rabbits, rats, mice and dogs with different grades of disease severity by varying the dose of alloxan used. As it has been widely accepted that alloxan selectively destroys the insulin-producing beta-cells found in the pancreas, hence it is used to induce diabetes in laboratory animals. The toxic action of alloxan on pancreatic beta cells involve oxidation of essential sulphydryl (-SH groups), inhibition of glucokinase enzyme, generation of free radicals and disturbances in intracellular calcium homeostasis ***Iranloye et al ., (2014).Ahmed et al.(2015)***reported that supplementation of lentil, chickpea significantly decreased fasting blood glucose levels and glycosylated hemoglobin (HbAc1) together with significant reduction of blood uric acid and total protein concentrations **.** Moreover, supplementation of okara in different forms to the normal or diabetic or fed-high fat diet rat/mice significantly reduced blood glucose levels**.** The blood glucose lowering effect may be attributed to inhibiting absorption of glucose in the intestine via interaction with membrane abundance of sodium-glucose transporter 1 (SGLT1) in experimental mice***Hosokawa et al.,(2016).***Okara is a pulp that consists of the insoluble parts of the soybean, which is a by-product of the production of soy milk and *tofu*. It is considered a functional food in Asia and contains three beneficial components: insoluble dietary fiber, soy proteins, and isoflavones. Although isoflavones and soy proteins have been shown to improve glucose tolerance ***Tachibana et al.,(2014).***Probiotics could be a supplementary therapeutic approach in type 2 diabetic patients to improve dyslipidemia and to promote better metabolic control. Probiotics appear to slow down digestion of carbohydrates, preventing blood sugar spikes that can lead to type 2 diabetes or exacerbate damage done by the disease, **Lactobacillus acidophilus and L. casei** improved glucose tolerance and hyperglycemia in animal studies ***Kesika et al.,(2019) and OkeseneGafa et al.,(2020).***

Table (2):

Effect of different concentration of lentil,chickpea ,okara and probiotic mixtures on blood glucose induced diabetic rats.(mean±SD)

Groups \ Parameter	Blood Glucose (mg/dl)
G1	100.07 [e] ±10.06
G2	279.55[a]± 11.44
G3	267.65[b]± 12.87
G4	245.98[c]± 7.99
G5	219.23[d]± 5.01

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

Effect of mixture of lentil, chickpea, okara and probiotic on serum lipids induced diabetic rats (Table 3).Serum HDL-c levels increased compared with the positive group(G2) but not significantly by the administration of the mixture of lentil, cicer ,okara and probiotics. Group 5 rats showed significantly higher levels of HDL-ccompared to control (+ve) group. The mean value of groups 3 and 4 were lower for HDL-c than control (-ve) group while it was higher for the other lipid parameters . The obtained results are similar to that of***Hasler (2002)***who found that okara can decrease LDL-c, total cholesterol and increase the level of HDL-c. Also,***Clydesdale (1997)***reported that probiotics reduce the absorption of lipids from diet .The present findings are similar to those of previous meta-analyses of randomized controlled trials considering the association between probiotics and lipid profiles. They reported that probiotics had the hypocholesterolemic effects of on total cholesterol and LDL cholesterol .*L. acidophilus* are able to survive in acid and bile environments and easily colonize the human intestinal tract.Thus, this strain is candidates for therapeutic dietary interventions for hyperlipidemia. However, these effects may be altered by the probiotic dose and delivery method (eg, fermented dairy products, freeze-dried bacteria). It has been suggested that probiotics may alter the pathways of cholesterol esters and lipoprotein transporters and may promote the excretion of cholesterol and bile acid rather than affecting hepatic cholesterol synthesis ***(Savard et al., 2011 and Ivey et al., 2015).***

Table (3):

Effect of mixture of lentil,chickpea ,okara and probiotic on serum lipids induced diabetic rats.

Serum lipids	G1	G2	G3	G4	G5
Total cholesterol	80.24[c] ±5.19	107.02[a] ±3.05	96.16[b] ±1.12	90.23[c] ±0.31	86.85[c] ±0.31
Triglycerides	85.48[e] ±6.03	180.8[a] 4.37±	165.68[b] ±5.93	140.96[c] ±9.56	129.06[d] ±10.06
HDL-cholesterol	47.94[a] ±2.12	36.87[d] ±1.05	39.92[c] ±0.03	41.04[c] ±0.05	44.94[b] ±0.05
LDL-cholesterol	15.2[d] ±0.17	33.9[a] ±0.34	23.31[b] ±1.01	19.1[c] ±4.41	16.01[d] ±3.65
VLDL-cholesterol	17.10[e] ±0.17	36.16 [a] ±1.34	33.14[b] ±0.91	29.19[c] ±0.91	25.81[d] ±0.91

