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Effect of sea buckthorn protein on the intestinal microbial community in streptozotocin-induced diabetic mice

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ABSTRACT

This study investigated the intestinal microbial community distribution of Type 2 diabetic mice and discussed the effects of the sea buckthorn protein on the regulation of gut microbes. Date was collected for 12 cases of normal mice (NC group), 12 cases of Type 2 diabetic mice (DC group), and 12 cases of highly concentrated sea buckthorn seed protein dosed mice (SSPH group). This study analysed fecal samples, measured faecal pH value, and cultivated and determined intestinal bacteria count. This investigation also included the extraction of faecal samples for genomic DNA, PCR amplification of bacterial V3 16S rDNA products by denaturing gradient gel electrophoresis, DGGE map analysis of intestinal flora, determination of intestinal bacteria richness, Shannon-Wiener index and evenness index, and image similarity cluster analysis with UPGMA clustering. This study analysed and elucidated differences between the normal mice group, diabetic mice group, and sea buckthorn protein supplemented group, and the structures of respective intestinal flora. The mice supplemented with sea buckthorn protein exhibited an obvious drop in body weight and blood glucose levels. The *Bifidobacterium*, *Lactobacillus*, *Bacteroides*, and *Clostridium coccoides* populations recovered. The amplification of the 16S rDNA gene V3 region revealed that the species of intestinal microbes in the treatment group were adjusted to a certain extent. Analysis by ARDRA confirmed that sea buckthorn protein could increase type 2 diabetes in mice intestinal microorganism diversity (H) and simpson (E).

1. Introduction

Diabetes may be brought on by a defect in the insulin secretion mechanism of the pancreas or through damage of its biological function, which is marked by a high blood sugar metabolic disease. Type 1 or Type 2 diabetes have familial tendencies and possess broad genetic heterogeneity [1–3]. Moreover, in recent years, with improvements in living standards, increased average human life span, lifestyle changes and continuous advances in detection, the prevalence of Type 2 diabetes has increased dramatically [3]. To date, there is no cure for diabetes; hence, it may be only controlled thorough a variety of treatments [4]. Although various medications and insulin drugs may be employed to treat diabetes, they all have limitations, including adverse side effects and toxicity. A variety of medicinal herbs and plants have been used as alternative agents for the treatment of diabetes on a global scale because of their effectiveness, reduced side effects and relatively low cost [5,6]. Sea buckthorn (SSP) is a medicinal plant that contains a variety of bioactive substances; sea buckthorn seed contains proteins, flavonoids, terpenoids, vitamins, and other nutrients and bioactive substances [7], and it exhibits anti-cance activity [8], immune enhancing activity [8], blood lipid regulating activity [9], anti-inflammatory activity [9] and protection of the retina etc. [10]. Eight types of essential amino acids are contained in SSP, where tyrosine (18.3%) and glutamic acid (16.8%) have the highest content and the tryptophan (0.31%) has the lowest content. The total amount of amino acids in the sea buckthorn protein was 83.41%, of which the total amount of essential amino acids was 67.57%, thus, it is close to being a complete protein [11]. Since the full range of protein amino acids and essential amino acid content in sea buckthorn is high, it is within a class of relatively high-quality plant protein resources [11]. Huang

Abbreviations: STZ, streptozotocin; NC, normal mice; DC, diabetic mice; SSPH, seabuckthorn seed protein high-dose; SCFA, short-chain fatty acid; DGGE, denatured gradient gel electrophoresis; UPGMA, unweighted pair-group method with arithmetic means; ARDRA, amplifed ribosomal DNA restriction analysis; SSP, seabuckthorn seed protein; SPO, seabuckthorn polysaccharide; SGOT, serum glutamic-oxaloacetic transaminase; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; SPR, seabuckthorn seed proanthocyanidins; SPL, seabuckthorn seed protein low-dose group; SSPM, seabuckthorn seed protein middle-dose group; SSPH, seabuckthorn seed prote in high-dose group; FBG, fasting blood glucose.

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et al. reported that sea buckthorn polysaccharide (SPO) may reduce high-fat diet-induced blood lipids and hyperglycemia and can significantly lower serum glutamic-oxaloacetic transaminase (SGOT) activity [12,13]. All of the above findings indicate that SSP is a class of natural medicinal food. For Type 2 diabetes in mice, SSP has been observed to have a significant blood sugar lowering capacity and insulin resistance. On the basis of previous research results, this paper investigates the effects of sea buckthorn protein on intestinal microbial communities of diabetic mice, in order to further elucidate the mechanism of action of sea buckthorn protein in the reduction of blood sugar.

