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FULL PAPER

Toxicology

Effects of burdock inulin-type fructans exposure on the physiological function of healthy mice and their filial generation

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ABSTRACT. Inulin-type fructans (ITFs) have been shown to possess various biological activities. However, studies on their safety and side effects are limited. Hence, we aimed to evaluate the possible effects of burdock ITFs on the physiological indices of healthy mice and their filial generation when fed for six months. Thirty-two C57BL/6J mice were randomly divided into two groups; a normal control (NC) and an ITFs group. The parental generations were kept in one cage with free access to a normal diet and double-distilled water (P-NC group) or burdock ITFs drinking water (P-ITFs group, 2% w/v). The filial generations (F-NC group and F-ITFs group) were kept separately and were fed as their parental generation. Behavior, organ/body weight, serum indices, histopathology, time of production, and number of pup births were observed. There were no significant adverse effects on these indices. Functional indices of the spleen, lung, heart, and pancreas of the ITFs groups were higher than those of the NC groups, respectively. Interestingly, the serum glucose (GLU), total cholesterol (TC), uric acid (UA) and creatine kinase (CK) levels of the ITFs groups were lower than those of the NC groups. Meanwhile, the pregnancy number and pup birth number of the P-ITFs group were more than those of P-NC group. Therefore, long-term consumption of burdock ITFs has no obvious adverse effects on the health of parental mice and their offspring, but may contribute to reproductive capacity, fatigue reduction, and risk reduction of renal disease.

KEYWORDS: burdock, inulin-type fructans, physiology, safety

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Worldwide, people live mainly on three starch-rich foods, rice, corn, and wheat, which represent over 60% of the world's food intake [9, 30, 35]. The reliance on single crops as staple foods poses a threat to population health in many countries [1]. The World Health Organization has recognized the widespread prevalence of inadequate nutrient intakes or nutrient imbalances, also termed "hidden hunger" [14, 18]. The United Nations Food and Agriculture Organization has reported that approximately 2 billion people around the world suffered from hidden hunger in 2019, of which 300 million were from China [9]. Excessive intakes of calories, fat, protein, and salt are the main cause of chronic non-communicable diseases including diabetes mellitus, cardiovascular disease, cancer, and chronic respiratory diseases, which represent a serious threat to human health [33]. In China, 70% of chronic diseases are associated with hidden hunger, accounting for 88% of all deaths. Chronic non-infectious diseases account for over 70% of the total disease burden in China [37]. Many studies have revealed that a lack of dietary fiber is linked with the onset of chronic non-infectious diseases [2]. Dietary fiber was declared as a nutrient by the Nutrition Labeling and Education Act in 1990 [11].

With the recent increased demand for prebiotic fiber and lower-energy foods, the use of non-starchy polysaccharides from the *Asteraceae* family such as Jerusalem artichoke (*Helianthus tuberosus* L.) tubers and chicory (*Cichorium intybus*) roots, which are known to contain high amounts of inulin-type fructans (ITFs; consisting of fructose subunits via β-(2→1) linkages with an α-glucose terminal unit) have been on the rise [4, 23, 34]. Due to the β-(2→1) glycosidic bonds, ITFs cannot be broken down by human digestive enzymes in the mouth, stomach, or small intestine, but can be fermented by gut bacteria into the major short-chain fatty

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acids (SCFAs) acetate, propionate, and butyrate in the colon. Changes in the intestinal flora caused by ITFs fermentation influence the immune function of the intestinal lymphoid tissue. Meanwhile, the changes in the intestinal flora on account of ITFs fermentation help to prevent and treat chronic non-infectious diseases. Due to these benefits, ITFs are eaten as a dietary fiber source by humans in their daily diet [3, 16, 21, 32].

Burdock (*Arctium lappa*) belongs to the *Asteraceae* family, as does chicory and Jerusalem artichoke [27, 42]. Burdock roots are known sources of prebiotic ITFs and are consumed raw or cooked in Asia, Europe, and the United States [8, 26]. Dried burdock root flour has been used as a prebiotic ingredient with whole wheat flour in the manufacture of bakery products and is available as a herbal tincture and tea in many Asian countries [38]. Burdock roots contain approximately 15% ITFs (approximately 80% moisture), which are the main bioactive components [27]. These ITFs exhibit hypolipidemic, hypoglycemic [40], antithrombotic [29], antitussive [17], free radical-scavenging, antioxidant [22], anti-inflammatory [41], and prebiotic properties [12, 27]. Therefore, burdock root is considered as a functional food with homology to medicine, which has led to it being widely planted in many countries [13]. Despite these reported positive properties, one month supplementation with ITFs had a negative effect on the liver morphology of non-diabetic rats and plasma glucose levels in both diabetic and non-diabetic rats [25]. ITFs have also been shown to cause adverse hypersensitivity reactions associated with the quality and purification of the product extracted from plants [6]. These studies demonstrate that not all sources and forms of ITFs are healthy and safe, and that a diet enriched with fermentable fibers displays different effects in different organisms [25]. Moreover, over-intake of ITFs induces an increase in gastrointestinal and/or other serious side effects such as inflammation, compensatory cell proliferation, and liver cancer [23, 34]. Few studies have evaluated the safety of burdock ITFs, though some have looked at chicory root and Jerusalem artichoke [6, 7, 34]. Therefore, we aimed to evaluate the long-term effects of burdock ITFs on the physiological indexes of healthy mice. The results presented here help span the knowledge gap on the dietary and medicinal applications of burdock root as a fiber source.