Values are mean±SD. Values in the same column sharing the same superscript letters are not statistically significantly different at (p≤0.05)

Effect of mixture of lentil, chickpea ,okara and probiotic on liver enzymes induced diabetic rats (Table 4) . The administration of 5% mixture of lentil, chickpea, okara and 2% probiotics significantly decreaseAST and ALT level when compared with the groups 2 and 3. There is no significant differences between group 2 and 3 for ALT and AST. While, there are significant changes between group 3 and the other treatment groups. Hyperglycemia, as evidenced in previous studies, can result in deterioration of liver and kidney functions which explains the increase in the activity of these enzymes in G2***Bai et al., (2013).*** In the present study, the activity of transaminases significantly decreased in G5 compared to G2 indicating that high fiber diet and high protein content have improved the liver function ***Cantero et al., (2017).*** From the above results, it could be noticed that crude fiber in legumes is a group of indigestible carbohydrates. It can improve the function of the alimentary tract and also lower blood glucose , cholesterol levels and improve liver functions in normal range***Roberfroid,(2000)***.Probiotics have been suggested as a treatment for the prevention of chronic liver damage, because they prevent bacterial translocation and epithelial invasion, and also inhibit bacterial mucosal adherence, and the production of antimicrobial peptides, while decreaing inflammation, stimulation of host immunity.Probiotics had significant effect on improving the level of liver enzymes and sonographic fatty liver. A 12-week course of probiotics studied was effective in improving nonal and coholic fatty liver disease and lipid profile of obese children***Xu et al., (2016)*****and***Miyake and Yamamoto (2013).*

Table (4):

Effect of mixture of lentil, chickpea, okara and probiotic on liver enzymes induced diabetic rats.

Parameters	G1	G2	G3	G4	G5
AST(U/L)	35.76^{d} ±5.07	54.2^{a} ±1.11	52.7^{a} ±6.05	49.1^{b} ±8.06	42.1^{c} ±0.56
ALT(U/L)	29.8^{d} ±3.87	50.9^{a} ±8.91	49.7^{a} ±7.25	46.23^{b} ±6.62	40.7^{c} ±6.99

Values are mean±SD. Values in the same row sharing the same superscript letters are not statistically significantly different at (p≤0.05).

Effect of mixture of lentil, chickpea, okara and probiotic on kidney functions in diabetic rats.(Table 5) .The effect of some protein sources on uric acid (UA) was presented in table (5) The effect of mixture of lentil, chickpea ,okara and probiotic on level of uric acid was 2 .95± 0 .01 mg/dl for control group which lower than all other groups and close to the negative control . The highest UA concentration was found in diabetic rats fed with basal diet as positive control as compared to the other rats (P ≤ 0.05) followed by the level of UA in group3, which fed on mixture of lentil, chickpea ,okara without probiotic . UA of groups 4 and 5 were significantly lower than the results were recorded in group 2 and 3 . However the levels of groups 4 and 5 were significantly higher than negative control group .For urea ,the negative control group showed significant difference with the other groups except group 5, which recorded non significant with group 1. There is no significant effect in creatinine levels in groups 4, 5 and negative control group , whereas serum creatinine concentrations of group 2 and 3 were significantly higher than the other groups .Alterations in dietary protein intake have an important role in prevention and management of several forms of kidney disease. Using soy protein instead of animal protein reduces development of kidney disease in animals. Reducing protein intake preserves kidney function in persons with early diabetic kidney disease.Okara intake significantly improved proteinuria and urinary creatinine ***Azadbakht and Esmaillzadeh, (2016).***Consumption of probiotics may improve gastrointestinal function and slow the progression of chronic kidney disease (CKD). The researchers also considered a study in which patients with CKD were given prebiotics and probiotics or a placebo. Blood urea nitrogen levels of patients with CKD were decreased. Treatment with symbiotic probiotics for six weeks led to significant decreases in mean blood urea levels of patients with CKD (chronic kidney disease) stages 3 and 4 compared to controls; effects were not seen in other renal function indicators. Probiotics can decrease the creatinine concentration by altering the gut microbiome, thereby increasing the extrarenal elimination of creatinine ***Ramezani and Raj, 2014 and Irouzi et al.,(2015).***

Table(5):
Effect of mixture of lentil, chickpea, okara and probiotic on kidney functions in diabetic rats.