The aetiology of diabetes has not been fully elucidated, and it is now thought that diabetes is not caused by a single genetic disease, but is a composite syndrome, including genetic, autoimmune disease, dietary and environmental factors. In recent years, mounting data suggests that intestinal flora and the occurrence of Type 2 diabetes, obesity and other metabolic conditions are closely linked [13,14]. A series of related diseases may be initiated by the disordered structure and function of the intestinal tract, and which are primarily manifested as metabolic and immune system diseases [14]. Further, investigations into the relationship between human symbiotic microorganisms and Type 2 diabetes will provide new strategies for its prevention and treatment. Intestinal microbes have been regarded as the key to the treatment of metabolic diseases [15].

According to a recent literature research survey, the scientific support of sea buckthorn protein for the regulation of intestinal microbial flora in Type 2 diabetic has not yet been recorded. The purpose of the present study was to examine the effects of the consumption of sea buckthorn protein in streptozotocin-induced diabetic rats, on various parameters related to the influence and regulation of intestinal flora. As a form of edible protein, SSP may reduce insulin-resistant serum concentrations of TC, TG and LDL-C, which were decreased in the serum of the SSP treatment group compared with that of the diabetes model group. Futher, SSP diets may reduce hyperglycaemia and inflammatory factor levels in diabetic mice [13,29]. Preliminary studies have indicated that sea buckthorn protein may bring relief for diabetes. The present study focused on the role of gut microbes in the effective regulation of blood glucose levels in diabetic mice with promising results.

2. Experimental

2.1. Materials

Sea buckthorn seeds were obtained from Qinghai KangPu Biotechnology Co., Ltd. Streptozotocin (STZ) was purchased from Sigma Chemical Co. (Nanjing, China). The buffer solution was purchased from the Pharmaceutical Group Co., Ltd. (Anhui, China). The remaining reagents and chemicals used were of analytical grade.

2.2. Preparation of sea buckthorn seed proteins

The preparation of sea buckthorn seed proteins was divided into deracination, flavonoids removal and extraction. The dried sea buckthorn seeds were treated with ether to remove oil ester and with ethanol to remove flavonoids. Then, the seeds were crushed with a grinder, using an 80-mesh sieve and the powder were collected. The powder was extracted with water (1:14, m/v), and sodium hydroxide solution was added to adjust the pH to 11.0. The solution was heated for 1 h at 60 °C, and then centrifuged at low speed. The supernatant was collected and hydrochloric acid was added to adjust the pH to 5.0. The solution was then centrifuged at low speed, and the supernatant was collected to obtain the precipitate. The precipitate was washed twice with water and the pH was adjusted to 7.0 to redissolve the precipitate. The solution was dried with a vacuum freeze dryer to obtain the sea buckthorn protein powder, which was collected and storage at 4 °C for later use.

2.3. Induction of Type 2 diabetes and treatments

This study included 60 ICR mice (male, 20 ± 2 g), which were purchased from Changzhou Cavens Co., Ltd. (Changzhou, China). The mice were maintained under controlled conditions in 12 h light/12 h dark cycles at 60% relative humidity at 25 ± 2 °C, with free access to food and water. Following seven days under general accommodation conditions, 12 mice received a normal chow diet and were randomly selected to serve as normal controls (NC group). The remaining mice were treated with a high-fat diet (consisting of 72% normal chow, 15% sugar, 10% lard and 3% yolk) for four weeks to induce insulin resistance and obesity. In the fourth week, they also received a streptozotocin injection (Sigma, USA, 30 mg/kg bw^{-1}). Successful model mice were divided into four groups: the diabetic control group (DC), SSPL (sea buckthorn seed protein low-dose group), SSPM (sea buckthorn seed protein middle-dose group) and SSPH (sea buckthorn seed protein high-dose group). The mice in the low-, middle- and high-dose groups were intragastrically administered 50, 100 and 200 mg/kg bw^{-1} SSP aqueous solutions,respectively, whereas mice in the normal and diabetic groups were treated with the same amount of distilled water. This experimental protocol was approved by the Animal Experiment Committee of China and followed the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

2.4. Body weight and fasting blood glucose

The body weight of all mice was determined every week. An Accu-check meter (Mannheim, Germany) was employed to measure the fasting blood glucose (FBG) levels of all mice, and blood samples were collected from the tail vein. Fasting blood glucose levels were measured prior to inducement to ensure that all experimental animals had normal FBG levels. During the induction period, the FBG levels were measured 30 days after the STZ injection. Subsequent to the four-week treatment, the FBG levels of all mice were measured in order to determine whether the treatments had a glucose lowering effect in diabetic mice.

2.5. Bacterial count analyses

The targeted bacterial species (*Bifidobacterium, Lactobacillus, Bacteroides and Clostridium coccoides*) were quantified by plate counting. Briefly, faecal samples were prepared by adding sterile deionized water (1 mL) directly into pre-weighed tubes containing fresh faeces, and these were further diluted to prepare samples with dilution factors ranging from 102 to 109. Subsequently, each dilution (0.1 mL) was spread on different selected media.

