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Hypoglycemic, hypolipidemic and antioxidant effects of green sprouts juice and functional dairy micronutrients against streptozotocin-induced oxidative stress and diabetes in rats

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Abstract

Hyperglycemia, the mark normal for diabetes and associated disorders are the main goals of natural diabetes therapies. In this context, the present research was designed to study the effects of fenugreek sprouts juice (FS), barley sprouts juice (BS), cell-free probiotic extract (cell-free PE), whey protein hydrolysate (WPH) and their mixture on diabetic rats. Free radical scavenging activity, total phenolic contents (TPC) and total flavonoid contents (TFC) of each item mentioned were determined. Diabetes was induced through the injection of male rats with a single intraperitoneal dose (45 mg/kg) of streptozotocin. After the development of diabetes, diabetic rats were orally administered daily with 1ml of with fenugreek

sprouts juice, barley sprouts juice, cell-free probiotic extract, whey protein hydrolysate or their mixture until the end of the study period (45 day). Oral administration of fenugreek sprouts juice, barley sprouts juice, cell-free probiotic extract, whey protein hydrolysate and their mixture to diabetic rats significantly reduced fasting blood glucose levels and improved the lipid profile. All the studied items limit the reductions of haemoglobin concentrations and plasma α -amylase activities. Also all the studied items suppressed the elevation of malondialdehyde values and the reduction of catalase activities. Histopathological investigation of pancreas, liver and kidneys of the diabetic rats showed histological alterations. On the other hand, supplementations with the tested materials lead to relieving these injuries. Results revealed that fenugreek sprouts juice, barley sprouts juice, cell-free probiotic extract, whey protein hydrolysate and their mixture had promising effects towards hyperglycemia and associated disorders.

Keywords: Food science, Metabolism

1. Introduction

Diabetes mellitus has been and remains one of the most prevalent diseases and one of the most common causes of morbidity and mortality [1, 2]. Although there are different hypoglycemic drugs, but these therapies cannot control the disorders (dyslipidemia, inflammation and excess production of the free radicals) that associated with the hyperglycemia and cause complications of diabetes [3]. In addition to the side effects of these drugs for example congestive heart failure, anaemia as well as sever hypoglycaemia [4]. Therefore, there is a critical need to find natural therapies for hyperglycemia and its related disorders to avoid the side effects of the hypoglycemic drugs.

Plant seeds germination produces sprouts, which contain high concentration of nutrients especially if consumed at the beginning of its growth. In addition, sprouts contain many substances beneficial to human health such as phytochemicals and antioxidants [5]. Fenugreek sprouts (*Trigonella foenumgraecum*) are characterized by its high content of phenolic antioxidants [6]. Also barley sprouts (*Hordeum vulgare* L.) are characterized by its high content of saponarin (flavone-C-glycosides) which possess antioxidant effect, policosanol polyphenol series, minerals and free amino acids [7, 8].

Cell-free probiotic extract, a new concept of probiotics utilization, is consider as an alternative to probiotic live cells and can be used to keep away from the problems caused by ingestion of living cell. Cell-free probiotic extract which contains the probiotic metabolites also can be used safely as a potent antioxidant ingredient in the nutritional and medicinal industries [9].

Whey protein hydrolysate (WPH) is generated from the hydrolyzing of whey protein. Enzymatic hydrolyzing is a way to improve the function of whey protein

[10]. Whey protein hydrolysates possess *in vitro* antioxidant effects [11]. These antioxidant properties may be attributed to presence of lactoferrin that play an important role in chelation of metals [12] and amino acids which scavenge free radicals [13]. So, the present work aimed to study the hypoglycemic, hypolipidemic and antioxidant effects of green sprouts juice (fenugreek and barley sprouts juice) and functional dairy micronutrients (cell-free probiotic extract and whey protein hydrolysate) against streptozotocin mediated oxidative stress and diabetes in rats.

2. Materials and methods

2.1. Materials

Fenugreek and barley sprouts were grown in local farm, Giza, Egypt. Starter cultures of *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* were obtained from Chr. Hansen's Laboratory, Copenhagen Denmark. Cheese whey powdered was purchased from Bahçivan Co., Turkey. The composition and nutritional values of whey used in this study per 100 g were as follows: moisture (3.25 %), total fat (0.5 %), total carbohydrates (65 %), protein (11%) and total ash (8.5%). The final concentrations of the prepared whey beverages solution were 20% as total solids. Chemicals and pure reagents were purchased from Sigma Chemical Companies (Sigma-Aldrich, St. Louis, MO, USA).

2.1.1. Animals

Male albino rats, of $138.14 \text{ g} \pm 6.98$ as Mean $\pm$ SD (8 weeks age) were used in this study. Animals were obtained from the Animal House of National Research Centre, Cairo, Egypt. The animals were kept individually in stainless steel cages at room temperature. Water and food were given *ad-libitum*.

2.1.2. Diets

A balanced diet was prepared based on the AIN-93 diet [14]. Balanced diet was prepared to contain 10% protein supplemented from casein, 10% corn oil, 10% sucrose, 60.5% maize starch, 5% fiber, 3.5% salt mixture prepared as the AIN-93 formulation (14) and 1% vitamin mixture prepared as the AIN-93 formulation [14]. The oil soluble vitamins were given weekly to rats separately from the diet.

2.2. Methods

2.2.1. Preparation of aqueous extracts of green sprouts juice

The aqueous extracts of green sprouts juice were prepared according to the method described by Abdel-Salam *et al.* [15] and Abdel-Salam and Al-Damegh [16]. Freshly

fenugreek and barley sprouts were cut into small to medium size cubes with a sharp knife then put in a high-speed electric blender to make juice. The final concentration of juice was reached to 50% by adding distilled water. The juice was filtered three times through cheese-cloth (50% cotton/50% polyester) and was preserved in sterile dark bottles (500 ml) at -4 °C until further used.

2.2.2. Preparation of whey protein hydrolysate

Generation of whey protein hydrolysate using Trypsin digestion of cheese whey beverage was carried out according to the method described by Mota *et al.* [17]. Cheese whey beverage was subjected to heat treatment to 70 °C/10 min after adjusting the pH value at 8. Trypsin hydrolysis was performed in a 1.0 L stirred, the final concentration was 20 gL⁻¹ and the ratio enzyme/substrate, pH and temperature were controlled. Trypsin enzyme (Sigma Chemical Companies, St. Louis MO, USA) was added to the solution at the 3% of trypsin to whey protein (w/w). The solution was incubated overnight at 37 °C. The reaction was stopped by immersion of the samples in a water bath at 90 °C and afterwards, the digest was filtered through a 45 micron cellulose acetate filter and stored at -20 °C until further used.

2.2.3. Preparation of cell-free probiotic extract inoculated in whey beverage

Whey beverage (20%) was pasteurized and cooled to 40 °C, then inoculated with probiotic bacteria *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* and incubated for 24 h at 42 °C. The inoculated solution was put into a high Ultrasonic cell disrupter equipped with microtip probe 400 W (Ultrasonic Get 750, USA) and afterwards centrifuged at 5000 rpm for 15 min. The cell-free probiotic supernatant was decant and stored at -4 °C until further used.

