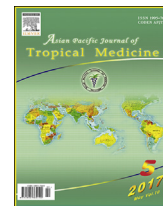




Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: <http://ees.elsevier.com/apjtm>Original research <http://dx.doi.org/10.1016/j.apjtm.2017.03.028>***Helianthus tuberosus* (Jerusalem artichoke) tubers improve glucose tolerance and hepatic lipid profile in rats fed a high-fat diet**Naoto Okada^{1,2,✉}, Shinya Kobayashi³, Kouta Moriyama³, Kohsuke Miyataka⁴, Shinji Abe¹, Chiemi Sato¹, Kazuyoshi Kawazoe¹¹Department of Clinical Pharmacy Practice Pedagogy, Institute of Biomedical Sciences, Tokushima University Graduate School, 1-78-1 Sho-machi, Tokushima, 770-8505, Japan²Department of Pharmacy, Tokushima University Hospital, 2-50-1 Kuramoto, Tokushima, 770-8503, Japan³Faculty of Pharmaceutical Sciences, Tokushima University, Shomachi-1, Tokushima, 770-8505, Japan⁴Faculty of Medicine, Tokushima University, 3-18-15 Kuramoto, Tokushima, 770-8503, Japan

ARTICLE INFO

Article history:

Received 16 Jan 2017

Received in revised form 15 Feb 2017

Accepted 14 Mar 2017

Available online 18 May 2017

Keywords:

Helianthus tuberosus

Glucose tolerance

Hepatic lipid profile

ABSTRACT

Objectives: To analyze the effects of feeding *Helianthus tuberosus* (HT) tubers on glucose tolerance and lipid profile in rats fed a high-fat diet (HFD).**Methods:** A normal HFD or HFD including 10 w/w% HT tubers (HFD + HT) was fed to F334/Jcl rats. After 10 weeks, organ weights, glucose tolerance, and lipid profile were analyzed.**Results:** The body weight, liver weight, and epidermal fat content in the HFD group were higher than those of the normal group, and similar to those of the HFD + HT group. The oral glucose tolerance test at 10 weeks revealed that the blood glucose level 30 min after beginning the test in the HFD + HT group was significantly lower than that in the HFD group. Liver triglyceride and total cholesterol levels in the HFD + HT group were significantly lower than those in the HFD group. Fecal triglyceride and total cholesterol levels in the HFD + HT group were higher than those in the HFD group. Histological analyses revealed that fat and glycogen accumulation increased in the HFD group, but decreased in the HFD + HT group.**Conclusions:** These results indicate that HT tubers have anti-fatty liver effects based on improvements in glucose tolerance and the hepatic lipid profile.**1. Introduction**

Non-alcoholic fatty liver disease (NAFLD) is a recognized condition that may progress to end-stage liver disease [1]. Previous studies have shown that 10%–40% of patients with NAFLD develop non-alcoholic steatohepatitis (NASH) [2]. Because NAFLD and NASH are risk factors for liver cancer, preventing their progression is important [3]. The progression of NAFLD and NASH is associated with type 2 diabetes mellitus and hyperlipidemia [4,5]. Therefore, preventing hyperlipidemia and changes in glucose tolerance is necessary to limit the progression of NAFLD and NASH.

Hyperlipidemia and type 2 diabetes mellitus are induced by excess intake of high-calorie and high-fat diets [6,7]. One way to prevent the progression of hyperlipidemia and type 2 diabetes mellitus is to refrain from ingesting such diets. However, maintaining a restricted diet is difficult for many people, and compliance with this restriction is poor. Alternatively, hyperlipidemia and type 2 diabetes mellitus can be prevented or treated with drugs or by consuming functional foods that have anti-hyperlipidemia or anti-type 2 diabetes mellitus effects. Of these options, functional foods have high safety and can be used as part of a normal diet. Therefore, functional foods that improve hyperlipidemia and glucose tolerance may be useful candidates for preventing the progression of NAFLD and NASH. However, such functional foods have not been reported.

Helianthus tuberosus (HT, Jerusalem artichoke) is a perennial plant whose tubers are used as a food worldwide; it grows well in the semi-arid tropics. HT tubers contain inulin, a non-degradable oligosaccharide polymer comprising 30–35 fructose units that are linearly arranged in β -1,2 linkages with an α -1,2-linked D-

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Peer review under responsibility of Hainan Medical University.

glucose at the non-reducing end [8–10]. Previous reports indicated that inulin improved glucose tolerance and liver lipid profile in rats fed a high-fat diet (HFD) [11,12]. Based on this background information, we hypothesized that feeding HT tubers might improve the hyperglycemia or hyperlipidemia induced by an HFD, suggesting their potential as a useful functional food for preventing the progression of NAFLD and NASH. In the current study, rats fed an HFD were used to assess the effect of feeding HT tubers on glucose tolerance and the hepatic lipid profile.