Parameters	G1	G2	G3	G4	G5
Uric acid(U/L) Mean ± SD	2.95±0.01^{c}	4.98±0.05^{a}	4.90±0.11^{a}	3.94±0.09^{b}	3.07±0.66^{b}
Urea Mean ±SD (U/L)	17.11±0.9^{c}	29.6±1.9^{a}	28.6±1.4^{a}	25.9±0.6^{b}	20.6±1.03^{c}
Creatinine(U/L) Mean ±SD	0.77± 0.057 ^{b}	1.96±0.085 ^{a}	1.74±0.075 ^{a}	0.98^{b}±0.094	0.94 ±0.11 ^{b}

Values are mean±SD. Values in the same row sharing the same superscript letters are not statistically significantly different at (p≤0.05).

Effect of mixture of lentil, chickpea, okara and probiotic on immunological productions in diabetic rats (Table 6) It could be observed that administration of the tested mixture and probiotics it affect rats activity (group 5). The mixture of lentil ,ckickpea ,okara and probiotics bacteria induced significant improvement in serum levels of immunological profile compared to positive control group. The tested mixture without probiotics and the low level which additives caused non significant changes in serum level of IgA (Immunoglobulin A) and IgG (Immunoglobulin G)productions.The same table showed the serum levels of IL-4(Interleukin 4) and IL-5(Interleukin 5) in diabetic rats and the other tested groups. IL-4 serum level was significantly higher in positive control group compared to those in the other groups . Likewise, the serum levels of IL-5 was significantly higher in positive control group than those in the others . There is no significant differences in the serum IL-5 and IL-4 for groups 2 and 3. Researchers found that adults with type 2 diabetes tended to have higher levels of the IgA and lower levels of IgG, IgE(Immunoglobulin E) and IgM (Immunoglobulin M) .The main antioxidant compounds in legumes are vitamin E, phenolic compounds. So, different studies have shown that they have a protective antioxidant effect on immunity status, cancer and cardiovascular diseases **(*Mallillinet al., 2008 and Murtyet al., 2010).***Probiotics are beneficial bacteria that live in the digestive tract and help digest food, synthesize vitamins and support the immune systems. Probiotics have been extensively used in improving the host health and for treating different infectious and non-infectious pathologies in animal models. Namely, protection against infections , relief of irritable bowel symptoms , inhibition of *Helicobacter pylori* growth , prevention of cancer , decrease in gut inflammatory response , and prevention of allergies . Probiotics such as *Lactobacillus rhamnosus* strain GG and *L. plantarum* showed the ability to inhibit attachment of enteropathogenic *Escherichia coli* in the GI tract and decrease in the cytokines production such as IL-4 and 5 and a slight increase in the mononuclear cell infiltration of small intestine was observed ***Park et al., (2017) and George et al., (2018).***

Table(6):

Effect of mixture of lentil, chickpea, okara and probiotic on immunological productions induced diabetic rats.

Immunological Profile IU/ml	G1	G2	G3	G4	G5
IgE	78.98d ±17.05	168.76a±12.2	159.87 a±10.88	135.76b±10.11	113.43c±9.11
IgM	120.2 a ±15.76	76.54e±8.95	86.13d±10.5	97.66c±12.5	108.41b±9.54
IgA	117.1d±1.1	173.5a ±6.99	169.4 a ±11.05	153.26 b ±6.72	136.99c±7.88
IgG	1113.05a±11.05	1002d±11.87	1015.43d ±25.16	1056.66c±11.27	1082.23b±21.76
IL-4 pg/ml	10.24 e ±0.52	17.13 a ±5.16	16.97a ±1.07	14.93 c±1.04	12.87 d±0.67
IL-5 pg/ml	7.92^{d} ±0.34	11.75^{a} ±1.28	11.05^{a}±0.99	9.95^{b}±1.04	8.95^{c}±0.55

Values are means±SD. Values in the same column sharing the same superscript letters are not significantly different at (p≤0.05)

Effect of feeding protein sourceson some metabolic hormones in diabetic rats(Table 7)the mean insulin hormone was the highest value in the negative control group and the lowest mean was in group 2, which fed on basal diet. There is no significant differences between groups (3 and 4).