2.6. Extraction of genomic DNA from faecal flora

Faecal sample were thawed on ice. According to manufacturer instruction of the DNA extract kit (yvduo, shanghai, China). Gut microbial genome was saved at −20 °C for later use [16].

2.7. Amplification of 16S rDNA gene V3 region

The primers used for the bacterial 16S rDNA V3 hypervariable region are shown in (Table 1). The total volume of the 50L reaction system included dd H_2O (41.25 L), 10× Buffer (5 L, 2 mM $MgCl_2$), dNTP (1 L, 10 mM), F357-GC (1 L, 10 M), R518 (1 L, 10 M) and Taq (5 U/l enzyme, 0.25 L, DNA 0.5L) template.

Table 1
PCR primer.

primer	sequence	Amplified fragment size
F357-GC	5′- CCTACGGGAGGCAGCAG -3′	About
R518	5′- ATT ACC GCG GCT GCT GG -3′	230bp

GC: CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCC.

The reaction procedures included: 94 °C for 4 min pre-denaturation, 94 °C for 0.5 min, 56 °C for 1 min, 72 °C for 0.5 min, 30 Cycles, extension 7 min, 72 °C.

Analysis of 3 μL of each PCR product was performed by agarose gel electrophoresis on a 1.5% agarose gel.

2.8. DGGE strip recovery

Select a more representative of the strip (number as shown), with a clean surgical blade will target DGGE with complete cut and loaded 1.5 mL centrifuge tube, according to the kit SK8131 recovery, backup.

3. Statistical analysis

3.1. Polymorphism analysis

ARDRA band as a OTU, Quantity One software will appear on the agarose gel DNA fragment into molecular weight information, according to the rule: I represents "appear" and 0 represents "does not appear" by statistics. Fuzzy bands and the molecular weight of less than 100 bp bands [17].

3.2. Diversity analysis

The Shannon-Wiener index (H) and richness index (S) were used to elucidate the microbial diversity in the intestinal tract. Calculating the formula for H: $H = -\sum P_i \ln P_i$; calculating the formula for E: $E = H/\ln S$. $Pi = Ni/N$. As Pi of an individual number, N *as* all the individual numbers of species in the community, S as the number of species, and individual Pi belongs to the type of i in all the proportions of the individual.

3.3. DEEG analysis

Reference Muyzer methods were used to extract total DNA from the faecal flora [18]. Faeces (0.1 g) were weighed and transferred to a 2 mL sterilized screw mouth tube (Bio-Spec), and the following was added: zirconium beads (0.4 g), bacterial lysis buffer (0.7 mL, 500 mmol/L NaCl, 50 mmol/L Tris–HCl, pH = 8.0, 50 mmol/L EDTA, 4% SDS), and a phenol/chloroform/isoamyl alcohol (25:24:1) solution (0.25 mL). The maximum speed in the Bead Beater™ Mini (Bio-Spec, USA) grinding machine was used for grinding the sample for 2 min. The sample was then centrifuged for 5 min after which the supernatant was transferred to a 1.5 mL centrifuge tube. Next, ammonium acetate (0.25 mL, 10 mol/L) was added to the centrifuge tube and mixed with ice for 5 min, and the sample then underwent centrifugation at 20,000 × *g* for 10 min with phenol/chloroform/isoamyl alcohol (25:24:1) solution (500 μL). The supernatant was twice extracted chloroform at 3 min each. The supernatant volume of pre-chilled isopropanol was evenly mixed and heated to 20 °C for 30 min centrifuged at 20,000 × g for 10 min then precipitated with 70% ethanol rinsing, and naturally dried. Subsequently, TE (50 μL, pH = 8. 0) and DNA-free-RNase (2 μL, 10 mg/ml) were added at 37 °C for 15 min of incubation. The DNA content of the samples was measured with a Nano DropND - 1000 nucleic acid quantitative instrument (Nanodrop technologies) using 1.0% agarose gel electrophoresis to verify the integrity of the DNA.

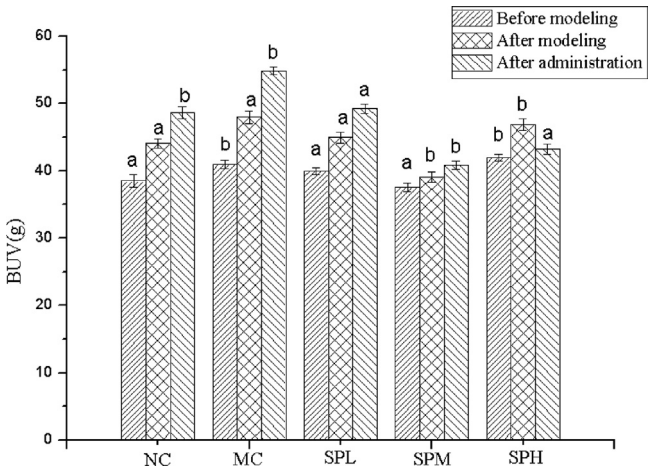

Fig. 1. Changes in body weight (n = 12) of the mice that received treatments. The body weight of the mice prior to and following modeling, and after treatment administration was measured, respectively. Values are shown as mean ± S.D and different superscript letters indicate a significant difference at $p < 0.05$.