MATERIALS AND METHODS

Animal ethics statement

The experimental animals, designs, and animal management in the present study were approved by the Laboratory Animals Ethical Committee of Wannan Medical College (Wuhu, China; approval number: LLSC-2022-041) and complied with the recommendations of the ARRIVE Guidelines for the Care and Use of Laboratory Animals.

Preparation and structural characterization of burdock ITFs

Fresh burdock root was purchased from a plantation based in Fengxian, Xuzhou city, Jiangsu province, China (Fig. 1). The obtained burdock root was classed as "Liuchuanlixiang," a representative variety of burdock that was widely planted in Fengxian and Peixian in Xuzhou City of China. To obtain a high concentration of ITFs, fresh burdock roots were harvested before first flowering and stored in polyethylene packages at 2°C [42]. ITFs were extracted from fresh burdock roots. The extraction process was performed as previously reported [31, 40]. Briefly, fresh burdock roots were washed, peeled, sliced into small pieces, submerged in water (1 g per 10 mL), and heated for 120 min at 70°C with constant stirring. The aqueous extracts were filtered using a vacuum water pump (SHZ-D (III), Gongyi Yuhua Instrument Co., Ltd., Gongyi, China). This extraction was repeated three times. The filtrates were combined and concentrated using a rotary evaporator (N-1100D-WB, EYELA, Tokyo, Japan) at 60°C. The concentrated extract was precipitated with a four-fold volume of 95% alcohol for 12 hr at 4°C. The precipitate was then collected and dissolved in distilled water, followed by centrifugation at 5,000 rpm. The supernatant was deproteinated using the Sevag method (chloroform:*n*-butanol, 4:1 v/v). The deproteinated polysaccharide aqueous extract was then decolorized with a D101-macroporous resin (Henghuibio Co., Ltd., Beijing, China), followed by concentration, dialysis (M_w cut-off: 1000 Da), and lyophilization. The obtained crude ITFs (10 mg/mL) were purified using a DEAE-52 chromatographic column (2.5 × 40 cm), wherein the sample volume was 20 mL and double-distilled water as the eluent (1.0 mL/min), and a G-75 chromatographic column (1.5 × 60 cm) where the sample volume was 5 mL (5 mg/mL) and double-distilled water was the eluent (0.5 mL/min). Total carbohydrates in the eluate (6 mL per tube) were measured using the phenol-sulfuric acid method. Polysaccharides from the same single peak were collected, dialyzed, and lyophilized to obtain pure burdock ITFs. The pure burdock ITFs were characterized using nuclear magnetic resonance (NMR) with a JNM-ECZ600R/S1 spectrometer (JEOL, Tokyo, Japan) at 600 MHz. Burdock ITFs (20 mg) were dissolved in 0.5 mL D_2O (99.9%), and One-dimensional ^1H (under water suppression), ^{13}C, and Distortionless Enhancement by Polarization Transfer 135 (DEPT-135) NMR spectra were recorded and analyzed using Mest ReNova software (version 6.1.0-6224; Mestrelab Research, Santiago de Compostela, Spain) [31].

Experimental animals, diets, and design

Thirty-two weanling C57BL/6J mice (six-weeks-old, 16 males and 16 females, average weight 22 g) were obtained from Sibf Biotechnology Co., Ltd. (Anyang, China) and housed at the specific pathogen-free Animal Center of Wannan Medical College. Mice were randomly divided into two groups: the normal control group and the ITFs-group (P-NC and P-ITFs groups, respectively). The experiment began with eight male and eight female mice per group

Fig. 1. Image of the commercial fresh burdock root.

as the parental generation. The parental generation was maintained in one cage with free access to a normal chow diet (lab mice diet #SWS9102, Jiangsu Syony Pharmaceutical Biological Engineering Co., Ltd., Nanjing, China) and double distilled water (P-NC group) or burdock ITFs drinking water (2% w/v, P-ITFs group) throughout the study [7, 20, 28]. The only difference between the groups was the burdock ITF supplement intake. The filial generations of both groups (termed F-NC and F-ITFs from the P-NC and P-ITFs groups, respectively) were kept separately in one cage and fed as their parental generation for six months. Food intake and water consumption were recorded daily. The environmental conditions of the animal room were at 23–25°C, 12 hr light/dark cycle, and 50 ± 10% relative humidity.

Sample collection and analysis

After six months, the four groups (P-NC, P-ITFs, F-NC, and F-ITFs) were fasted overnight. All animals were weighed, anesthetized with pentobarbital sodium (40 mg/kg, 0.036 g/mL), and then sacrificed. The nose-to-tail tip lengths of all animals were recorded. Blood samples were collected from the retro-orbital sinus, and the serum was separated via centrifugation for 10 min at 1500 rpm and 4°C. Serum was then stored at −80°C for the future measurement of total protein (TP), albumin (ALB), globulin (GLOB), total bilirubin (TBIL), direct bilirubin (DBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), urea nitrogen (Bun), creatinine (Cr), uric acid (UA), glucose (GLU), total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), creatine kinase (CK), lactate dehydrogenase (LDH), amylase (AMY), calcium (Ca), phosphorus (P), and magnesium (Mg), which were measured using an automatic biochemical analyzer (7080, HITACHI, Tokyo, Japan).

The brain, lung, heart, liver, spleen, kidneys, pancreas, colon, and abdominal fat were collected. The selected organs from the four groups of animals were weighed immediately and the organ/body weight ratio was calculated. After being weighed, the specimens were frozen in liquid nitrogen or fixed in neutral-buffered formalin for morphological analysis. Paraffin sections (5 µm) of the kidney and liver samples were stained with hematoxylin and eosin for histological analysis. Photomicrographs (400× magnification) of the stained tissues were evaluated for steatosis and tissue damage using an Olympus BX53M optical microscope (Olympus Corp., Tokyo, Japan) and a 40× objective lens.