While data reflected the effect of mixture of lentil, chickpea, okara and probiotic on leptin hormone for tested rats showed the highest mean value was in the groups fed basal diet as positive control and the lowest mean was in the group fed basal diet as a negative control. There is no significant differences between groups 4and 5 which fed on mixture of lentil, chickpea, okara and probiotic . Data for T3, T4 and TSH hormones of tested rats showed that the highest mean value was in the negative control group and the lowest mean was in diabetic group fed on basal diet.There is no significant differences between group4 and 5 . Diabetes mellitus led to significantly increase leptin and TSH hormones concentration during the study. While it led to decrease the levels of insulin, T3 and T4 .Generally, plasma concentrations of leptin have been shown to be proportional to body fat mass, because leptin plays a major physiologic function in informing the central nervous system about the amount of energy that is stored to regulate satiety and energy expenditure***(Grossmann, 2011).***

George et al.(2018)reported that dietary proteins have an insulinotropic effect and thus promote insulin secretion, which indeed leads to enhanced glucose clearance from the blood. In the long term, however, a high dietary protein intake has been associated with an increased risk of type 2 diabetes and this matched with the obtained results.Thyroid hormones (TH) play a crucial role in the differentiation, growth, development and function of nearly all tissues. They have long been recognized as a key regulator of oxygen consumption and the basal metabolic rate . These effects have been attributed to the direct actions of TH on the heart and metabolically active tissues, including liver, white and brown adipose tissue and skeletal muscle. Thyroid hormones are also involved in the central regulation of energy balance at the level of the hypothalamus . A normal Total T4 level in adults ranges from 5.0 to 12.0µg/dl. A normal Total T3 level in adults ranges from 80-220 ng/dl and TSH was between 0.4- 4.0 milli-international units/liter***Dandona and Dhindsa, (2011).***The source of consumed protein has a significant impact on the thyroid hormone status., thyroid hormones stimulate the synthesis as well as the degradation of proteins, whereas in supraphysiological doses protein catabolism predominates. Okara protein is commonly used due to its antioxidant, antitumoral, and immunity increasing features and its effect on protein synthesis.

Supplementation of probiotics showed beneficial effects on thyroid hormones and thyroid function in general. A literature research was performed to examine the interplay between gut microbiota and thyroid disorders that should be considered when treating patients suffering from thyroid diseases. Probiotics are non-pathogenic microorganisms that can reach the colon alive, having beneficial health effects for their host. In hypothyroidism and hyperthyroidism, *Lactobacillaceae* and *Bifidobacteriaceae* are often reduced. Lactobacillus reuteri supplementation proved to benefit the thyroid function in mice by increasing free T4, thyroid mass, and physiological parameters, like more active behavior. This effect could be triggered by interleukine-10 and subsequent enhanced T-regulatory cells. In broiler chickens, two studies examined increased T3 and T4 levels after the supplementation of probiotics. However, when conferring animal studies, it must be considered that microbiota in different animal species are not obligatory comparable to humans ***Parketal.,(2017) and George et al.,(2018).***

Table (7):
Effect of feeding protein sources on some metabolic hormones induced diabetic rats.

Animal Groups	Insulin hormone (mIU/L)	Leptin (ng/ml)	Thyroid hormones		
			T3 (ng/dl)	T4 (µg/dL)	TSH (milli-international units /liter)
(G1)	25.15 ± 0.321 [a]	3.87 ± 0.59 [d]	85.75 ±5.55 [a]	6.05 ±0.03 [a]	0.43 ±0.002 [c]
(G2)	10.41 ± 2.267 [d]	15.15 ± 2.07 [a]	67.76 ±4.35 [d]	2.12 ±0.43 [c]	1.95 ±0.003 [a]
(G3)	14.11 ±1.49 [c]	12.15 ± 1.83 [b]	75.01 ±4.96 [c]	4.22 ±0.96 [b]	2.35 ±0.002 [a]
(G4)	14.91 ± 2.467 [c]	6.90 ± 1.71 [c]	80.80 ±3.63 [b]	5.07 ±0.72 [a]	0.89 ± 0.001 [b]
(G5)	19.431 ± 2.341 [b]	6.09 ± 1.08 [c]	81.38 ±3.63[b]	5.82 ±0.55 [a]	0.73 ±0.001 [b]

Values are mean±SD. Values in the same column sharing the same superscript letters are not statistically significantly different at ($p \le 0.05$).