3.4. Degeneration gradient gel electrophoresis (DGGE)

The D-Code mutation detection system for DGGE analysis of samples was used, where the polyacrylamide gel concentration was 8% (acrylamide/methylenebisacrylamide, 37.5:1), and the denaturant concentration was from 30% to 60% (100% of the denaturant urea 7 mol/L, 40% formamide), under a voltage of 60 V and temperature of 60 °C, and 1× TAE in electrophoresis for 16.0 h. After electrophoresis using ultra-pure water for rinsing, the samples were glued in the dye-containing EB, placed in the rocking bed after dyeing for 30 min, and photographed using the UVI imaging system.

4. Results and discussion

4.1. Body weight

To induce obesity and a pre-diabetic state, all of the mice, except for the normal controls, received a high-fat diet and weighed significantly more than the normal control mice ($p < 0.05$) following the diabetic state inducement [20], as shown in Fig. 1. After four weeks of treatment with different concentrations of sea buckthorn protein, different effects on the body weight of diabetic mice were found and were consistent with findings from previous studies [21]. After modelling and administration, a significant weight gain was found between the NC and DC groups. Similar results to the NC group were seen for the SSPL, SSPM and SSPH groups, where the body weight of the treatment group was close to that of the NC group, and was positively correlated with the concentration, which indicated that sea buckthorn protein can negate weight loss caused by diabetes.

4.2. Fasting blood glucose levels

Fasting blood glucose levels are the most direct indicator of an anti-insulin condition. STZ-induced diabetic mice showed a significant ($P < 0.05$) increase in blood glucose levels than did the mice of the NC group. Fasting blood glucose levels of the mice in the SSPL, SSPM and SSPH groups were significantly ($p < 0.05$) lower than that of the diabetic mice, as shown in Table 2; this was particularly exhibited by the mice of the SSPH groups, potentially caused by the high SSP content. These findings were consistent with previous