2.2.4. Determination of radical scavenging activities, total phenolic and total flavonoid contents

2.2.4.1. Samples extraction

The samples were extracted with ethanol (70% v/v), shaken together for one hour and then filtered with Whatman No. one paper as described by Bloor [18]. The extracts were labeled and kept for laboratory analysis.

2.2.4.2. DPPH (2,2-diphenyl-1-picrylhydrazyl)-radical scavenging activities (%)

Electron-donating capacity of each extract was determined by implying DPPH radical-scavenging assay as described by Brand-Williams *et al.* [19]. Aliquots 1

ml of sample extract was mixed with 1ml of 0.2 mM DPPH in methanol. The control sample contained all the reagents except the extract. The reaction mixture was shaken well and allowed to react for 30 min at room temperature. The remaining DPPH free radical was determined by absorbance measurement at 517 nm against methanol blanks. The percentage scavenging effect was calculated from the decrease in absorbance against control according to the following equation:

$$\text{Radical Scavenging activity \%} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

2.2.4.3. Determination of total phenolic contents (TPC)

The total phenolic substances of each extract were determined colorimetrically, using the Folin-Ciocalteu method, as described by Singleton *et al.* [20]. Aliquots of 0.5 ml of each extract were added to 0.5 ml of Folin-Ciocalteu reagent, followed by addition of 0.5 ml of an aqueous 7.5% solution of sodium carbonate. The mixture was stirred and allowed to stand for 30 min. The absorbance at 765 nm, blank sample consisting of water and reagents was used as a reference. The results were expressed as milligrams of gallic acid equivalents per ml extract (mg GAE/ml) by reference to the gallic acid calibration curve.

2.2.4.4. Total flavonoid contents (TFC) assay

The total flavonoid contents were determined according to the technique of Mohdaly *et al.*, [21]. A 100 μL aliquot of 2% AlCl_3 ethanolic solution was added to 100 μL of each extract and mixed well. After keeping for one hour at room temperature, the absorbance at 420 nm was measured. A yellow color indicates the occurrence of flavonoids. The total flavonoid contents were expressed as milligram quercetin equivalents (QE).

2.2.5. Design of the animal study

Forty-two rats were divided into seven groups of six rats each. The first group was considered as the normal healthy group (normal control). The remained rats were served as diabetic rats. To induce diabetes, rats were fasted overnight and injected intraperitoneally with 45 mg/kg body weight (w/w) of streptozotocin (STZ) (from Sigma chemical Co) in citrate buffer, pH 4.5 then given glucose solution (5%) for 48 h after the injection to prevent hypoglycemia. To ensure occurrence of diabetes in rats, blood samples were withdrawn 72 h after streptozotocin injection then the blood glucose levels were determined. Rats with blood glucose higher than 200 mg/dl were considered diabetic and used in the following procedures. After the development of diabetes the diabetic rats were divided into six groups of six rats each as follows:

Group I: Untreated diabetic rats (diabetic control), **Group II:** Diabetic rats orally administered with 1ml of fenugreek sprouts juice (diabetic FS), **Group III:** Diabetic rats orally administered with 1ml of barley sprouts juice (diabetic BS), **Group IV:** Diabetic rats given oral dose equivalent to 1ml of cell-free probiotic extract (diabetic cell-free PE), **Group V:** Diabetic rats given oral dose equivalent to 1ml of whey protein hydrolysate (diabetic WPH) and **Group VI:** Diabetic rats given oral dose equivalent to 1ml of freshly prepared mixture of fenugreek sprouts juice, barley sprouts juice, cell-free probiotic extract and whey protein hydrolysate (diabetic Mix).

All rats groups were fed on balanced diet all over the study time (45 days). Throughout the experiment, body weight and food intake were recorded weekly. At the end of the study, total food intake, body weight gain and food efficiency ratio (Body weight gain/total food intake) were calculated. Blood samples were withdrawn from all rats after an overnight fast. A portion of the whole blood was analyzed for haemoglobin (Hb) concentration according to Drabkin [22]. The remaining blood was centrifuged and the plasma was analyzed for fasting blood glucose levels according to Trinder [23], α -amylase according to Caraway [24], total cholesterol according to Watson [25], high-density lipoprotein cholesterol (HDL-C) using the method of Burstein *et al.* [26], low-density lipoprotein cholesterol (LDL-C) according to Schriewer *et al.* [27] and triglycerides according to the method of Megraw *et al.* [28]. Cholesterol/HDL-C ratio was calculated. The levels of creatinine and urea were determined depending on Larsen [29] and Fawcett and Scott [30] in succession as indicators of kidneys function. The activities of aspartate transaminase (AST) and alanine transaminase (ALT) were determined according to Reitman and Frankel [31]. Malondialdehyde (MDA) was determined according to Ohkawa *et al.* [32]. Catalase activity was assayed according to the method of Aebi [33]. After blood sampling rats were dissected and the liver, kidneys and pancreas were immediately separated from each rat and weighed then immersed in 10% formalin solution for histopathological examination. The study protocol was reviewed and approved according to the instructions of the ethical committee of National Research Centre. Animal procedures were performed in accordance with the ethics committee of National Research Centre (Ethics Committee, National Research Centre, Cairo, Egypt) and according to the Guide for the Care and Use of Laboratory Animals of the National Institute of Health (Publication No. 85-23, revised 1985). Animals were kept under standard conditions of temperature and humidity along the experimental period.

2.2.6. Histopathological examination

A portion of pancreatic, liver and kidney tissues preserved in a 10% neutral buffered formalin solution at room temperature for 24 hour were dehydrated in ascending series of ethyl alcohol, cleared in xylene and embedded in paraffin.

The sections were cut (5 micrometer) using Leica microtome. Then, sections were deparaffinized in xylene and rehydrated in descending series of ethanol and rinsed in water. Slides were stained in hematoxylin and eosin, mounted in DPX, cover-slipped and examined under a light microscope (Leica, Germany) connected to a camera and computer [34].

2.2.7. Statistical analysis

The results of animal experiments were expressed as the mean $\pm$ SE and they were analyzed statistically using the one-way analysis of variance ANOVA followed by Duncan's test. In all cases $p < 0.05$ was used as the criterion of statistical significance [35, 36].

3. Results

The data presented in Table 1 showed that barley sprouts juice recorded the highest value of DPPH-radical scavenging activity (84.39%), followed respectively by fenugreek sprouts juice, cell-free probiotic extract and whey protein hydrolysate. Fenugreek sprouts juice showed the highest content of total phenols 46.05 mg/ml followed by barley sprouts juice 33.70 mg/ml. While, significant increase in the content of total flavonoids 21.48mg/ml was recorded by the barley sprouts juice followed by fenugreek sprouts juice 22.15 mg/ml.

Concerning the growth performance parameters of normal and diabetic rats, data tabulated in Table 2. The data disclosed that the untreated diabetic rats showed the lowest body weight gain (30.5g) in comparison to the other experimental groups. On the other hand, the oral dosage of all the studied items lead to improvement in the body weight gain and food efficiency ratio with different degrees compared with untreated diabetic rats. Moreover, diabetic rats administered orally whey protein hydrolysate showed increase in the body weight gain more than the normal control rats as

Table 1. The DPPH-radical scavenging activities, total phenolic and total flavonoid contents of fenugreek sprouts juice, barley sprouts juice, cell-free probiotic extract and whey protein hydrolysate.