2. Materials and methods

2.1. Animals and diets

Four-week-old F344/Jcl rats were purchased from CLEA (Tokyo, Japan). The rats were acclimated for one week before use. The animals were cared for according to approved procedures, and the experiments received institutional animal approval (approval number: 13137). Normal diets and HFDs were purchased from the Oriental Yeast Company (Tokyo, Japan). Components of the HFD were milk casein (256 g/kg), L-cystine (3.6 g/kg), maltodextrin (60 g/kg), α -cornstarch (160 g/kg), sucrose (55 g/kg), soybean oil (20 g/kg), lard (330 g/kg), cellulose (66.1 g/kg), calcium carbonate (1.8 g/kg), and choline bitartrate (2.5 g/kg). HT tuber powder was provided by the Tokushima Prefectural Government. HT tuber components were carbohydrates (325 g/kg), inulin (452 g/kg), protein (92 g/kg), ash (68 g/kg), water (54 g/kg), and fat (9 g/kg). The HFD + HT diet was prepared by mixing the HFD diet with HT tuber powder. The final concentration of HT powder was 10% (w/w). The basic composition of each diet is as follows, normal diet; carbohydrate: 553 g/kg, protein: 231 g/kg, fat: 51 g/kg, HFD diet; carbohydrate: 248 g/kg, protein: 230 g/kg, fat: 350 g/kg, HFD + HT diet; carbohydrate: 256 g/kg, protein: 216 g/kg, fat: 316 g/kg, inulin: 45 g/kg. Rats ingested each diet and water freely. Eight rats were included in each group.

2.2. Measurements of body and organ tissue weights

Body weight in each group was measured weekly for 10 weeks. After 10 weeks, urethane (2.5 g/kg) was administered intraperitoneally and liver and epididymal fat were sampled and weighed. These samples were stored at -80°C before use. Slices of the liver were preserved in formalin for histological analyses. Caloric intake was calculated by measuring the total feed consumed by each group.

2.3. Plasma glucose level measurements and oral glucose tolerance test (OGTT)

After 10 weeks, blood glucose was measured in each group of rats using blood drawn from the tail vein after 16 h of fasting. The OGTT was performed by administering 1 g/kg glucose to each rat and harvesting blood samples from the tail vein intermittently. After centrifugation ($2000 \times g$, 10 min), blood glucose was measured in the serum. The blood glucose measurement kit was purchased from Sanwa Kagaku (Aichi, Japan). The measurements were performed according to the manual provided by the manufacturer.

2.4. Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) measurement

Blood samples used for fasting glucose measurements were also used for measurement of serum AST and ALT levels. AST color endpoint assay kit and ALT color endpoint assay kit were purchased from Empire Genomics (Buffalo, NY, USA). Measurements were performed according to the manual provided by the manufacturer.

2.5. Lipid profile analyses

Blood samples used for fasting glucose measurements were also used for measurement of serum triglyceride (TG) and total cholesterol (TC) levels. Total lipids of liver tissue and feces between 8 and 9 weeks of feeding were extracted by the Folch method [13]. Briefly, liver tissue and feces were homogenized in phosphate buffer. Folch solution (chloroform/methanol: 2/1) was added to this lysate and vortexed. These samples were centrifuged at $1500 \times g$, for 15 min and the lower layer was collected. Sodium chloride solution (0.5%) was added and centrifuged at $1500 \times g$ for 15 min. The lower layer was collected and concentrated using a rotation evaporator. The obtained lipids were dissolved in 2-propanol and used for measurement of TC and TG. TC and TG measurement kits were purchased from Wako (Osaka, Japan). Measurements were performed according to the manual provided by the manufacturer.

2.6. Histological analyses

Liver tissues were embedded in paraffin and cut into sections ($4 \mu\text{m}$ thick). Sections were stained with hematoxylin and eosin (H&E) or 1% periodic acid-Schiff's (PAS) solution as described previously [14].

2.7. Statistical analyses

Results are shown as the mean \pm SD. Data were subjected to a one-way analysis of variance and differences between groups were determined using the Holm's sequential Bonferroni procedure. All analyses were performed using Microsoft Excel (Redford, WA, USA). All P -values were two-sided. Differences with $P < 0.05$ were considered significant.