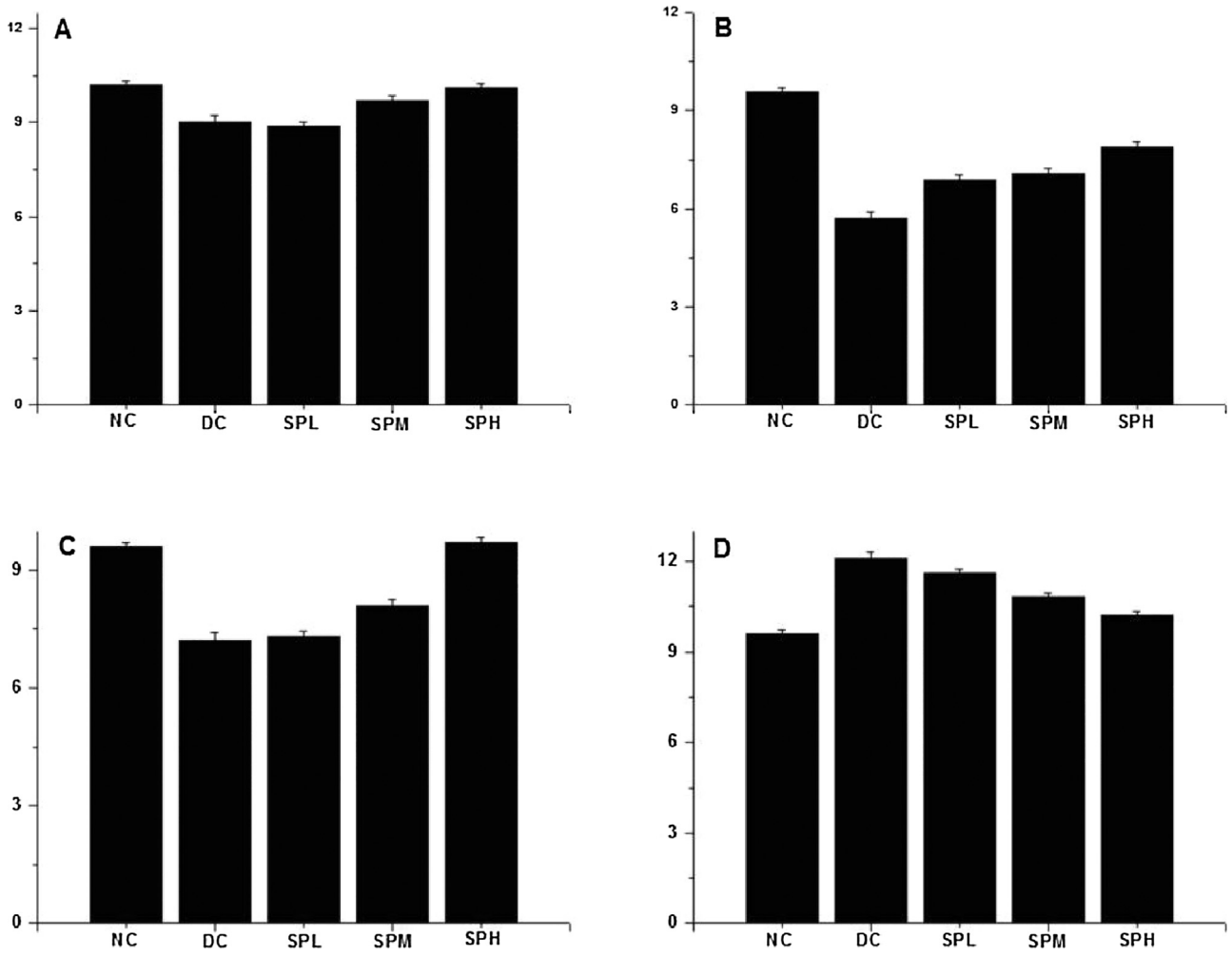

Fig. 2. Effect of SRRS on fecal *Bifidobacterium* (A), *Lactobacillus* (B), *Bacteroides* (C) and *Clostridium coccoides* (D) following four-week-treatment (n = 12). Values are shown as means ± S.D.% difference compared to the normal control ($p < 0.05$).

Table 2
Glucose values in each group of mice.

Group	Fasting blood glucose level			
	0 days	7 days	14 days	28 days
NC	8.7 ± 0.4^{a}	7.9 ± 0.5^{a}	8.7 ± 0.9^{a}	9.1 ± 1.2^{b}
DC	16.5 ± 0.2^{ab}	18.9 ± 1.4^{a}	20.5 ± 1.1^{b}	24.0 ± 0.8^{ab}
SSPL	17.3 ± 0.4^{b}	15.9 ± 1.1^{a}	19.0 ± 0.8^{b}	22.8 ± 0.9^{a}
SSPM	15.8 ± 1.7^{a}	15.0 ± 0.9^{b}	17.6 ± 0.4^{ab}	20.12 ± 1.1^{a}
SSPH	17.8 ± 0.6^{a}	14.2 ± 1.2^{ab}	17.0 ± 0.9^{a}	19.6 ± 1.2^{b}

This was a single factor experiment, where each value in the table represents the mean values ± standard; a indicates ($P < 0.05$) compared to the model group.

studies, which showed that SSP could improve serum lipid levels, and that SSP may reduce glucose levels in diabetic mice [21,22]. The lower levels of blood glucose and body weight in the mice that were fed SSP were not unexpected, as a number of studies have reported the ability of plant protein from oilseeds to lower blood glucose levels [23]. The hypoglycemic effect was considered to result from its competitive inhibition of the sodium-glucose symporter, which is located in the mucosa of the small intestine.

4.3. Effects of SSP on faecal bacteria in diabetic mice

Faecal bacteria were quantified by analysing the percentage of difference compared with the normal control; the results are shown in Fig. 2. Significant differences in all targeted microorganism species (*Bifidobacterium, Lactobacillus, Bacteroides* and *Clostridium coccoides*) were found between the SSP-treated mice and the diabetic control mice ($P < 0.05$). Compared with the NC mice, the DC mice showed resistance.

Bifidobacterium and *Lactobacillus* are beneficial bacteria that play an important role in the stimulation of the decomposition of sugar, the reduction of blood and so on. *Clostridium coccoides* is one of the predominant microflora that flourishes within the intestine. It has been recently suggested that it may promote the host's capacity to absorb energy from food, which may lead to abnormal energy metabolism that is related to the occurrence and development of metabolic diseases such as obesity and diabetes mellitus [20]. *Clostridium coccoides* facilitates the degradation of cellulose polysaccharide, which the human body cannot typically absorb, there by increasing energy sources. Nadja et al. found this to be the case with *Bacteroides* in obese populations [24]. The same strain has the ability to degrade other polysaccharides, and the absorption and utilization of nutrients in the human body show that the energy generating efficiency of the host may be greatly improved. A study by Zhang and Ye also found that changes in the composition of intestinal microbiota could be positively correlated with blood glucose levels [25]. As a result, the presence of thick-walled phylum of bacteria, including *Bacteroides* and *Clostridium coccoides,* within the intestine is more effective than that of *Bacteroides*, leading to a more efficacious absorption of energy from food. The most likely mechanism is that proteins were fermented to form short chain