Extracts	Scavenging activity %	Total phenols (mg/ml) as gallic acid	Total flavonoids (mg/ml) as quercetin
Barley sprouts juice	84.39 ^a $\pm$ 0.91	33.70 ^b $\pm$ 0.59	21.48 ^a $\pm$ 0.37
Fenugreek sprouts juice	72.63 ^b $\pm$ 0.89	46.05 ^a $\pm$ 0.10	18.41 ^b $\pm$ 0.49
cell-free probiotic extract	62.62 ^c $\pm$ 1.12	ND	ND
Whey protein hydrolysate	37.67 ^d $\pm$ 0.53	ND	ND

In each column same letters means non-significant difference; different letter means the significance among the extracts at 0.05 probability.

Table 2. Growth performance parameters of different experimental groups.

Groups	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Total food intake (g)	Food efficiency ratio
Normal control	138.00 ^a ± 3.53	196.67 ^d ± 2.64	58.67 ^d ± 4.25	673.50 ^{cd} ± 11.00	0.09 ^{cd} ± 0.006
Diabetic control	138.17 ^a ± 2.48	168.67 ^a ± 4.95	30.50 ^a ± 2.59	684.17 ^d ± 5.22	0.04 ^a ± 0.003
Diabetic FS	138.17 ^a ± 3.11	176.33 ^{ab} ± 5.97	38.17 ^{ab} ± 4.79	665.00 ^{cd} ± 6.43	0.06 ^{ab} ± 0.007
Diabetic BS	138.00 ^a ± 2.77	179.50 ^{abc} ± 3.60	41.50 ^{abc} ± 5.67	655.00 ^{abc} ± 6.95	0.06 ^{abc} ± 0.008
Diabetic cell-free PE	138.17 ^a ± 4.14	194.17 ^{cd} ± 6.87	56.00 ^{cd} ± 7.28	636.17 ^{ab} ± 4.71	0.09 ^{cd} ± 0.011
Diabetic WPH	138.17 ^a ± 1.72	199.67 ^d ± 6.93	61.50 ^d ± 6.19	656.67 ^{bc} ± 6.29	0.09 ^d ± 0.009
Diabetic Mix	138.17 ^a ± 3.26	187.67 ^{bcd} ± 5.99	49.50 ^{bcd} ± 5.31	634.17 ^a ± 6.91	0.08 ^{bcd} ± 0.007

In each column, same letters means non-significant difference; different letter means the significance among the tested groups at 0.05 probability. (*n* = 6 in each group).

also illustrated in Fig. 1 that shows the growth curve of different experimental groups. As illustrated the weight of streptozotocin injected rats decreased a week after injection then increased to varying degrees depending on the type of treatment.

The weight of the internal organs (liver, kidney and pancreas) and the percentage of these organs weight are illustrated in Table 3. As for the liver weight, slight changes were observed in the liver weights between the different groups. There are no significant differences in the weights of neither the kidney nor the pancreas among the different groups.

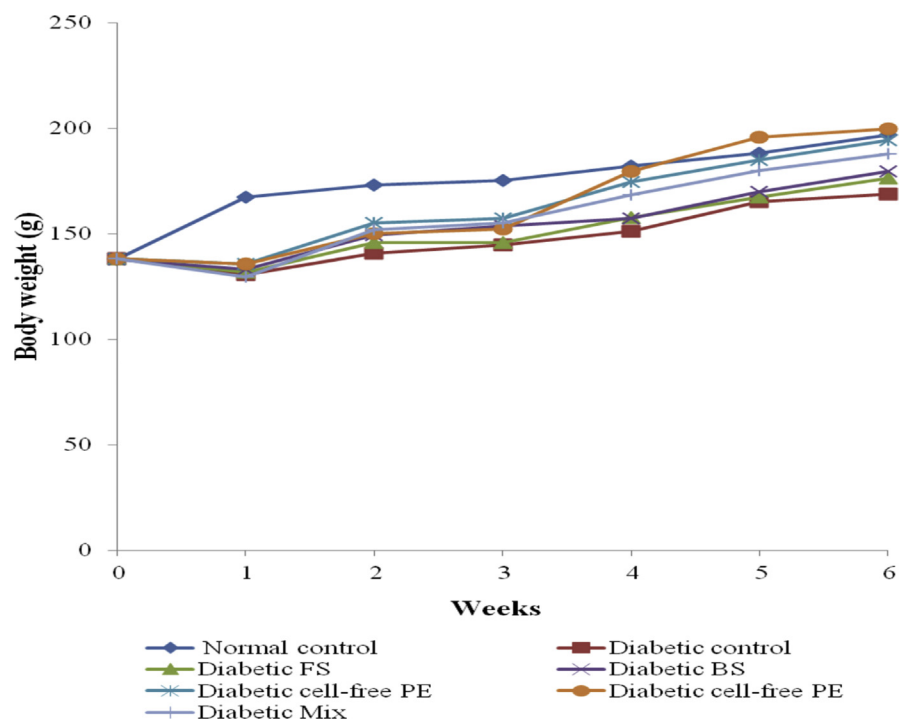
**Fig. 1.** Growth curve of different experimental groups. (*n* = 6 in each group).

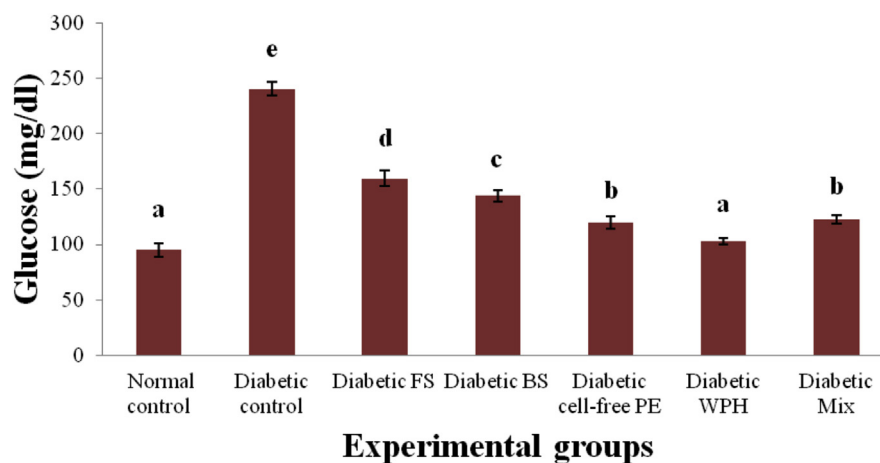
Table 3. Organs weight and organs/body weight ratio of different experimental groups (Mean $\pm$ SE).