3. Results

3.1. Changes in body and organ tissue weights

The average body weight of rats in the HFD group was significantly higher than that in the normal diet group from 3 to 10 weeks, but the same as that in the HFD + HT group throughout the experiment (Figure 1). Caloric intake was identical between the HFD and HFD + HT groups (Table 1). The liver and epididymal fat weights of rats in the HFD group were higher than those of the normal group rats, but identical to those of the HFD + HT group rats (Table 1). These data indicated that intake of HT tubers did not affect the changes in body and organ tissue weights induced by the HFD.

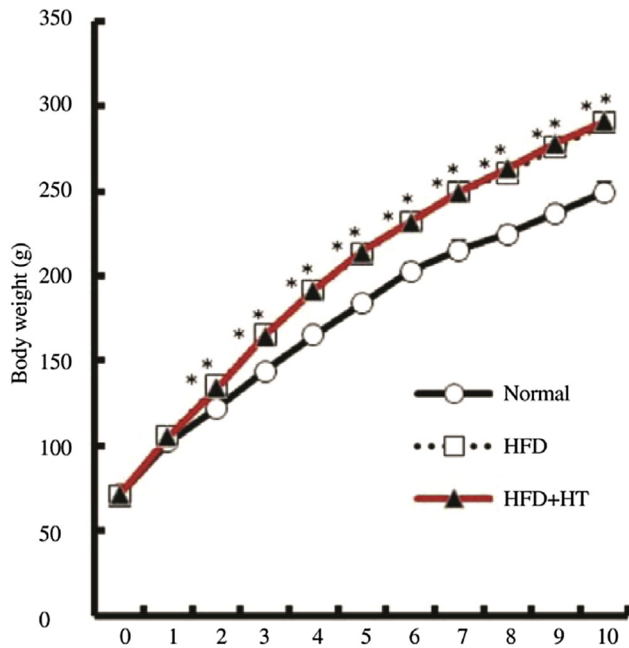


Figure 1. Changes of body weights in each dietary treatment group. Body weights of rats fed a normal diet (○), HFD (□), or HFD + HT (▲). * $P < 0.05$ compared with control. Data are expressed as means \pm SD. $n = 8$. HFD: high-fat diet, HT: *Heliantus tuberosus* tubers.

3.2. Effect on glucose tolerance and plasma AST and ALT level

The fasting blood glucose level of rats in the HFD group was higher at 10 weeks than that of the normal group. Importantly, the fasting blood glucose level of rats in the HFD + HT group decreased compared to that in the HFD group (Table 1). The OGTT showed a significant but transient increase of blood glucose in the HFD group compared to that in the normal group (Figure 2). In contrast, the blood glucose level 30 min after the OGTT in the HFD + HT group decreased significantly compared to that in the HFD group. These data indicate that intake of HT tubers inhibited the transient increase in blood glucose induced by an acute glucose load. However, the plasma AST and ALT levels at 10 weeks were not different among the groups (Table 1).

3.3. Effect on lipid profiles in blood, liver, and faeces

Blood TG and TC levels were similar among the three treatment groups (Table 1). However, liver TG and TC levels in the HFD group increased compared to those in the normal diet group. The liver TG level of the HFD + HT group decreased significantly compared to that in the HFD group. Fecal TG and TC levels in the HFD group significantly increased compared to those in the normal group, and the levels in the HFD + HT group significantly increased compared to those in the HFD group. These data indicate that the intake of HT tubers decreased liver TG and TC content and significantly increased the content of fecal TG and TC.

3.4. Histopathological analyses of hepatocytes

H&E and PAS-stained hepatic sections are shown in Figure 3. Accumulation of fat and glycogen was observed in the

Table 1

Physiological parameters in each dietary treatment group, Effect of HT tubers on blood glucose, AST and ALT level, and Lipid profiles in each dietary group.

Group	Plasma (mg/dL)		Liver (mg/g liver)		Faeces (mg/g faeces)		Total intake calorie (kcal)	Liver weight(g)	Epidymal fat(g)	Glucose (mg/dL)	AST (unit/L)	ALT (unit/L)
	TG	TC	TG	TC	TG	TC						
Normal	108.0 \pm 9.9	57.7 \pm 14.5	9.2 \pm 2.2	0.9 \pm 0.8	1.0 \pm 0.2	1.3 \pm 0.2	3235 \pm 223	6.9 \pm 0.7	4.9 \pm 0.8	71.4 \pm 6.1	47.8 \pm 7.8	288.0 \pm 50.1
HFD	105.0 \pm 3.9	43.9 \pm 15.1	36.1 \pm 5.9 ^a	4.5 \pm 1.7 ^a	1.8 \pm 0.2 ^a	1.5 \pm 0.2	4018 \pm 198 ^a	8.4 \pm 0.6 ^a	9.4 \pm 0.9 ^a	89.5 \pm 15.7 ^a	65.2 \pm 4.9	248.3 \pm 33.7
HFD + HT	105.0 \pm 7.3	49.9 \pm 16.3	23.4 \pm 3.4 ^{ab}	3.2 \pm 1.0 ^a	2.2 \pm 0.2 ^{ab}	3.1 \pm 0.3 ^{ab}	3994 \pm 206 ^a	8.7 \pm 0.5 ^a	9.0 \pm 0.7 ^a	76.2 \pm 12.6	53.7 \pm 21.9	232.0 \pm 35.1

^a $P < 0.05$ compared with control group.