Histopathological examination of liver of the negative control rats revealed normal histological picture of hepatic lobule which consists of central vein surrounded by normal hepatocytes as shown in (photo. 1). Examination of liver of group (2) showed of hepatocytes and infiltration of leucocytes in hepatic sinusoid (photo. 2). Liver of rats given the mixture without probiotic showed little vacuolar degeneration of hepatocytes and some improvement in degeneration (photo. 3). In addition, portal edema and few leucocytes infiltration in hepatic lobule were observed in group (4) which fed on the mixture and 1% probiotic (photo. 4). Liver in (G5) showed marked improvements with no observed pathological lesions (photo 5). These results were according to ***Canteroet al. (2017) and George et al.(2018)***who found that probiotic at level 2% and the tested mixture can keep the liver tissue in normal status without any changes and improve the cells structure more than the other diabetic rats

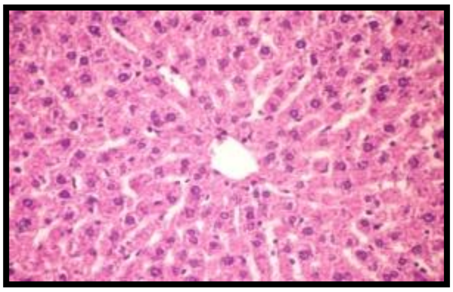

Photo (1):
Liver of normal rat received basal diet (negative control)

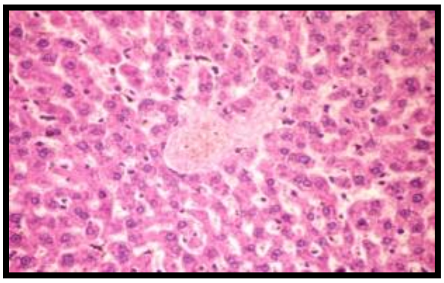

Photo (2):
Liver of diabetic rat received basal diet (positive control)

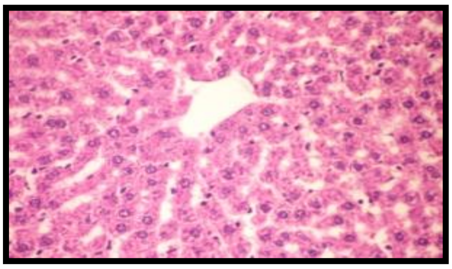

Photo (3):
Liver of diabetic rat received basal diet and the mixture without the probiotic

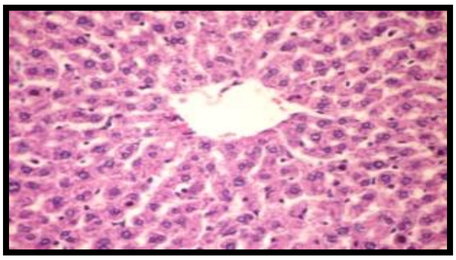

Photo (4):
Liver of diabetic rat received basal diet and the mixture with 1% probiotic

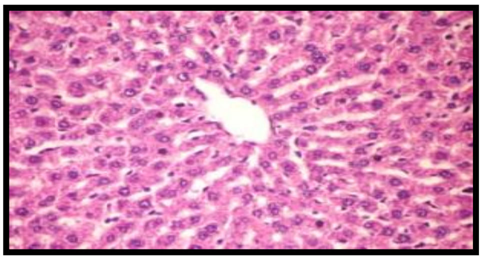

Photo (5):
Liver of diabetic rat received basal diet and the mixture with 2% probiotic

Conclusion On the basis of the present results, it could be concluded that mixture at 5% of lentil ,chickpea ,okara and 2% probiotics may have synergistic effect and its intake is useful for product high protein food supplemented with probiotic bacteria (*lactobacilli*) lead to reduce feed intake and body weight and improves serum lipid profile, liver function and immunological activity in diabetic rats. Moreover, this mixture has a promising effect on the liver tissues as it ameliorates the histopathological lesions seen in this organ of diabetic rats.

References

Abbasi, B.; Mirlohi, M.; Daniali, M. and Ghiasvand, R. (2018):
Effects of probiotic soymilk on lipid panel in type 2 diabetic patients with nephropathy: A double-blind randomized clinical trial. Prog. Nutr**.,** 20: 70–78 .

Ahmed, B.; Al-Howiriny, T. A. and Siddiqui, A. B. (2015):
Antihepatotoxic activity of seeds of phyllanthus. J. Ethnopharmacol., 87: 237-240.

Allain, C. C.; Poon, L. S. and Chan, C. S. (1974):
Enzymatic determination of serum total cholesterol. Clin. Chem., 20: 470-475.

Anand, G.; Vasanthakumar, R. and Mohan, V.(2014):
IL-4 and decreased IL-5serum levels are associated with increased Th1 and suppressed Increased Th2 cytokine profile in patients with diabetic nephropathy (CURES-134). Int. J. Clin. Exp. Pathol.,7(11):8008-15.