Groups	Liver (g)	Liver (%)	Kidney (g)	Kidney (%)	Pancreas (g)	Pancreas (%)
Normal control	5.68 ^{ab} $\pm$ 0.29	2.89	1.56 ^a $\pm$ 0.04	0.80	0.60 ^a $\pm$ 0.07	0.31
Diabetic control	6.09 ^{ab} $\pm$ 0.16	3.62	1.53 ^a $\pm$ 0.03	0.91	0.41 ^a $\pm$ 0.05	0.24
Diabetic FS	5.62 ^{ab} $\pm$ 0.23	3.19	1.53 ^a $\pm$ 0.03	0.87	0.49 ^a $\pm$ 0.05	0.27
Diabetic BS	5.44 ^a $\pm$ 0.28	3.04	1.52 ^a $\pm$ 0.03	0.85	0.50 ^a $\pm$ 0.07	0.28
Diabetic cell-free PE	5.72 ^{ab} $\pm$ 0.29	2.97	1.59 ^a $\pm$ 0.03	0.81	0.60 ^a $\pm$ 0.02	0.31
Diabetic WPH	5.90 ^{ab} $\pm$ 0.37	2.95	1.60 ^a $\pm$ 0.04	0.80	0.58 ^a $\pm$ 0.08	0.29
Diabetic Mix	6.34 ^b $\pm$ 0.11	3.39	1.5 ^a $\pm$ 0.08	0.80	0.55 ^a $\pm$ 0.04	0.29

In each column, same letters means non-significant difference; different letter means the significance among the tested groups at 0.05 probability. ($n = 6$ in each group).

Data illustrated in Fig. 2 declared that the fasting blood glucose levels of different diabetic rats elevated significantly compared with the normal healthy control rats. The highest elevation in fasting blood glucose level (240.73 mg/dl) was recorded by the untreated diabetic rats. On the other side, the highest reduction in the fasting blood glucose level (102.56 mg/dl) was recorded by the diabetic rats administered orally with whey protein hydrolysate. Also the diabetic rats treated with cell-free probiotic extract showed high reduction in the fasting blood glucose (119.85 mg/dl) compared with untreated diabetic rats.

As declared in Fig. 3 a significant decrease in haemoglobin concentration was recorded by the untreated diabetic rats. Whereas, the oral administration of different items under the study limit the reduction of haemoglobin concentrations.

**Fig. 2.** Fasting blood glucose of different experimental groups. Same letters means non-significant difference; different letter means the significance among the tested groups at 0.05 probability. ($n = 6$ in each group).

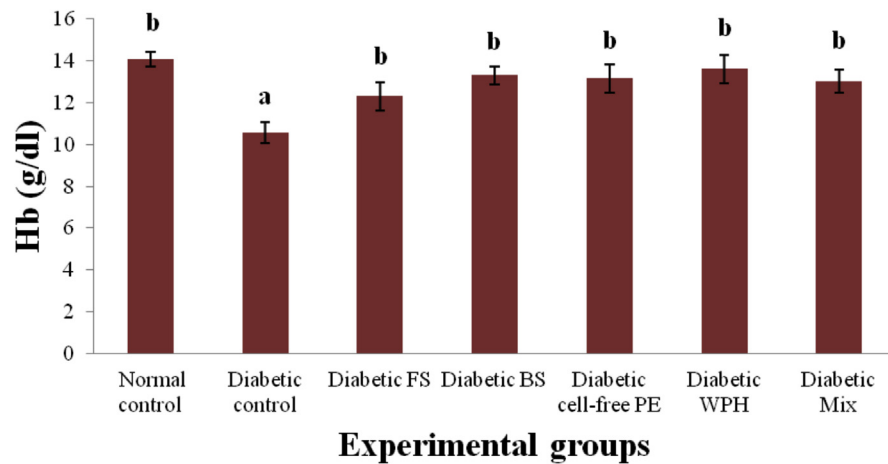


Fig. 3. Haemoglobin concentrations of different experimental groups. Same letters means non-significant difference; different letter means the significance among the tested groups at 0.05 probability. ($n = 6$ in each group).

As declared in Fig. 4 a significant decrease in α -amylase activity was recorded by the untreated diabetic rats. Whereas, the oral administration of different items under the study limit the reduction of α -amylase activities.

Results in Table 4 point out to the changes in the lipid profile. Total cholesterol, low density lipoprotein cholesterol (LDL-C) as well as triglycerides levels of the diabetic rats increased significantly whereas high density lipoprotein (HDL-C)

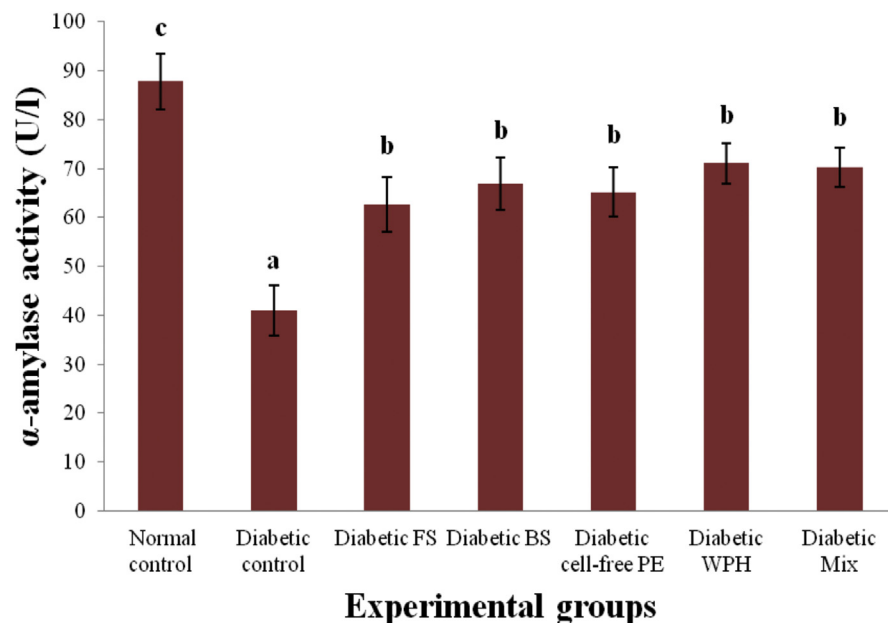


Fig. 4. α -amylase activities of different experimental groups. Same letters means non-significant difference; different letter means the significance among the tested groups at 0.05 probability. ($n = 6$ in each group).

Table 4. Lipid profile of different experimental groups (Mean $\pm$ SE).