^b $P < 0.05$ compared with HFD group. Data are expressed as means \pm SD. $n = 8$. HFD: high-fat diet, HT: *Heliantus tuberosus* tubers, TG: triglyceride, TC: total cholesterol.

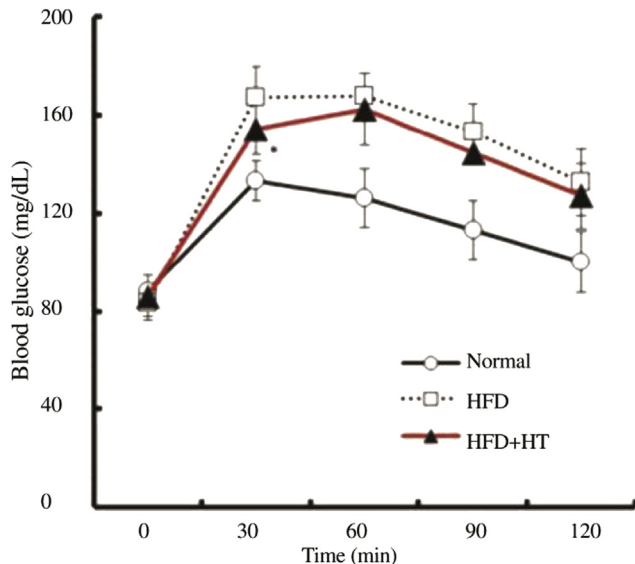


Figure 2. The effect of *Heliantus tuberosus* tubers (HT) on blood glucose levels after OGTT.

Blood glucose levels after OGTT in the normal (○), HFD (□), and HFD + HT (▲) groups. * $P < 0.05$ compared with HFD. Data are expressed as means \pm SD. $n = 8$. HFD: high-fat diet.

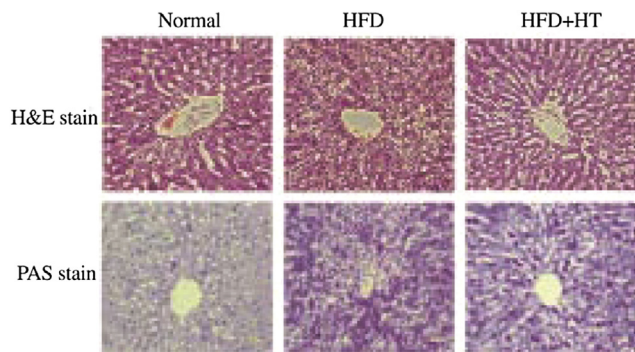


Figure 3. Histopathological analyses of liver sections.

HFD, high-fat diet; HT, *Heliantus tuberosus* tubers; H&E, hematoxylin and eosin; PAS: Periodic acid-Schiff.

liver of rats fed an HFD diet, but not in the liver of rats fed a normal diet. Slight accumulation of fat and glycogen was observed in the livers of HFD + HT rats, although this accumulation was weaker than that in the HFD group. These data indicate that the HFD induced accumulation of fat and glycogen in the liver and that this accumulation was inhibited by intake of HT tubers.

4. Discussion

The results of this study indicate that intake of HT tubers increased the excretion of TG and TC into the feces and decreased TG and TC levels in the liver. Moreover, the OGTT showed that intake of HT tubers improved glucose tolerance. Histological studies indicated that intake of HT tubers inhibited the accumulation of fat and glycogen in the liver.

We speculate that the positive effect of HT tubers on lipid profile and glucose tolerance can be attributed to their inulin content. Inulin is a water-soluble dietary fiber that has anti-metabolic syndrome effects. It can affect serum glucose and lipid levels by delaying gastric emptying or modifying the

secretion of gut hormones such as glucagon-like peptide 1 [12]. Previous studies showed that inulin inhibited absorption of TG and TC via increased excretion of TG and TC to the feces [9–11]. In our study, the amount of TG and TC in the liver of rats in the HFD + HT group was less than that reported for the HFD group. Moreover, the fecal amount of TG and TC in the HFD + HT group was higher than that of the HFD group. These results indicate that the inulin in HT tubers facilitated the excretion of TG and TC to the feces.