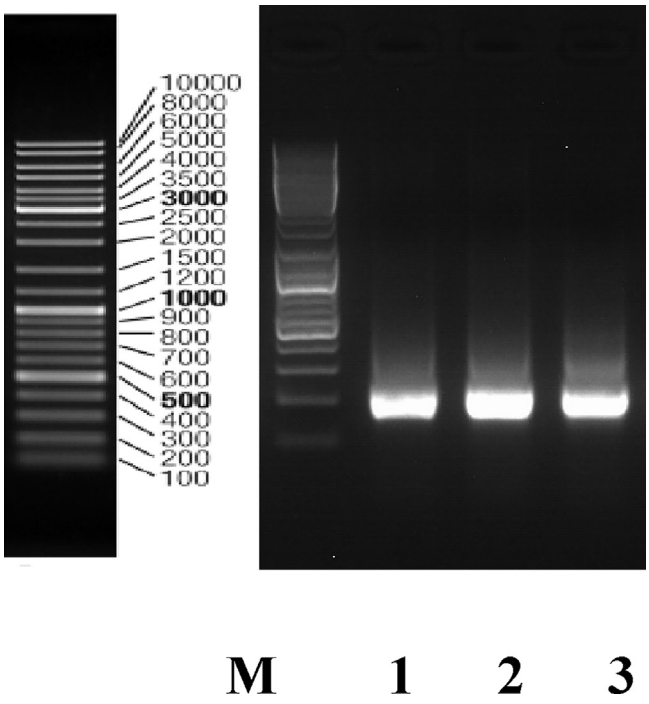

Fig. 3. Intestinal flora in mice macro16S rDNA amplification of genomic DNA electrophoresis figure M: Mark; 1: NC group; 2: DC group; 3: SPH group.

fatty acids (SCFAs) and a lower pH, with an apparent beneficial impact on gastrointestinal health; caecal bifidobacteria and total anaerobes were found to be higher in Type 2 diabetic mice [22]. Further, Donskey et al. confirmed a correlation analysis between SCFAs and the *Bifidobacterium*, *Lactobacillus, and Bacteroides* that were present in the faecal content of diabetic mice, and observed that the abundance of these intestinal probiotics significantly and positively correlated with total SCFAs and butyrate [26]. Therefore, SSP, which is the functional component of sea buckthorn, may be beneficial for individuals who are suffering from obesity and Type 2 diabetes.

4.4. Analysis of PCR-DGGE spectra of intestinal flora

A genetic fingerprinting technique, referred to as DGGE, is extensively employed in the study of intestinal flora. Identical lengths and sequences of microbial 16S rDNA may produce diverse PCR amplification products due to variable regional sequence degeneration concentrations. In corresponding positions of the DGGE gel, the migration and band dispersion may be stopped, where the brightness of the bands and the number, respectively, reflect the quantity and species of bacteria [27]. The results of the PCR were observed and analysed by agarose gel electrophoresis. The results showed that the length of *Lactobacillus* 16S rDNA was ~380 bp, and the bands were stable and clear after comparing with Mark.

4.5. ARDRA analysis of the enzyme digestion of PCR products from the macro genome of intestinal flora

Quantity One software analysis was performed, with the results shown in Fig. 3, which reduced to the same OUTs, and each DNA restriction fragment length shows polymorphism; OUTs multifunction performance estimates the number of bacteria isolated in the existence of a minimum number of OUTs in each group (see Table 3). ARDRA bands that are rich in community diversity revealed a stronger signal, which indicated a larger number of species (the NC group had 26 bands, the DC group had 23 bands, and the SSPH group had 25 bands). The faecal content and number of bacteria species in the normal mice was significantly higher than that of the Type 2 diabetic mice [27]. Through the analysis of intestinal microflora of Type 2 diabetic mice, Lin found that the occurrence and development of diabetes are critical factors that lead to structural changes in intestinal flora [28]. Using STZ modelling, it was shown that the intestinal flora of mice reduced the amount of probiotics, and diversity was decreased; the SSP group had more diversity than the DC group, but less than that of the NC group. It was revealed that with the sea buckthorn protein treatment, the intestinal flora of mice recovered to a certain extent, but did not attain normal levels. The SSPH group exhibited seven unique bands, while the other groups were not found in this band, which indicated that the treatment did indeed have an effect on the gut bacteria. In further studies we will identify the seven unique bands that emerged through the change of flora.

Table 3
Number of OTUs of intestinal in different treatment groups.

Group	NC group	DC group	SSPH group
OTUs	26	23	25

Table 4
Diversity (*H*) and Simpson (*E*) indices of microorganisms in mice under different treatments.