Statistical analysis

Data variables were expressed as the mean ± standard deviation and were analyzed using GraphPad Prism 7.0 software (GraphPad Software, Inc., San Diego, CA, USA). Comparisons between P-NC and P-ITFs groups, or F-NC and F-ITFs groups were performed using one-way analysis of variance and the Student's *t*-test. A value of $P<0.05$ was considered statistically significant.

RESULTS

Structural characteristics of burdock ITFs

^{1}H, ^{13}C, and DEPT-135 NMR spectra were recorded to confirm the structure of ITFs. In the ^{1}H NMR spectrum (Fig. 2A), the peaks in the region of δ 4.7–5.5 ppm are attributed to the *α*-glycosidical configuration anomeric carbon hydrogen signals, and the *β*-glycosidical configuration anomeric carbon hydrogen signals usually appear in the δ 4.4–4.8 ppm region. In the anomeric region, the weak peaks at δ 5.33 ppm (d, *J*=3.6 Hz) were assigned to the H1 signal of *α*-D-Glc*p* (1→. The strong signals in the region of δ 3.20–4.30 ppm were sugar-ring-proton peaks. The peaks at δ 3.82 ppm (d, *J*=10.8 Hz), δ 3.60 ppm (d, *J*=10.8 Hz), δ 4.16 ppm (d, *J*=9.0 Hz), δ 3.99 ppm (t, *J*=8.4 Hz), and δ 3.65 ppm (q, *J*=6 Hz) were characteristic H1a, H1b, H3, H4, and H6 signals of D-Fru*f*, respectively. The H5 and H6a signals of the fructosyl hydrogen were assigned between δ 3.73–3.76 region. The weak signals in a similar region are attributed to the *α*-D-Glc*p* (1→. Based on these reports, the chain length of burdock ITFs was estimated at 14 according to the integration ratio of the H-3 signal of Fru*f* and the H-1 signal of Glc*p* from the ^{1}H NMR spectrum, in accordance with literatures [28, 31]. In the ^{13}C NMR spectrum (Fig. 2B), the *α*-glycosidical configuration anomeric carbon chemical shift ranged from δ 95–101 ppm, while the *β*-glycosidical bond anomeric carbon chemical shift was in the region of δ 101–112 ppm. Six predominant carbon signals were assigned to the carbon chemical shifts of *β*-D-Fru*f*. The strong peak at δ 103.14 ppm corresponds to the C2 signal of (2,1)-*β*-D-Fru*f*. The methylene group (CH_2) peaked at δ 60.79 and 62.07 ppm for C1 and C6 of (2,1)-*β*-D-Fru*f*, respectively. The C-H group peaks at δ 74.12, 76.93, and 81.02 ppm were assigned to C4, C3, and C5 of (2,1)-*β*-D-Fru*f*, respectively. The weak peak at δ 92.40 ppm in the anomeric carbon region is the C1 signal of *α*-D-Glc*p* (1→ residue. These were confirmed by the DEPT 135 experiment (Fig. 2C). The quaternary carbon had no peak, the tertiary carbon (CH) had a positive peak, and the secondary carbon (CH_2) had an inverted peak.

Effects of burdock ITFs supplementation on behavior and weight

The P-ITFs and F-ITFs groups received free access to a normal chow diet and burdock ITFs drinking water (2% w/v) throughout the study. The P-NC and F-NC groups were fed a normal chow diet and double distilled water. No deaths were observed during the study. The animals in the ITFs groups were more active. When handled by their tails, the male mice were more likely to bite the handler. The increased aggression was observed during the experiment. No abnormalities were observed in the general state of the mice. It was concluded that ITFs did not affect the general health of the mice. A slight increase in the average feed intake of the ITFs groups was observed. After six months, all mice gained weight and length, and the weights and lengths were similar among the groups as shown in Fig. 3A and 3B. A slight decrease in abdominal fat was observed in the P-ITFs and F-ITFs groups compared to the P-NC and F-NC groups, respectively (Fig. 3C).