Groups	Total-Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-Ch (mg/dl)	LDL-Ch (mg/dl)	Cholesterol/HDL ratio
Normal control	91.86 ^a $\pm$ 4.24	84.54 ^a $\pm$ 2.36	40.23 ^c $\pm$ 0.72	34.73 ^a $\pm$ 3.39	2.28 ^a $\pm$ 0.09
Diabetic control	137.75 ^d $\pm$ 5.47	106.85 ^c $\pm$ 2.21	29.92 ^a $\pm$ 0.84	86.46 ^d $\pm$ 5.13	4.62 ^d $\pm$ 0.20
Diabetic FS	121.55 ^c $\pm$ 5.42	94.79 ^b $\pm$ 2.06	36.81 ^b $\pm$ 0.64	65.79 ^c $\pm$ 5.27	3.31 ^c $\pm$ 0.13
Diabetic BS	99.29 ^{ab} $\pm$ 4.63	88.83 ^{ab} $\pm$ 2.50	39.47 ^c $\pm$ 0.75	42.06 ^{ab} $\pm$ 5.35	2.52 ^{ab} $\pm$ 0.11
Diabetic cell-free PE	111.86 ^{bc} $\pm$ 4.94	91.43 ^{ab} $\pm$ 2.44	37.78 ^{bc} $\pm$ 0.79	55.79 ^{bc} $\pm$ 4.90	2.96 ^{bc} $\pm$ 0.13
Diabetic WPH	109.01 ^{bc} $\pm$ 5.69	90.13 ^{ab} $\pm$ 2.56	39.31 ^c $\pm$ 0.93	51.67 ^{bc} $\pm$ 6.45	2.79 ^b $\pm$ 0.18
Diabetic Mix	112.81 ^{bc} $\pm$ 4.74	93.41 ^b $\pm$ 3.14	38.96 ^{bc} $\pm$ 0.86	55.17 ^{bc} $\pm$ 4.75	2.90 ^{bc} $\pm$ 0.12

In each column same letters means non-significant difference; different letter means the significance among the tested groups at 0.05 probability. ($n = 6$ in each group).

decreased significantly in comparison to the normal healthy rats. Diabetic rats showed significant elevation in cholesterol/HDL-C ratio (Atherogenic index) which related to the increase in total cholesterol. Each of fenugreek sprouts juice, barley sprouts juice, cell-free probiotic extract and whey protein hydrolysate improved the lipid profile but to some extent, barley sprouts had the best effect in improving the lipid profile.

Concerning malondialdehyde levels (as indicator to lipid peroxidation) as well as catalase activities, results presented in Table 5 disclosed that malondialdehyde level significantly increased in the untreated diabetic rats while catalase activity decreased significantly in this group compared with the normal healthy control rats. This increasing in MDA level and decreasing in catalase activity revealed that diabetes is associated with elevation of lipid peroxidation and oxidative stress. Each of fenugreek sprouts juice, barley sprouts juice, cell-free probiotic extract and whey protein hydrolysate alleviated lipid peroxidation and oxidative stress that was evident by the reduction of MDA levels and the increasing of catalase activities.

Table 5. Malondialdehyde levels and catalase activities of different experimental groups (Mean $\pm$ SE).

Groups	Malondialdehyde (nmol/ml)	Catalase (U/l)
Normal control	5.25 ^a $\pm$ 0.31	343.65 ^c $\pm$ 4.76
Diabetic control	9.78 ^c $\pm$ 0.24	275.20 ^a $\pm$ 6.36
Diabetic FS	6.38 ^b $\pm$ 0.27	317.31 ^{cd} $\pm$ 6.36
Diabetic BS	5.32 ^a $\pm$ 0.21	320.76 ^d $\pm$ 4.72
Diabetic cell-free PE	7.04 ^b $\pm$ 0.36	290.09 ^{ab} $\pm$ 5.61
Diabetic WPH	6.65 ^b $\pm$ 0.36	306.48 ^{bcd} $\pm$ 5.38
Diabetic Mix	6.98 ^b $\pm$ 0.25	302.48 ^{bc} $\pm$ 6.53

In each column, same letters means non-significant difference; different letter means the significance among the tested groups at 0.05 probability. ($n = 6$ in each group).

The changes in the kidney functions (creatinine and urea values) and liver functions (AST and ALT activities) are illustrated in Table 6. As shown creatinine and urea values of the untreated diabetic rats increased significantly compared with the normal healthy control rats. Each of fenugreek sprouts juice, barley sprouts juice, cell-free probiotic extract and whey protein hydrolysate retarded these increases in creatinine and urea values. While there were no significant changes neither in AST activities nor ALT activities between the different experimental groups.

Concerning the histological examination, the pancreas sections of the normal control rats showed normal proportions of the exocrine and endocrine components. The extremely stained acinar cells which coordinated in lobules with prominent nuclei were obvious. The islet cells embedded within the acinar cells and encompassed by a fine capsules (Fig. 5A).

As shown in Fig. 5B. Breakdowns of micro-anatomical features were observed in the pancreas section of diabetic group including degenerative, necrotic changes, and shrunken in the pancreatic islet of Langerhans, β -cell degranulation, pycnotic β -cell nuclei. Also a decrease in the islet cellular density as well as a severe reduction in the number of cells in the islets and mononuclear cellular infiltration were observed. The normal appearance of pancreatic acinar epithelium, ductal and connective tissues could be observed.

On the other hand, pancreas sections of diabetic rats given oral doses of fenugreek sprouts juice, barley sprouts juice, cell free probiotic extract and whey protein hydrolysate showed normal structure of the exocrine and endocrine components (Fig. 5C, D, E, and F, respectively). While, diabetic rats given oral doses of the mixture (Fig. 5G). showed less improvement than the other treatments.

The liver section of the control rats showed normal architecture of the hepatic lobule. The central vein lied at the centre of the lobule surrounded by the hepatocytes with

Table 6. Kidney and liver functions of different experimental groups (Mean $\pm$ SE).

Groups	Urea (mg/dl)	Creatinine (mg/dl)	ALT (U/l)	AST (U/l)
Normal control	29.18 ^a $\pm$ 1.13	0.64 ^a $\pm$ 0.05	30.73 ^a $\pm$ 1.87	73.87 ^a $\pm$ 1.33
Diabetic control	36.19 ^b $\pm$ 1.24	0.89 ^b $\pm$ 0.07	34.76 ^a $\pm$ 1.76	79.12 ^a $\pm$ 2.84
Diabetic FS	30.88 ^a $\pm$ 1.10	0.72 ^a $\pm$ 0.05	32.62 ^a $\pm$ 2.65	75.76 ^a $\pm$ 2.12
Diabetic BS	30.65 ^a $\pm$ 1.33	0.69 ^a $\pm$ 0.05	31.00 ^a $\pm$ 1.69	74.75 ^a $\pm$ 2.67
Diabetic cell-free PE	29.60 ^a $\pm$ 0.79	0.67 ^a $\pm$ 0.04	31.27 ^a $\pm$ 1.49	76.49 ^a $\pm$ 2.61
Diabetic WPH	31.18 ^a $\pm$ 1.16	0.69 ^a $\pm$ 0.05	32.08 ^a $\pm$ 1.28	76.78 ^a $\pm$ 2.22
Diabetic Mix	31.96 ^a $\pm$ 1.00	0.70 ^a $\pm$ 0.05	32.56 ^a $\pm$ 1.64	75.47 ^a $\pm$ 1.31

In each column, same letters means non-significant difference; different letter means the significance among the tested groups at 0.05 probability. ($n = 6$ in each group).