Group	*H*	*E*
NC group	3.367	0.966
DC group	2.890	0.944
SSPH group	3.332	0.964

4.6. Intestinal microbial diversity index and similar coefficients in each group of mice

The Shannon-Wiener diversity index (*H* value) was used to estimate the diversity of bacterial communities, and it contains two factors: the number of species, that is, richness (*S* value), and species in the individual distribution of the average, or uniformity, (*E* value) which were once used to describe the relative richness, or proportion of individuals in a species [19]. Table 4 shows that following a high-fat feed diet and STZ induction, there was significant difference between the intestinal microflora of the DC group and the NC group, where *H* and *E* values were decreased, which suggested that the DC group had affected diversity. Subsequent to the high-dose sea buckthorn seed protein treatment, the SSPH's indicators were increased. This result indicated that diabetes initiated a certain degree of damage to the intestinal microbial diversity and species evenness in mice, and mice intestine microbial diversity was restored by treatment with sea buckthorn seed protein. In general, following treatment with sea buckthorn seed protein, the SSPH group became more similar to the NC group, than the DC group in terms of intestinal microbial diversity. This revealed that SSP may have the capacity to alleviate and repair intestinal disorders caused by diabetes mellitus to some extent, however, it cannot be completely cured.

4.7. Cluster analysis and principal component analysis of intestinal bacteria in each group of mice

To process the DGGE fingerprint using Quantity One 4.6.2 software, the lane background was removed, the detection position of each band migration and brightness were quantified, a two-dimensional matrix with position and brightness was obtained, and a phylogenetic tree analysis was undertaken between each lane clustering using the UPGMA clustering method. The UPGMA

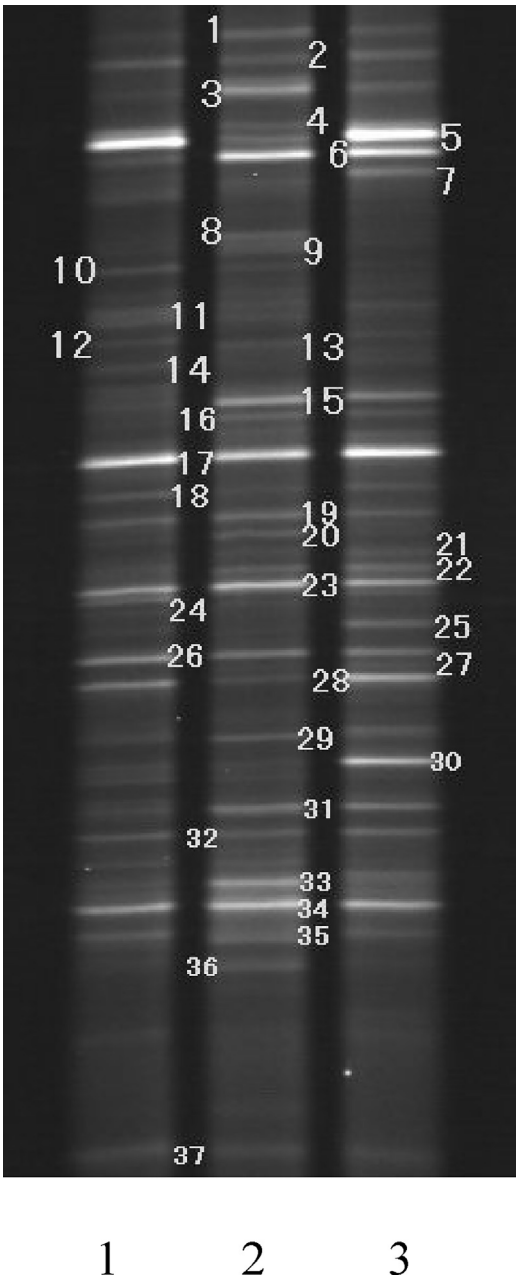

Fig. 4. Intestinal flora in mice 16S rDNA amplification of genomic DNA electrophoresis Fig. 1: NC group; 2: DC group; 3: SPH group.

clustering analysis method is more commonly used, and the first employed to solve the classification problem; the tree generated by the UPGMA method may be considered to be a simple manifestation of tree species. The use of UPGMA analysis to assess similarities between microbial community profiles has become very common [29].

As the results show in Fig. 4, two groups may be clustered. For the first group of NC and SSPH, the similarity between samples was ~77%. In the three intestinal flora of the phylogenetic tree of the mice, the DC group was clustered at different locations, and the NC and the SSPH groups were clustered in a similar position. This was similar to that reported in papers by Xueran Mei who found the similarity of fingerprints between the diabetic group treated with phlorizin and the NC group increased from 57% to 85% after the phlorizin administration for 10 weeks [13]. However, there was a significant difference between the DC and the first group. In the diabetic mice following treatment, it was demonstrated that the intestinal flora had been adjusted; however, the intestinal flora had not completely recovered to nominal levels. This suggested that there was a considerable difference between the intestinal microflora of the NC and the DC groups. Further, it may be seen that the intestinal flora of the diabetic mice following treatment by sea buckthorn protein were adjusted and restored; thus, SSP had an important effect on the intestinal flora (Fig. 5).