Arora, A.; Behl, T.; Sehgal, A.; Singh, S.; Sharma, N.and Bhatia, S. (2021):
Unravelling the Involvement of Gut Microbiota in Type 2 Diabetes Mellitus. *Life Sci.,* 273: 11931.

Azadbakht, L. and Esmaillzadeh, A. (2016):
Soy-protein consumption and kidney-related biomarkers among type 2 diabetics: a crossover, randomized clinical trial.J. Ren. Nutr.,19(6):479-86.

Bai, F.; Jiang, F.; Lu, J.; Ma, S.; Peng, Y.; Jin, Y. Xu, W.; Cheng, J. and Wu, H.(2013):
The Impact of hyperglycemic emergencies on the kidney and liver. J. Diabetes Res., 967097

Bancroft, D.; Steven, A. and Tunner, R. (1996):
Theory and Practices of Histological Techniques, 4th Ed. Churchill LivingstoneEdinburg, London, Melbourne

Belloque, J.; Garcia, M.; Torre, M. and Marina, M.(2002):
Analysis of Soybean Proteins in Meat Products. Critical Reviews in Food Science and Nutrition, 42(5):507–532.

Cantero, I.; Abete, I.; Monreal, J.; Martinez, J. and Zulet, M. (2017):
Fruit fiber consumption specifically improves liver health status in obese subjects under energy restriction. Nutrients, 9: 667 .

Clydesdale, F.(1997):
A proposal for the establishment of scientific criteria for health claims for functional foods. Nutr. Rev., 55:413–22.

Considine, R.V.; Sinha, M.K.; Heiman, M.L. and Kriunas, A. (1996):
Serum immunoreactive- leptin concentration in normal weight and obese humans. N. Engl. j. Med., 334(5):292-295.

Dandona, P. and Dhindsa, S.(2011):
Update: Hypogonadotropic hypogonadism in type 2 diabetes and obesity. Journal of Clinical Endocrinology and Metabolism , 96 :2643–2651.

Defronzo, R.A.; Tobin, J.D. and Andres, R. (1979):
Glucose clamp technique: A method for quantifying insulin secretion and resistance. Am. J. Physiol., 237: 214-223.

Desai, A. and Bhide, M. (1985) :
Hypoglycemic effect ofHanitonisuavecolens .Idian. J. Med., 81: 86 – 91.

Engvall, E. and Perlmann, P. (1971):
Enzyme-Liked immuno. Sorbent (ELISA): Qualitative assay of Immunoglobulins. Immuno. Chem., (8): 817.

Evan, **J. R. and Heather, J. L. (2020):**
An Afternoon Hummus Snack Affects Diet Quality, Appetite, and Glycemic Control in Healthy Adults. J. Nutr.,150(8):2214-2222.

Fassati, P. and Prencipe, L. (1982):
Triglyceride enzymatic colorimetric method. J. Clin. Chem., (28):2077- 2078.

Fei, L.; Zhenkun, C. and Yang, L. (2013):
Okara dietary fiber and hypoglycemic effect of okara foods. Bioactive Carbohydrates and Dietary Fiber, 2:126–132.

Friedewald, W. T.; Levy, R. I. and Friedrickson, D. S. (1972):
Estimation of plasma or serum low density lipoprotein cholesterol concentration without use of preparaline ultracentrifuge. Clin. Chem., 18: 499-502.

George, K. R.; Patra, J.K. and Gouda, S. (2018):
Benefaction of probiotics for human health: a review. J. Food Drug Anal., 26: 927–939.

Grossmann, M. (2011):
Low testosterone in men with type 2 diabetes: significance and treatment. Journal of Clinical Endocrinology and Metabolism , 96: 2341–2353.

Gurung, M.; Li, Z.; You, H.; Rodrigues, R.; Jump, D. B. and Morgun, A. (2020):
Role of Gut Microbiota in Type 2 Diabetes Pathophysiology. *E.Bio.Medicine,* 51 : 102590.

Hasler, C. M. (**2002**):
The cardiovascular effects of soy products. Cardiovascular Nursing,16(4):50–63.

He, J.; Zhang, F. and Han, Y.(2017):
Effect of probiotics on lipid profiles and blood pressure in patients with type 2 diabetes: A meta-analysis of RCTs. Medicine, **96**: e9166 .

Hosokawa M,Katsukawa M, Tanka H , Fukuda H , Okuno S , Tsuda K,IritaniN.Okara ameliorates glucose tolerance in GK rats .G.Clin.Biochem.Nutr.(2016); 58:216-222

Iranloye, B.O.; Arikawe, A.P.; Rotimi, G. and Sogbade, A.O.(2014): Anti-diabetic and antioxidant effects of *Zingiber Officinale* on alloxan-induced and insulin-resistant diabetic male rats. Niger J. Physiol. Sci. 26:89-96.