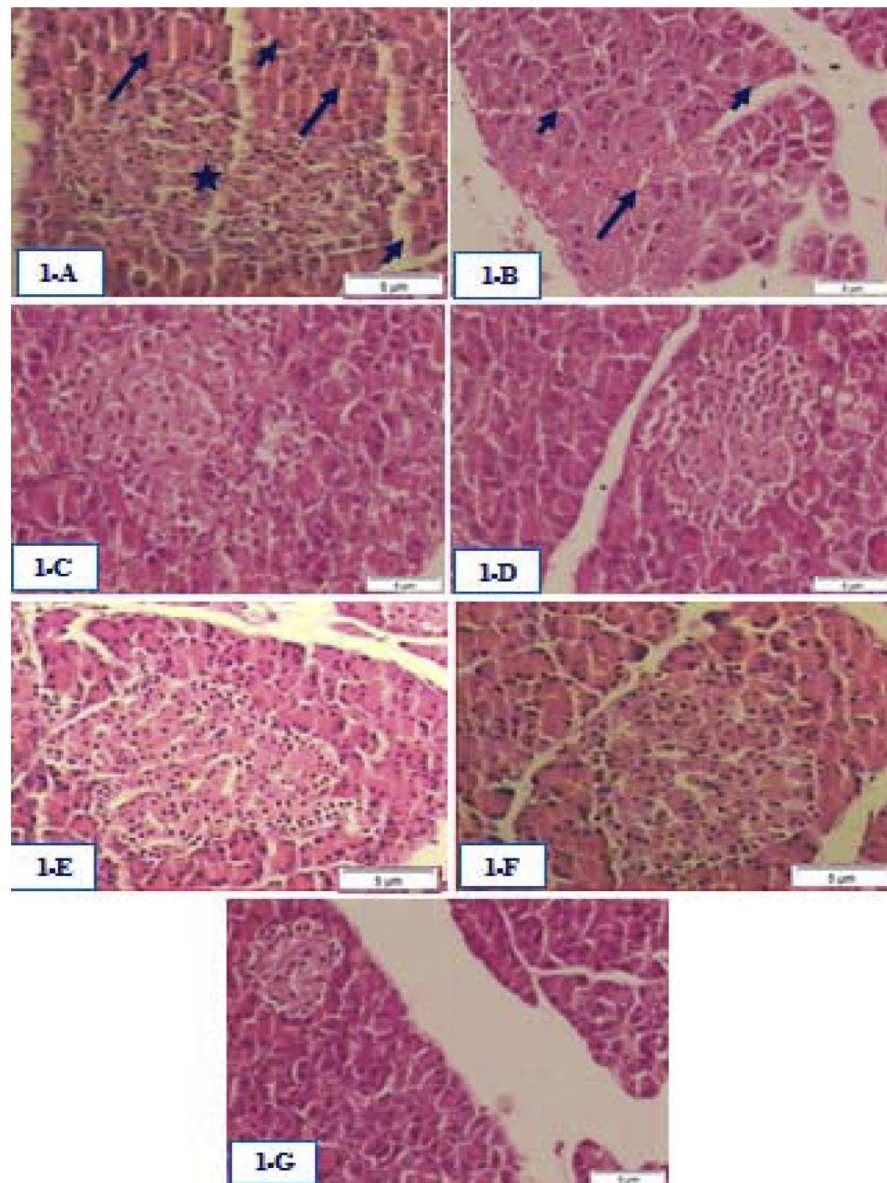


Fig. 5. Pancreas sections of A) control rat showing the normal structure of the exocrine and endocrine component. The exocrine component consisting of closely packed acini (arrows). The interlobular ducts (arrowhead) appear surrounded with the supporting tissue. The endocrine tissue of the pancreas, islet of Langerhan (asterisk), is scattered throughout the exocrine tissue; B) diabetic rat showing the acinar cells around the islets though seem to be in normal proportion does not look classical. A breakdown of micro-anatomical features including degenerative and necrotic changes, and shrunken in the pancreatic islet of Langerhans, β -cell degranulation, pyknotic β -cell nuclei, decreased islet cellular density and mononuclear cellular infiltration are found; C, D, E, and F respectively) diabetic rats given oral doses of fenugreek sprouts, barley sprouts, cell free probiotic and whey protein showing normal structure of the exocrine and endocrine components; G) diabetic rat given oral dose of the mixture showing degenerative and necrotic changes, and shrunken in the pancreatic islet of Langerhans, β -cell degranulation, pyknotic β -cell nuclei, decreased islet cellular density (H & E; Scale Bar: 5micrometer).

powerfully eosinophilic granulated cytoplasm and distinct nuclei. The hepatic sinusoids can be seen between the strands of hepatocytes (Fig. 6A). On the other hand, the liver section of diabetic rats showed disturbance in the hepatic lobule. Focal necrosis and hydropic degeneration were noticed (Fig. 6B). In addition, diabetic rats showed portal tracts congestion associated with mild inflammatory infiltration (Fig. 6C). Administration of diabetic rats with oral doses of fenugreek sprouts juice, barley sprouts juice, cell free probiotic extract or whey protein hydrolysate revealed normal hepatic lobules (Fig. 6D, E, F, and G, respectively). However, in case of diabetic rats given oral doses of the mixture, foci of necrotic hepatocytes associated with inflammatory infiltration were seen (Fig. 6H).

The kidney section of control rats showed normal structure of the renal corpuscles and renal tubules (Fig. 7A). While, the kidney section of diabetic rats showed degeneration of renal corpuscles and both distal renal and proximal tubules (Fig. 7B). Diabetic rats also showed wide areas of intracellular hemorrhage (Fig. 7C). On the other side, diabetic rats given oral doses of fenugreek sprouts, barley sprouts, cell free probiotic and whey protein exhibited normal structure of the renal corpuscles and the renal tubules (Fig. 7D, E, F, and G respectively). While diabetic rats given oral doses of the mixture showed disturbance of the structure of renal corpuscles and tubules as compared with the control one (Fig. 7H).

4. Discussion

Fenugreek sprouts juice, barley sprouts juice, cell-free probiotic extract, whey protein hydrolysate or their mixture were evaluated for their hypoglycemic, hypolipidemic and antioxidant activities in diabetic rats. The injection with streptozotocin was used in the present study to induce diabetes in male rats. Diabetic rats presented high fasting blood glucose levels and low body weight compared with non diabetic rats. Oral administration of Whey protein hydrolysate exhibited the best effect in alleviation of the fasting blood glucose and elevation of the body weight gain. Whey protein hydrolysate that generated by the enzymatic hydrolysis possess several biological activities that are associated with bioactive peptides. These bioactive peptides can be converted from inactive form to active form through the hydrolysis by the digestive enzymes [37]. Morato *et al.* [38] suggested that whey protein hydrolysate might be useful in the management of diabetes since whey protein hydrolysate increased the translocation of GLUT-4 to the plasma membrane as well as the glycogen concentration. Jakubowicz and Froy [39] disclosed that oral administration of whey proteins hydrolysates exhibited insulinotropic effect in humans and the same effect were described in diabetic animal by Gaudel *et al* [40]. Jakubowicz and Froy [39] stated that the secretion of gut hormones has been catalyzed in vitro via the bioactive peptides and amino acids generated from the hydrolysis of whey proteins. Also the inhibition of