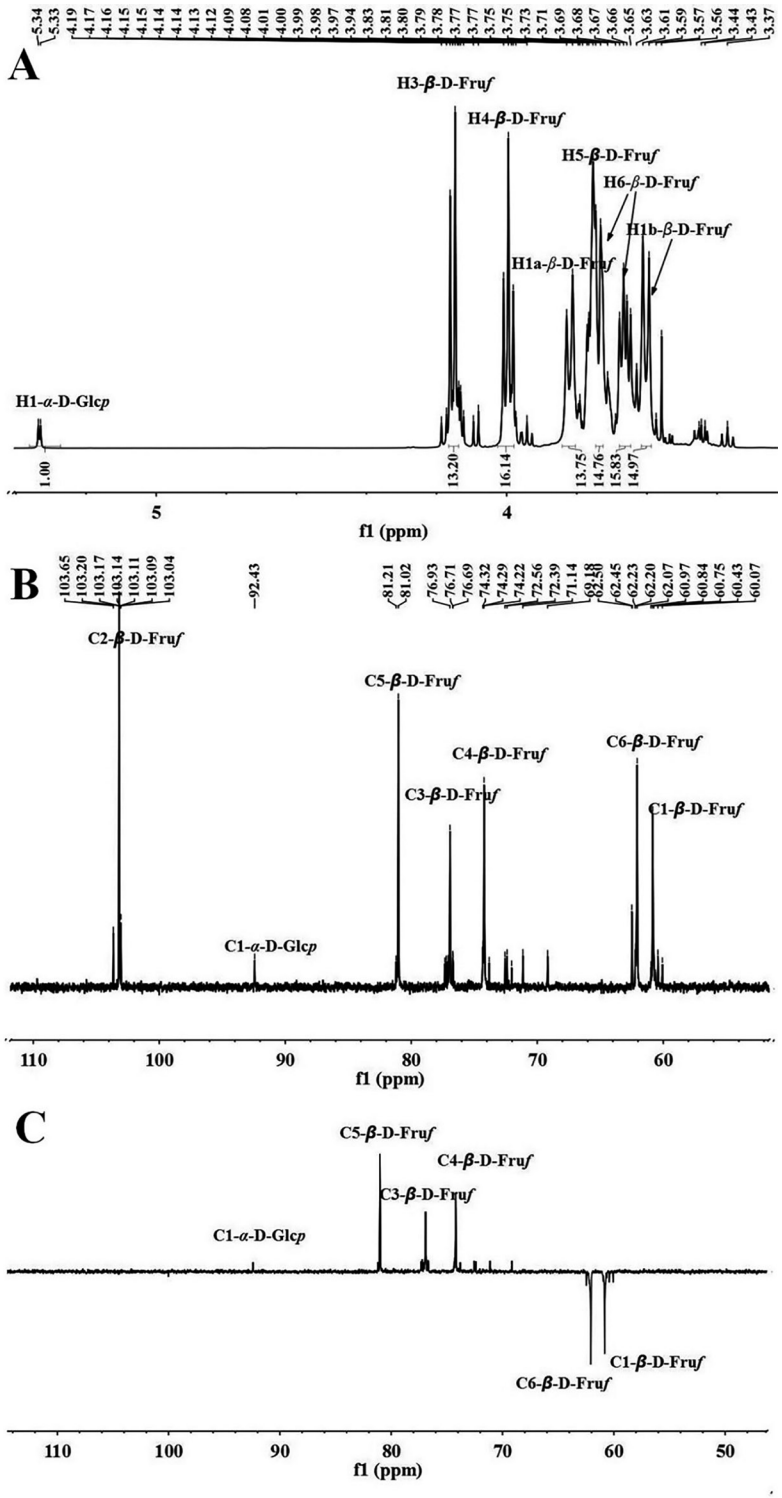

Fig. 2. Nuclear magnetic resonance (NMR) spectra of burdock inulin-type fructans (ITFs). (**A**) ^{1}H, (**B**) ^{13}C, and (**C**) Distortionless Enhancement by Polarization Transfer 135 (DEPT-135).

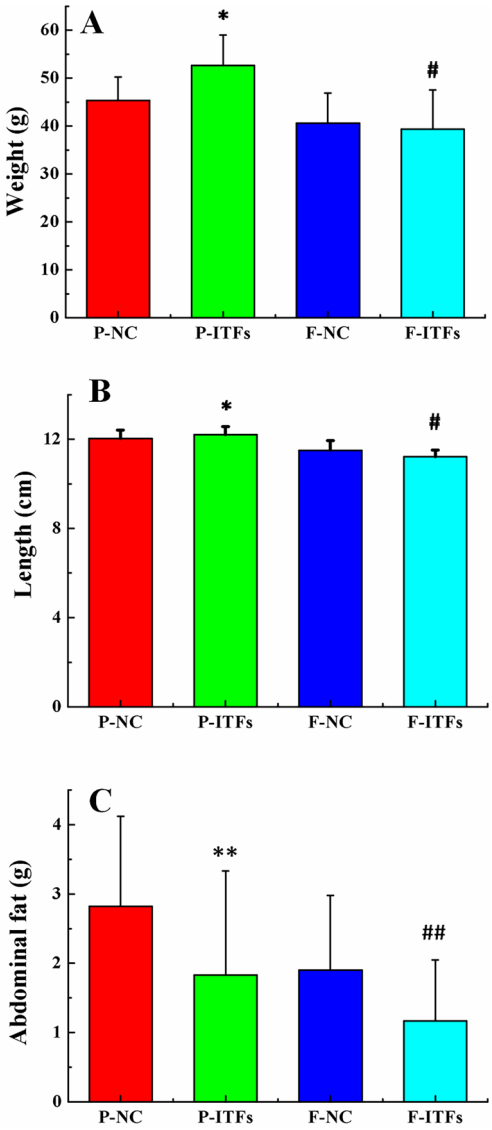

Fig. 3. Terminal body weight (**A**), Length (**B**), and abdominal fat (**C**) of the normal control and experimental groups and their filial generations. *P<0.05, **P<0.01, compared to the P-NC group; $^{\#}P$<0.05, $^{\#\#}P$<0.01, compared to the F-NC group. P-NC: parental normal control mice; P-ITFs: parental burdock inulin-type fructans supplemented mice; F-NC: filial normal control mice; F-ITFs: filial burdock inulin-type fructans supplemented mice.

Effects of burdock ITFs supplement on organ weight

No significant differences were observed between the normal control and experimental groups with respect to their filial generations, as illustrated by the organ/body weight ratios (Table 1). However, the P-ITFs and F-ITFs groups showed a trend of slight increase in the spleen, lung, heart, and pancreas when compared to the P-NC and F-NC groups, respectively (Fig. 4).