In this study, the amount of food intake in the HFD + HT group was approximately (5–10) g/day. This consumption was equivalent to (0.25–0.50) g/day of inulin intake. Because the average body weight of rats in the HFD + HT group during the test was approximately 200 g, the inulin intake was (1.25–2.50) g/kg/day. Based on the conversion formula of the US FDA according to body surface area, the human-equivalent dose of inulin for which this HT effect was reported in our study was approximately (0.2–0.4) g/kg/day (BW; for 60 kg adult body weight; conversion coefficient: 6.2) [15]. Inulin has been reported to cause gastrointestinal problems, and this adverse effect occurred with inulin intake over 30 g/day in a human study [15]. The inulin dose in our study was lower than that at which adverse effects have been reported to develop. Moreover, loose stools were not observed in the HFD + HT group (data not shown). These results indicate that gastrointestinal problems induced by HT intake did not occur in our study. HT tubers are used as food worldwide and their safety is well established. Therefore, we may be able to obtain the effects observed in our rat study in humans using the conventional intake of HT tubers.

The current results indicate that intake of HT tubers inhibited the accumulation of fat and glycogen in the liver. Previous histological studies demonstrated the accumulation of excess fat and glycogen in the liver of NAFLD or NASH patients [16]. Because the progression of NAFLD and NASH is related to the presence of type 2 diabetes mellitus and hyperlipidemia, improvement of the lipid profile and glucose tolerance by feeding HT tubers might inhibit fatty liver changes such as NAFLD or NASH. However, HT intake did not decrease the plasma AST and ALT levels in our study. This indicates that HT suppressed fatty liver degeneration, but may not suppress liver damage. In fact, Zaky *et al.* reported that HT tubers decreased fasting blood glucose level, but did not decrease plasma AST and ALT level in a diabetic rat model [17]. Overall, this is the first report describing the possibility of HT tubers inhibiting the progression of fatty liver disease.

Water-soluble dietary fibers, such as inulin, can modify levels of short-chain fatty acids (SCFAs) that are produced from colonic fermentation of prebiotics and change the gut microbiome [18]. Takemura *et al.* reported that inulin prolongs the survival of probiotics in mice fed an HFD [19]. We speculated that HT may change the gut microbiome in our study; however, the details were unclear. Inulin also promotes the production of SCFAs such as propionate or butyrate [20]. SCFAs have an inhibitory effect on the production of inflammatory cytokines [18]. Because the induction of an inflammatory response is involved in exacerbating aberrant glucose tolerance and fatty liver, the anti-inflammatory effect of SCFAs may also be involved in the suppression of inflammation by HT tubers. A previous study showed that inulin increased SCFA production and improved liver lipid metabolism

[21]. It is necessary to analyze the correlation between changes in the gut microbiome and SCFA content, fatty liver degeneration, and impaired glucose tolerance.

In NAFLD and NASH patients, levels of oxidative stress markers are increased in the liver [22]. Wang *et al.* reported that *Monordica charantia*, which improved the liver lipid profile, decreased oxidative stress in the liver induced by a HFD [23]. Inhibition of fatty liver changes by HT tubers may also decrease oxidative stress in the liver. Expression of fatty acid synthase is associated with accumulation of fat in the liver [24]. Future studies should investigate changes in the expression of genes involved in fatty acid synthesis. Moreover, analyses of the detailed mechanism by which glucose tolerance is improved by HT tubers should also be performed using measurements of serum insulin content or HOMA-R index [25].

A previous study reported that inulin did not inhibit the increase of body weight in rats fed a normal diet [26]. Therefore, we did not assess the effects of HT on weight gain in rats fed a normal diet. Plasma TG and TC levels did not increase in the HFD group. Because weight gain and increased epididymal fat mass were observed in the HFD group, an appropriate HFD load could be determined. Analyses using rats fed an HFD for a prolonged period may be required to determine the effect of HT tubers on plasma TG and TC contents.

In our study, feeding rats an HFD containing HT tubers decreased the TG and TC contents in the liver induced excretion of TG and TC into the feces, improved glucose tolerance, and inhibited the accumulation of fat and glycogen compared to that in rats fed the HFD without HT tubers. Although further analyses are needed to confirm our results, these results indicate that HT tubers have the ability to improve glucose tolerance and hepatic lipid profile, and may be a useful functional food to prevent the progression of fatty liver to NAFLD or NASH.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgment

This study was supported by the Tokushima prefectural government.

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