Irouzi, S. Mohd-Yusof, B.N. ;Majid, H.A.; Ismail, A. and Kamaruddin, N.A.(2015): Effect of microbial cell preparation on renal profile and liver function among type 2 diabetics: a randomized controlled trial. BMC Complement. Altern. Med. 15:433–433.

Ivey, K.L.; Hodgson, J.M. and Kerr, D.A. (2015): The effect of yoghurt and its probiotics on blood pressure and serum lipid profile; a randomised controlled trial. *Nut. Metab. Cardiovasc. Dis.* 25:46–51.

Jonklaas, J.; Bianco, A.C.; Bauer, A.J.; Burman, K.D.; Cappola, A.R. and Celi, F.S. (2014): Guidelines for the treatment of hypothyroidism: prepared by the American thyroid association task force on thyroid hormone replacement. Thyroid, 24(12):1670–751.

Kesika, P.; Sivamaruthi, B.S. and Chaiyasut, C.(2019): Do Probiotics Improve the Health Status of Individuals with Diabetes Mellitus? A Review on Outcomes of Clinical Trials. Biomed. Res. Int., 14:1531567.

Kumar, G. and Baojun, X.(2018): Polyphenol-Rich Lentils and Their Health Promoting Effects.Crit. Rev. Food Sci. Nutr., 58(8):1359-1374.

Lisa, M.; Lukas, S.; Georg, H. and Cem, E. (2018): The effect of vegetarian diets on iron status in adults: A systematic review and meta-analysis. Int. J. Mol. Sci., 18(11): 2390.

Lopez, M.F. (1977): HDL- cholesterol colorimetric method. J. Clin. Chem., 230: 282.

Mallillin, A.C.; Trinidad, T.P. and Raterta, R. (2008): Dietary fiber and fermentability characteristics of root crops and legumes. The British Journal of Nutrition, 100 (3):. 485-488.

Miyake, Y. and Yamamoto, K. (2013): Role of gut microbiota in liver diseases. Hepatol. Res. ,43(2):139–46

Morelli, L.(2014): Yogurt, living cultures, and gut health. Am. J. Clin. Nutr., 99 : 1248S-1250S.

Murty, C.M.; Pittaway, J.K. and Ball, M.J. (2010):
Chickpea supplementation in an Australian diet affects food choice, satiety and bowel health. Appetite, 54(2):282-8.

NDDG (National Diabetes Data Group) (1994):
Classification and diagnosis of diabetes mellitus and other catogiries of glucose intolerance. Journal of diabetes, 28:1039-1057.

Okesene-Gafa, K.A.; Moore, A.E.; Jordan, V.; McCowan, L. and Crowther, C.A.(2020):
Probiotic treatment for women with gestational diabetes to improve maternal and infant health and well-being. Cochrane Database Syst. Rev. ,6(6):CD012970.

Ostermann, P.; Quiroga, P. and Rinaldoni, A.N. (2017):
Incorporation of Okara into gluten-free cookies with high quality and nutritional value. *Journal of Food Quality,*8.:234-242.

Park, M.S.; Kwon, B. and Ku, S. (2017):
The efficacy of *bifidobacterium longum* BORI and *lactobacillus acidophilus* AD031 probiotic treatment in infants with rotavirus infection. Nutrients , 9:E887.

Patrono, C. and Peskar, B.A. (1987):
Radioimmunoassay in basic and clinical pharmacology. handbook of experimental pharmacology, springer-verlag, Berlin, Germany.Edn., 15 : 376.

Ramezani, A. and Raj, D.S.(2014):
The gut microbiome, kidney disease, and targeted interventions. J. Am. Soc. Nephrol,. 25:657–670.

Reeves, P. G.; Nielson, F. H. and Fahey, G. C. (1993):
AIN- 93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr., 123: 1939-1951.

Salminen, S. ; Bouley, C.; and Boutron, M .C. (1998):
Functional food science and gastrointestinal physiology and function. Br. J. Nutr. ,80(suppl):S147–71.

SAS. (2002).
Statistical Analysis System SAS /Stat User's Guide version 9 SAS Institute . Inc, Gary, NC, USA.