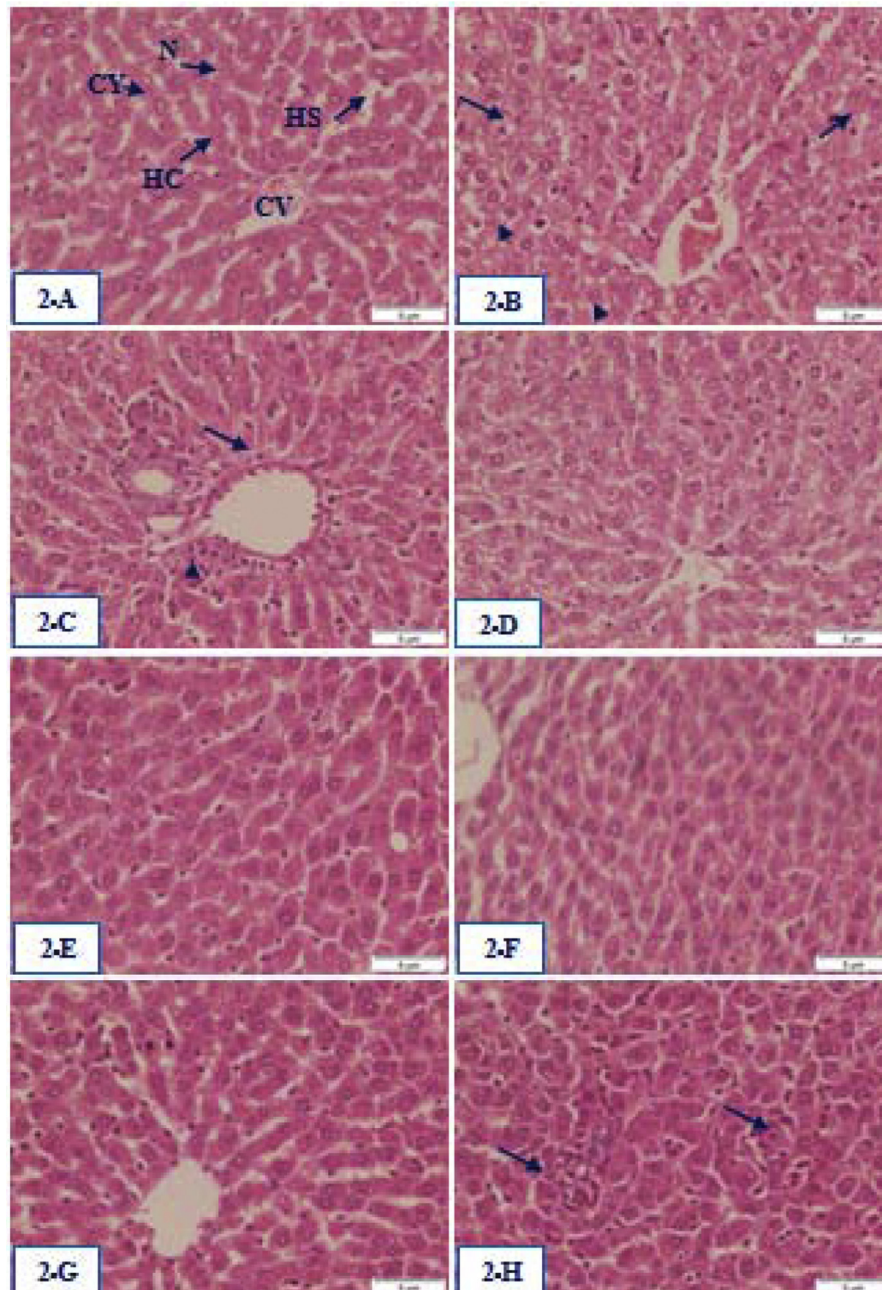


Fig. 6. Liver sections of A) control rat showing the normal architecture of the hepatic lobule. The central vein (CV) lies at the centre of the lobule surrounded by the hepatocytes (HC) with strongly eosinophilic granulated cytoplasm (CY) and distinct nuclei (N). Between the strands of hepatocytes, the hepatic sinusoids (HS) are shown, B) diabetic rat showing disturbance of the hepatic lobule. Notice focal necrosis (arrows) and hydropic degeneration (arrowhead); C) diabetic rat showing congestion of portal tract (arrowhead) that associated with mild inflammatory infiltration (arrowhead); D, E, F, and G respectively) diabetic rats given oral doses of fenugreek sprouts, barley sprouts, cell free probiotic and whey protein showing the hepatic lobule appears more or less like normal; H) diabetic rat given oral dose of the mixture showing foci of hepatocytes necrosis associated with inflammatory infiltration (arrows) (H & E; Scale Bar: 5micrometer).