Table 1. Organ/body weight ratios of the normal control and experimental groups with their filial generations

Parameter	P-NC group	P-ITFs group	F-NC group	F-ITFs group
Liver (mg/g)	53.77 ± 7.95	56.69 ± 13.81*	57.66 ± 9.90	51.97 ± 8.04$^{\#}$
Spleen (mg/g)	2.97 ± 1.96	3.18 ± 1.58*	2.77 ± 1.26	3.45 ± 1.69$^{\#}$
Left kidney (mg/g)	9.36 ± 1.21	10.44 ± 1.64	10.12 ± 2.29	8.90 ± 2.22
Right kidney (mg/g)	9.49 ± 1.64	10.61 ± 1.52	11.19 ± 2.69	9.09 ± 2.84
Lung (mg/g)	7.28 ± 2.74	8.32 ± 2.34*	7.12 ± 1.86	7.65 ± 1.80$^{\#}$
Heart (mg/g)	4.83 ± 0.39	5.36 ± 0.42**	5.11 ± 0.50	5.43 ± 0.80$^{\#\#}$
Brain (mg/g)	13.90 ± 1.63	11.96 ± 1.35*	14.66 ± 1.79	15.53 ± 2.62$^{\#}$
Pancreas (mg/g)	13.87 ± 6.40	16.52 ± 2.56**	9.87 ± 3.46	13.98 ± 3.95$^{\#\#}$
Mesenterium (mg/g)	28.29 ± 9.95	19.56 ± 6.79	13.16 ± 7.42	17.57 ± 15.90
Colon (cm)	9.69 ± 1.68	11.33 ± 1.78	10.93 ± 0.68	7.81 ± 1.12

Mean ± SD, N=6. *P<0.05, **P<0.01, compared with P-NC group. $^{\#}P$<0.05, $^{\#\#}P$<0.01, compared with the F-NC group. P-NC: parental normal control mice; P-ITFs: parental burdock inulin-type fructans supplemented mice; F-NC: filial normal control mice; F-ITFs: filial burdock inulin-type fructans supplemented mice.

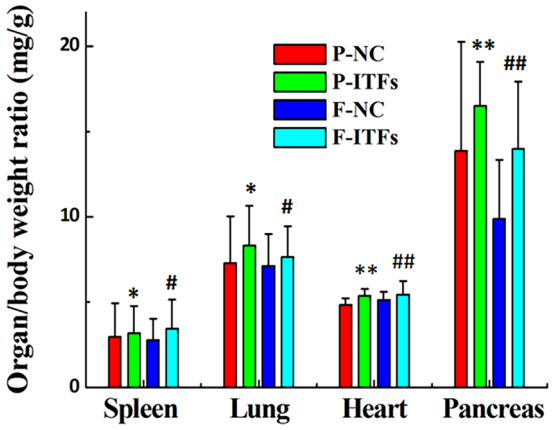

Fig. 4. Organ/body weight ratios of normal control and experimental groups and their filial generations. *P<0.05, **P<0.01, compared to the P-NC group; $^{\#}P$<0.05, $^{\#\#}P$<0.01, compared to the F-NC group. P-NC: parental normal control mice; P-ITFs: parental burdock inulin-type fructans supplemented mice; F-NC: filial normal control mice; F-ITFs: filial burdock inulin-type fructans supplemented mice.

Effects of burdock ITFs supplementation on serum indexes

The serum biochemical indices are shown in Table 2. No significant differences were observed in serum parameters. Most serum indices for the P-ITFs and F-ITFs groups were similar with those of the P-NC and F-NC groups. As shown in Fig. 5, we observed a trend of decrease in serum GLU, TC, UA, and CK levels in the P-ITFs and F-ITFs groups compared to those in the control groups.

Effects of burdock ITFs supplementation on morphology of liver and kidney

After a six-month exposure to burdock ITFs, macroscopic analysis of the liver and kidney were carried out (Fig. 6). The liver cells of all groups presented a normal appearance, size, color, and consistency without vacuoles. The portal triad showed a venule (represented by the acronym, V), bile duct (indicated by a red arrow), and sinusoid capillaries (indicated by a green arrow). Round hepatocyte nuclei (stained blue) were distinguished in the liver tissue. There was no hepatocyte cytoplasmic vacuolization, condensed nuclear chromatin, or sinusoidal widening damage (Fig. 6B1 and 6D1). Normal convoluted proximal and distal tubules (represented by the acronyms, PT and DT, respectively) with cellular debris in the lumen, blood vessels (indicated by a blue arrow), and renal bodies (represented by the acronym, RB) were observed in the kidney tissues of all groups. Kidney cells were arranged closely and were uniform in size and shape without loosening, edema, vacuoles, and necrosis (Fig. 6B2 and 6D2). Morphologically, the analyzed liver and kidney did not show any alterations in the P-ITFs and F- ITFs groups as compared to both P-NC and F-NC groups, respectively. These confirmed that the consumption of burdock ITFs caused no toxicity to the mouse liver and kidney.

Effects of burdock ITFs supplementation on development and reproduction

The total number of pregnancies and pup birth number of the P-ITFs group were higher than those of the P-NC group (Fig. 7).

DISCUSSION

Burdock (*Arctium lappa*) belongs to the *Asteraceae* family, known as the sources of prebiotic ITFs. The most prominent nutrients in burdock root are ITFs [27]. The burdock ITFs are linear chains of fructose linked together by *β*-D-(2→1) bonds with a glucose bound to the free end of the chain by an *α*-D-(1→2) bond. Its molecular size is represented by the degree of polymerization, DPn, where n represents the number of fructose molecules. The average n of burdock ITFs is 12–14 [27]. These chemical shifts we observed were characteristic of a typical fruit residue according to previous reports [39]. All chemical shifts observed were uniform to the structural characteristics of ITFs, *α*-D-Glc*p* (1→2)-*β*-D-Fru*f* $_{n-1}$- (1→2)- *β*-D- Fru*f* [28], as shown in Fig. 8.