Savard, P.; Lamarche, B. and Paradis, M.E. (2011):
Impact of *Bifidobacterium animalis* subsp. lactis BB-12 and, *Lactobacillus acidophilus* LA-5-containing yoghurt, on bacterial fecal counts of healthy adults. *Int. J. Food Microbiol .,* 149:50–57.

Schermer, S. (1967):
The Blood Morphology of Laboratory Animal. Longmans, Printed in Great Britain, Green and Co. LTd, P. 350.

SPSS(2000):
Statistical package for the social sciences,version14.0.chicago,il:SPSS

Sun, Z. *(*2019*):*
Using probiotics for type 2 diabetes mellitus intervention: Advances, questions, and potential. Crit. Rev. Food Sci. Nutr*.,* **60**: 1–14 .

Tachibana, N.; Yamashita, Y. and Nagata, M. (2014):
Soy β-conglycinin improves glucose uptake in skeletal muscle and ameliorates hepatic insulin resistance in Goto-Kakizaki rats. Nutr. Res.,34:160–167.

Taylor, C.; Robert, M. and Kathleen, M. Z.(2016):
The Nutritional Value and Health Benefits of Chickpeas and Hummus. Nutrients, 8(12): 766.

Tietz, N. W. (1976):
Fundamentals of Clinical Chemistry. Philadelphia. B.W. Standers, P.243.

Uotila, M.; Ruoslahti, E. and Engvall, E. (1981):
Two-site sandwich enzyme immunoassay with monoclonal antibodies to human alpha-fetoprotein. J. Immunol.Methods, 42:11-15.

Xu, M. Wang, B. and Fu, Y. (2016):
Changes of fecal Bifidobacterium species in adult patients with hepatitis B virus-induced chronic liver disease. Microb. Ecol*.,*63(2):304–13.

Yen PM Physiological and molecular bass of thyroid hormone action. PhysicologicalReviews . (2001) ;
81:1097-10142.

Yound, D.S. (1975):
Determination of GOT. Clin. Chem., 22(5): 21.

Zoke, C.(2020):
The importance of the human intestinal microbiota. Dtsch. Med. Wochenschr., 139 (24) : 1282-1283.

تأثيرالعدس والحمص والاوكارا مع بكتيريا البروبيوتيك على الحالة الصحية للفئران المصابة بداء السكري

هبة سعيد عبد الحليم[1] ، باسم علي زوين[2]

[1]المعهد القومي للتغذيه - القاهره
[2] مستشفيات جامعة القاهرة – القصر العيني

الملخص العربي

تم دراسة تأثيرالنسب المختلفة من العدس والحمص والاوكارا مع بكتريا البروبيوتيك (Lactobacillus acidophilus) كمخاليط على بعض العوامل البيولوجية والنسيجية للفئران البيضاء المصابة بداء السكري. تم توزيع ثلاثين أنثى من الفئران البالغة على خمس مجموعات،المجموعة الأولى كانت سلبية،والثانية كانت الفئران المصابة بداء السكري التي تغذت على النظام الغذائي الأساسي كمجموعة ضابطة إيجابية ،بينما تم تغذية المجموعات الثلاث الأخرى المصابة بالسكري على علائق مختبرة بوجود بكتيريا بروبيوتيك وبدونها. 28 يومًا. أظهرت النتائج أن زيادة وزن الجسم كانت أعلى بشكل ملحوظ خاصة في خليط البروبيوتيك (2٪) مقارنة بالفئران الأخرى المصابة بداء السكري. كم أدى هذا الخليط إلى انخفاض تركيزات الكوليسترول الكليو الدهون الثلاثية وLDL-cوVLDL-cوانخفاض إنزيماتASTو ALTتقريبًا إلى المستويات الطبيعية. علاوة على ذلك،تسبب الخليط في زيادة معنوية فيHDL-cوإنتاج الجلوبولين المناعي الكلي (IgG، IgM،IgE،IgA) والتي كانت أعلى من خليط 5٪ عدس،حمص،اوكارا بدون بروبيوتيك.

أظهر الفحص المرضي للنسيج تحسنًا في الآفات النسيجية المرضية التي لوحظت في كبد الفئران التي تلقت مزيجًا من المواد المختبرة بمستويات مختلفة مع البروبيوتيك. لذلك،يمكن التوصية بتناول 5٪ من خليط العدس والحمص والاوكارا مع 2٪ من البروبيوتيك لمرضى السكري لتحسين التحليل البيوكيميائي وزيادة إنتاج المناعة.

الكلماتالمفتاحية: إناثالفئران،العدس،الحمص،الاوكارا،البروبيوتيك،التركيبةالنسيجيةالمرضية.