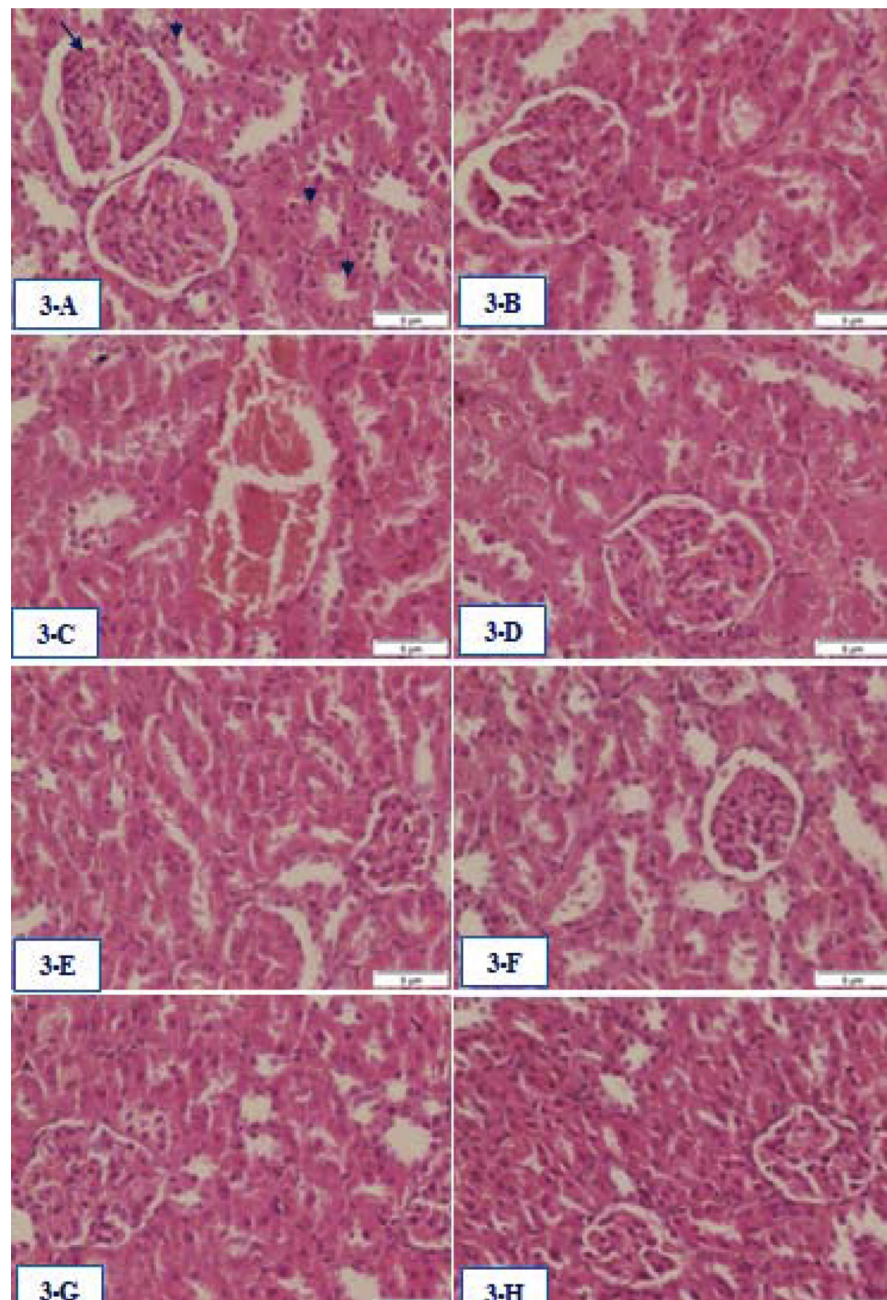


Fig. 7. Kidney sections of A) control rat showing the normal structure of the renal corpuscles (arrows) and renal tubules (arrowheads); B) diabetic rat showing degeneration of both distal renal and proximal tubules; C) diabetic rat showing intracellular hemorrhage; D, E, F, and G respectively) diabetic rats given oral doses of fenugreek sprouts, barley sprouts, cell free probiotic and whey protein revealed normal structure of the renal corpuscles or the renal tubules; H) diabetic rat given oral dose of the mixture showing disturbance of the structure of renal corpuscles and tubules as compared with the control one (H & E; Scale Bar: 5micrometer).

dipeptidyl peptidase IV (DPP IV) has been performed via these bioactive peptides and amino acids. The hypoglycemic and hypolipidemic effects of fenugreek sprouts may be due to the presence of biogenic amines cadaverine and putrescine [41]. Takano *et al.* [42] disclosed that barley leaf in a powder form alleviated the postprandial blood glucose level through elevation of digesta viscosity. Cell-free probiotic extract contains the probiotic metabolites such as organic acids, bacteriocins and peptides [43]. Bacteriocins able to manage the disturbances in the gut microbiota thus many diseases that are caused by these disturbances for examples obesity, diabetes and inflammatory bowel disease can be prevented [44].

Hyperglycaemia is associated with the reduction of haemoglobin concentration due to either glycosylation of red blood cells membrane proteins [45] or haemolysis of red blood cells by increased lipid peroxides [46]. Schmatz *et al.* [47] explained that the oxidative stress associated with hyperglycemia causes inhibition of aminolevulinate dehydratase (δ -ALA-D, the second enzyme of the heme pathway) which known also as uroporphobilinogen synthase. Oral administration of fenugreek sprouts juice, barley sprouts juice, cell-free probiotic extract, whey protein hydrolysate or their mixture increased haemoglobin concentrations compared to the untreated diabetic rats. These effects may be attributed to the antioxidant activities of these treatments in addition to their abilities to reduce lipid peroxidation (MDA) as was evident from the present study results.

Burski *et al.* [48] determined the amylase activity as an indicator of pancreas activity in alloxan induced diabetic rabbits and concluded that the decreased stimulating insulin effect on exocrine pancreatic cells results in decrease in the amylase activity while, the regeneration of the pancreatic tissue results in increase in the amylase activity. Since oral administration of fenugreek sprouts juice, barley sprouts juice, cell-free probiotic extract, whey protein hydrolysate or their mixture increased the amylase activity compared to the untreated diabetic rats, it is possible that these treatments act as pancreatic regenerative.

Usually diabetes is accompanied with dislipidaemia due to the absence or the insufficient of the insulin which contribute to the regulation of the lipid metabolism [49]. Oral administration of barley sprouts juice exhibited the best effect in improving the lipid profile may be as a result of the presence of polyphenols which lower lipids [50] and β -glucan (a water-soluble dietary fiber) which impairs the intestinal absorption and encourages the excretion of fat, thus the blood lipid metabolism can be improved and the tissue lipid accumulation can be suppressed [51].

Hyperglycemia is associated with elevation in the production of reactive oxygen species (ROS) which cause the cells damage and activate several pathways which in turn cause dysfunction of the endothelial [52]. Also hyperglycemia is associated with the reduction of antioxidant enzymes (especially catalase) gene expression [53]. The antioxidant activities of fenugreek sprouts juice, barley sprouts juice,

cell-free probiotic extract, whey protein hydrolysate not only shown via the results of radical scavenging activity but also *in vivo*. Since the oral administration of mentioned items suppressed the elevation of malondialdehyde values and the reduction of catalase activities. Results of the present study declared that increased kidney functions (urea and creatinine) were associated with diabetes. These results in agreement with Fernandes *et al.* [54] who stated that the elevation of lipid peroxidation in diabetic rats resulted in renal dysfunction.

Histopathological investigation of pancreas, liver and kidneys of the diabetic rats showed histological alterations. On the other hand, supplementations with the tested materials lead to relieving these injuries.

It is known that streptozotocin attacks the pancreatic β -cells via GLUT 2 (the glucose transporter) results in damage of the β -cells by DNA alkylation. In addition, the production of both superoxide radicals with the help of xanthine oxidase and nitric oxide free radicals contributes in the damage of β -cells. Therefore, free radicals play an important role in the development of diabetes mellitus by causing the partial destruction of β -cells [55].

Also, the damage of the liver may be attributed to STZ induced free radical production. The GSH stores can be depleted via the reactive free radicals allowing the reactive intermediate to react with and destroy hepatic cells [56].

In the present work, diabetic group showed glomerular atrophy, widening of urinary spaces, swelling of cells, hydropic degeneration of tubules, congestion of capillaries, and tubular necrosis. Matsubara *et al.* [57] reported that one of the major complications in kidney that associated with diabetes resulted from the expansion of mesangial cells, a hallmark of diabetic rats. The results of Balakumar *et al.* [58], indicated to several changes in the kidney tissues of diabetic rats among them, thickening of the glomerular and tubular basement membranes, tubulointerstitial fibrosis, glomerulosclerosis and renal endothelial dysfunction in addition to the decreasing in the glomerular filtration rate. It was reported that the oxidative stress in kidney caused due to the excessive generation of reactive oxygen species and the reduction in the activities of antioxidant enzymes [59].

In view of that, it was hypothesized that due to the free radicals scavenging properties of fenugreek sprouts juice, barley sprouts juice, cell-free probiotic extract, whey protein hydrolysate or their mixture they can ameliorate diabetes and its associated complications.

5. Conclusion

It can be concluded that fenugreek sprouts juice, barley sprouts juice, cell-free probiotic extract, whey protein hydrolysate or their mixture have promising

effects towards hyperglycemia and associated disorders precisely deslipidemia and oxidative stress.

Declarations

Author contribution statement

Rasha S. Mohamed, Ahmed M. Abdel-Salam: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Diaa A. Marrez, Salah H. Salem, Ahmed H. Zaghloul, Ihab S. Ashoush: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Abdel Razik H. Farrag: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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