According to the literature, prebiotic fiber ITFs can improve diet-induced obesity by regulating antimicrobial peptides [3, 27]. We observed a decrease in abdominal fat in ITFs-supplemented mice. This suggests that the administration of burdock ITFs could prevent the occurrence of obesity due to its low caloric value (approximately 1.5 kcal/g) and a decrease in the intake of carbohydrates and lipids due to dietary ITFs content [19]. In addition, some studies have shown that the inhibition of lipogenesis and stimulation of

Table 2. Serum biochemical values of the normal control and experimental groups with their filial generations

Parameter	P-NC group	P-ITFs group	F-NC group	F-ITFs group
TP (g/L)	58.47 ± 5.47	59.22 ± 5.70*	58.83 ± 2.05	52.74 ± 5.60$^{\#}$
ALB (g/L)	19.17 ± 3.67	19.7 ± 4.54*	20.27 ± 1.27	20.09 ± 2.01$^{\#}$
GLOB (g/L)	39.3 ± 4.35	39.52 ± 4.67*	38.57 ± 1.31	32.66 ± 4.29
ALB/GLOB	0.49 ± 0.10	0.51 ± 0.14	0.53 ± 0.03	0.62 ± 0.07
TBIL (μmol/L)	1.01 ± 0.55	1.11 ± 0.92*	1.50 ± 0.76	0.72 ± 0.21$^{\#\#}$
DBIL (μmol/L)	0.56 ± 0.27	0.53 ± 0.45	0.63 ± 0.25	0.51 ± 0.21
ALT (U/L)	66.33 ± 68.13	34.5 ± 14.73	38 ± 4	33.71 ± 10.56
AST (U/L)	190.5 ± 137.31	114.67 ± 31.69	127 ± 21.70	122.71 ± 69.46
AST/ALT	3.57 ± 1.52	3.65 ± 1.37	3.36 ± 0.69	3.97 ± 2.53
GGT (U/L)	2.33 ± 0.82	2 ± 1.26	3.33 ± 1.15	2.14 ± 0.69
ALP (U/L)	60.5 ± 21.37	51.33 ± 19.39**	84 ± 31.32	82 ± 21.77$^{\#}$
Bun (mmol/L)	6.54 ± 2.14	8.03 ± 1.73	7.54 ± 2.81	7.41 ± 1.59
Cr (μmol/L)	5.88 ± 0.73	5.57 ± 0.63	6.5 ± 0.2	6.56 ± 1.46
UA (μmol/L)	297.65 ± 126.17	281.87 ± 98.19*	287.27 ± 11.85	202 ± 43.09$^{\#}$
GLU (mmol/L)	5.51 ± 2.05	4.51 ± 1.82*	4.90 ± 0.82	4.11 ± 0.62$^{\#}$
TC (mmol/L)	2.49 ± 0.83	2.26 ± 0.32**	2.62 ± 1.14	1.88 ± 0.41$^{\#\#}$
TG (mmol/L)	0.56 ± 0.20	0.62 ± 0.32	1.05 ± 0.61	0.71 ± 0.57
HDL (mmol/L)	1.76 ± 0.46	1.75 ± 0.19	2.00 ± 0.98	1.40 ± 0.24
LDL (mmol/L)	0.41 ± 0.27	0.23 ± 0.10	0.28 ± 0.09	0.29 ± 0.17
CK (U/L)	1,295.83 ± 479.24	754 ± 257.99*	1,106.67 ± 132.08	754 ± 163.83$^{\#\#}$
LDH (U/L)	1,378.33 ± 550.51	1,051 ± 502.08**	1,202.67 ± 482.15	1,062.71 ± 155.78$^{\#}$
AMY (U/L)	3,135.5 ± 1,580.72	3,195 ± 739.72*	2,544.33 ± 243.10	3,357 ± 1,411.34$^{\#\#}$
Ca (mmol/L)	2.19 ± 0.24	2.18 ± 0.19	2.24 ± 0.04	2.14 ± 0.08
P (mmol/L)	2.12 ± 0.38	2.16 ± 0.46	2.54 ± 0.42	2.16 ± 0.23$^{\#\#}$
Mg (mmol/L)	1.24 ± 0.13	1.18 ± 0.10	1.29 ± 0.15	1.13 ± 0.10

Mean ± SD, N=6. *P<0.05, **P<0.01, compared with P-NC group. $^{\#}P$<0.05, $^{\#\#}P$<0.01, compared with the F-NC group. P-NC: parental normal control mice; P-ITFs: parental burdock inulin-type fructans supplemented mice; F-NC: filial normal control mice; F-ITFs: filial burdock inulin-type fructans supplemented mice; total protein: TP; albumin: ALB; globulin: GLOB; total bilirubin: TBIL; direct bilirubi: DBIL; alanine aminotransferase: ALT; aspartate aminotransferase: AST; gamma glutamyl transpeptidase: GGT; alkaline phosphatase: ALP; urea nitrogen: Bun; creatinin: Cr; uric acid: UA; glucose: GLU; total cholesterol: TC; triglycerides: TG; high density lipoprotein: HDL; low density lipoprotein: LDL; creatine kinase: CK; lactate dehydrogenase: LDH; amylase: AMY; calcium: Ca; phosphorus: P; magnesium: Mg.