5. Conclusion

Diabetes is a metabolic disease characterized by high blood sugar. Hyperglycaemia is caused by the destruction of insulin producing β-cells of the pancreas or by the loss of the biological effects of these cells. Streptozotocin is employed to selectively destroy the pancreatic cells stimulating deficiency of insulin leading to diabetes. This study explored the effect of sea buckthorn protein on intestinal microbial community in streptozotocin-induced diabetic mice.

High fasting blood glucose leavels and decreasing body weight indicate that the mice are suffering from diabetes.The present study proves that SSP can increase the number of *Bifidobacterium*, *Lactobacillus* and *Bacteroides* and reduce the number of *Clostridium coccoides*. previous studies had shown *Bifidobacterium, Lactobacillus* and *Bacteroides* as beneficial bacteria that can directly apply to the immune system of the host, induce intestinal immunity, stimulate the thymus, spleen and other immune organs, promote macrophage activity, play a role in specific immune activity by enhancing B and T lymphocytes to increase the reactivity of the antigen, and finally, enhance the immune function of the body [30,31]. Short chain fatty acids (SCFAs) include acetate, propionate, and butyrate. SCFAs are produced in high concentrations by the decomposition of intestinal microbes or by the metabolism of proteins and amino acids, and are subsequently released in the bloodstream [30,31]. The order of action on cells is first butyric acid, followed by propionic acid, and then finally acetic acid. In the superior colon, CO_2 and ketone bodies are produced by the metabolism of butyric acid as precursor substances for the synthesis of mucosal lipids. In the posterior colon, butyric acid enters the TCA cycle and provides energy for colon epithelial cells. The health of colon epithelial cells is dependent on butyric acid. Butyric acid can inhibit the growth of colon cancer cells [32,33]. A slightly acidic environment can promote the growth of probiotics, where the fermentation produces SCFAs which can reduce intestinal pH and maintain the balance of intestinal flora [34,35]. It may prove that SSP increases the number of dominant flora, causing the number of SCFAs to increase and regulate the balance of intestinal microflora in diabetic mice. This may increase blood sugar decomposition and promote the body to effectively use the energy provided by food, so that energy metabolism to return to a normal state [36], and thus this may solve the obesity and metabolic diseases caused by diabetes.

The findings indicated and established significant correlations in diabetic mice and SSP, including lower body weight and lower blood glucose levels in the SSPH mice, in contrast to the DC mice. An analysis of the PCR-DGGE spectra of the intestinal flora suggested that the damaged intestinal environment of the Type 2 diabetic mice underwent a certain level of recovery. In future research we will investigate the different bands that are representative of specific strains. In relation to the effects of SSP on the faecal bacteria in diabetic mice, the Shannon-Wiener diversity index and cluster analysis provided strong evidence to show that the similarity of fingerprints between the DC group and SSPH group was increased, and the intestinal formation of both was closer. The work presented

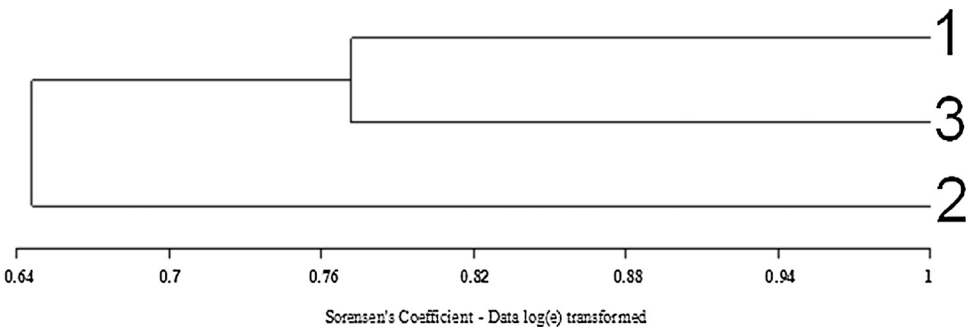

Fig. 5. Dendrogram of microbial flora in mice under sea buckthorn protein treatment.

here highlights the diversity of the gut microbial community during recovery following treatment with sea buckthorn protein.

The association between faecal metabolites and intestinal flora may provide significant information on bacterial activity; therefore, we can take this as an excellent opportunity to develop and optimize the potential microorganisms based on the prevention and treatment of diabetes. In summary, as a natural food that has been deemed as healthy and safe, sea buckthorn protein might act as a beneficial therapeutic diet strategy for diabetic patients. In future studies, the intestinal microbiota regulation mechanism of sea buckthorn protein certainly warrants significant attention.

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