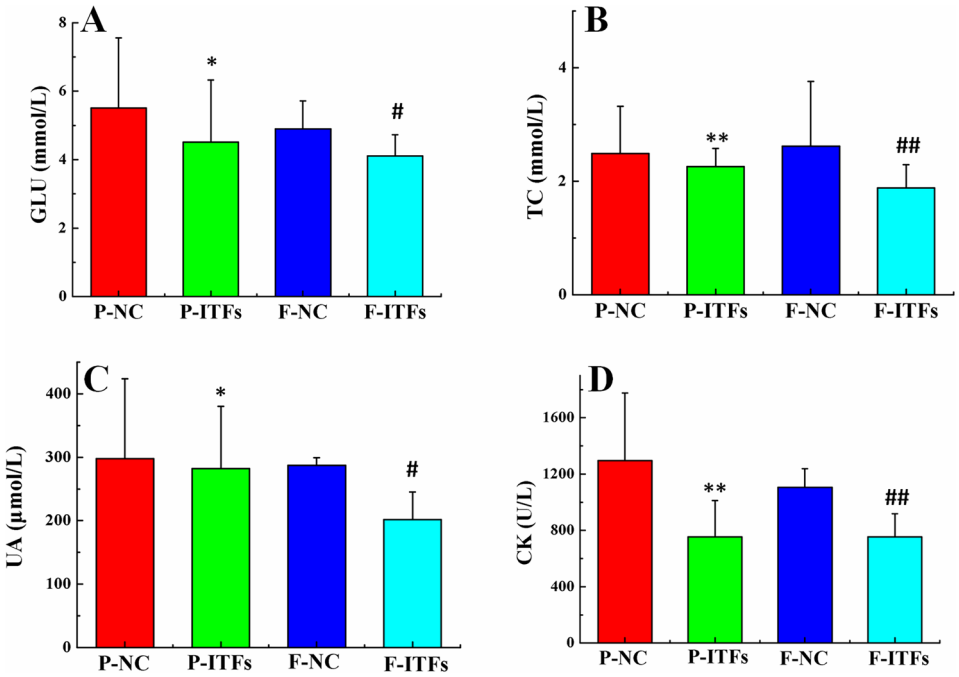

Fig. 5. Levels of serum glucose (GLU) (**A**), total cholesterol (TC) (**B**), uric acid (UA) (**C**), and creatine kinase (CK) (**D**) of normal control and experimental groups and their filial generations. *P<0.05, **P<0.01, compared to the P-NC group; $^{\#}P$<0.05, $^{\#\#}P$<0.01, compared to the F-NC group. P-NC: parental normal control mice; P-ITFs: parental burdock inulin-type fructans supplemented mice; F-NC: filial normal control mice; F-ITFs: filial burdock inulin-type fructans supplemented mice.

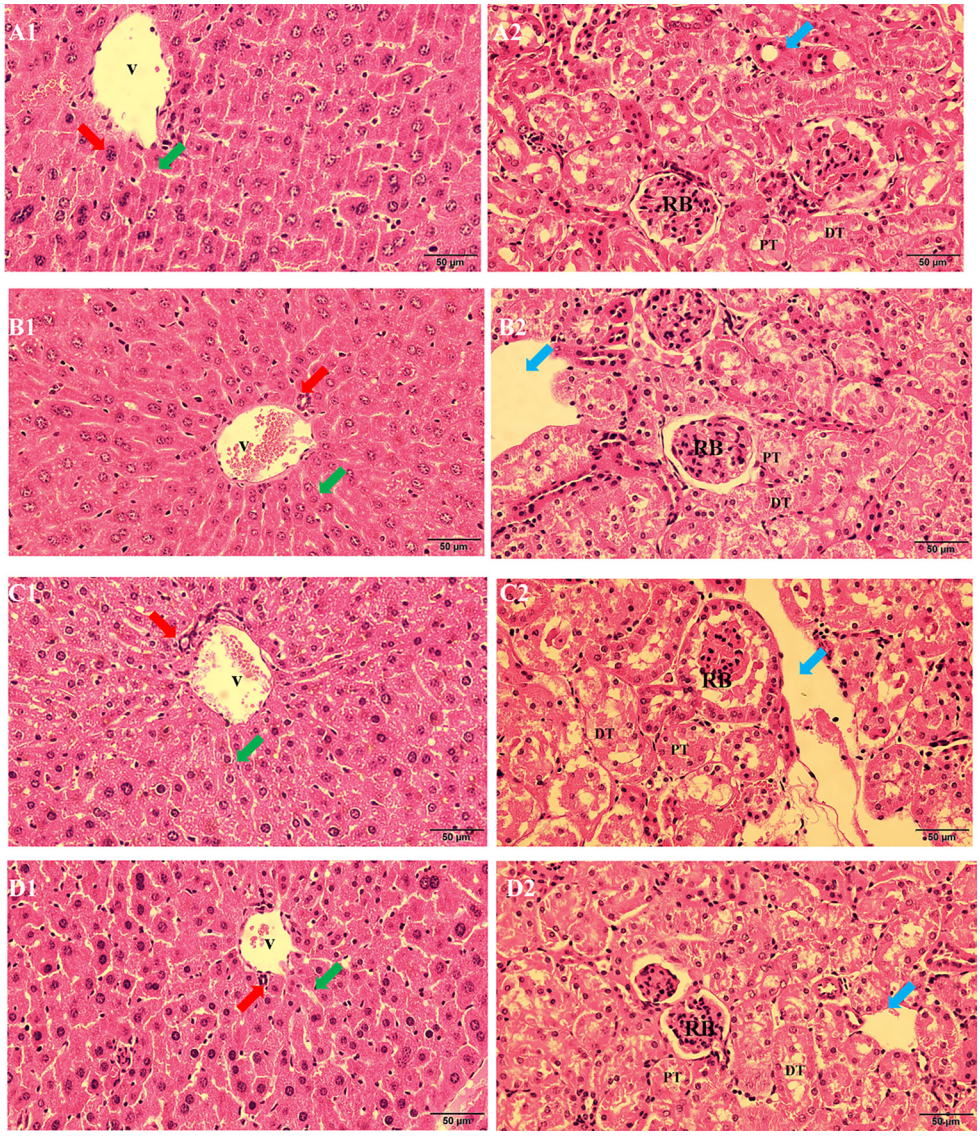

Fig. 6. Hematoxylin and eosin staining (×400) of the liver and kidney tissues of C57BL/6J mice (bar=50 μm). Liver (1) and kidney (2) tissues within the P-NC (**A**), P-ITFs (**B**), F-NC (**C**), and F-ITFs (**D**) groups. Bile ducts are indicated by red arrows, sinusoid capillaries are indicated by green arrows, and blood vessels are indicated by a blue arrow. V: venule; PT and DT: proximal and distal tubules; RB: renal bodies; P-NC: parental normal control mice; P-ITFs: parental burdock inulin-type fructans supplemented mice; F-NC: filial normal control mice; F-ITFs: filial burdock inulin-type fructans supplemented mice.

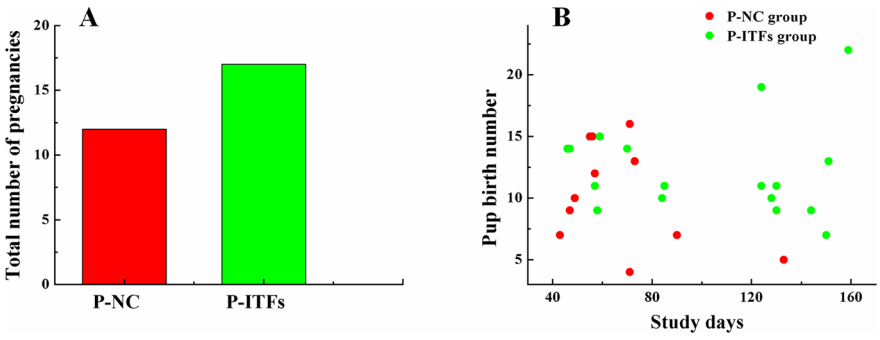

Fig. 7. Total number of pregnancies (**A**) and pup birth number (**B**) of the parental normal control and experimental groups in a six-month exposure study. P-NC: parental normal control mice; P-ITFs: parental burdock inulin-type fructans supplemented mice.

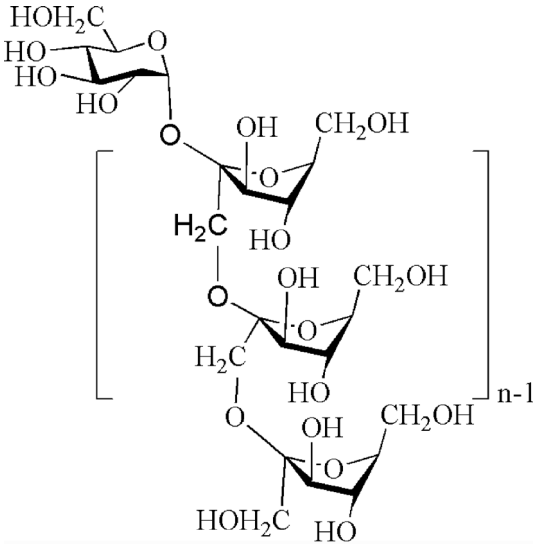

Fig. 8. Chemical structure of burdock inulin-type fructans (ITFs).

catabolism are the crucial cause for obesity reduction after administration of burdock extracts [8].

While we observed no physical differences in organ sizes when comparing parental and filial mice receiving the same treatment, ITFs-supplemented mice had increased spleen, lung, heart, and pancreas sizes when compared with controls of the same generation. In agreement with literature, these results reveal the contribution of burdock ITFs to body health [12, 26, 27].

Generally, the long-term consumption of burdock ITFs has a positive health effect on the body. Burdock ITFs have been shown reduce postprandial blood GLU and TC levels, endowing hypoglycemic and hypolipidemic activities as the result of the SCFAs [5, 27, 36, 40]. The results presented here suggest that burdock ITFs could reduce serum UA levels in mice originating from UA degradation by the intestinal microbiota [15]. Burdock ITFs may have a protective effect on renal function and lower the risk of renal disease, although further studies are needed to confirm this. Further, in mice, burdock ITFs decreased levels of CK, a muscle injury biomarker, showing a potential effect of burdock ITFs on preventing fatigue and elevating exercise performance [10].

Burdock ITFs exhibited positive effect on developmental and reproductive capacity of parental mice. According to previous reports, burdock ITFs enhance the body's immune system, and improve the intestinal environment and body health [26]. Based on previously published literature, maternal intestinal microbiota can influence the bacterial colonization and intestinal properties of newborns and infants directly. Therefore, the maternal intake of prebiotics and dietary fiber during pregnancy and lactation can improve the health of both the mother and the offspring [24].

Here, we evaluated the effects of burdock ITFs on the physiological function indices of healthy mice and their filial generation. As expected, the results of serum biochemical parameters and liver and kidney pathological morphology revealed that no adverse effects on the development of the parental mice or their offspring in a six-month *in vivo* study. Additionally, our results suggest potential roles for ITFs in improving reproductive parameters, preventing renal disease, and aiding in combating fatigue. These positive results provide useful data for deeper research and development of burdock ITFs as functional foods and medicines. Further biochemical and pharmacological studies are needed to support this hypothesis.

CONFLICT OF INTEREST. The authors have no conflicts of interest.

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