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Physiological Response to Diets Fortified with Jerusalem Artichoke Tubers (*Helianthus tuberosus* L.) Powder by Diabetic Rats

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Abstract: The present study aimed at evaluating the biological, biochemical and histopathological parameters of diabetic rats fed on Jerusalem artichoke tubers. Liver function, kidney function, total protein, total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) in diabetic rats serum were estimated. The obtained results indicated that the diets fortified with Jerusalem artichoke tuber powder (JAT) at different levels (5, 10 and 15%) improved the body weight gain, feed efficiency ratio of alloxan-injected diabetic rats relative to positive control group. The liver, kidney, heart and spleen weight of rats fed on JAT at the above three mentioned levels had similar mean values and lower levels than these of the rats of the control (+) group. Also, a significant decrease occurred in serum glucose levels for all groups with different levels (5, 10 and 15%) when compared with alloxan-induced diabetic rats. A significant increases were observed in urea and uric acid. However a decrease was found in serum creatinine for rats fed on different diets relative to the diabetic rats. On the other hand diabetic rats had a higher value of ALT, AST activities and total protein than rats fed on basal diet and JAT at different levels (5, 10 and 15%). pronounced decrease occurred in serum total cholesterol, triglycerides, HDL, LDL and VLDL-cholesterols with rats fed on different levels (5, 10 and 15%) of JAT compared with the positive control. However, the histopathological picture of liver, kidney and spleen indicated that addition of Jerusalem artichoke tubers to the diets of alloxan-induced diabetic rats had slight effects on the microscopic structure. Thus diet containing Jerusalem artichoke tubers would reduce serum glucose levels, triglycerides, total cholesterol and LDL-cholesterol in the hyperglycemic rats. Also, the obtained results indicate some improvement in liver and kidney functions as a result of these additives.

Key words: Jerusalem artichoke tuber (JAT) · Diabetic · Hyperglycemic · Lipids profile · Cholesterol

INTRODUCTION

Considerable interest has been generated in Jerusalem artichoke tubers (*Helianthus tuberosus* L.) (JAT), mainly because this crop is an excellent source of both soluble and insoluble fibers. Fructo-oligosaccharides (FOS), the soluble fibers components have been identified as an important substrate for desirable intestinal flora, especially bifido bacteria [1, 2]. Also used for prevention of cancer [3].

In general soluble fibers decrease serum cholesterol and low density lipoprotein (LDL) cholesterol without affecting serum triglycerides. Often consumption of these soluble fibers is accompanied by distinct reductions in serum high density lipoprotein (HDL) cholesterol concentrations. Soluble fibers such as inulin appear to exert their principal effects on cholesterol metabolism through a decrease in bile acid absorption in the small intestine [4].

Jerusalem artichoke tubers (JAT) helps in stabilizing blood sugar level in the human. The effect of JAT as reported by Alegria and Vivanco [5] is due to optimum quantity of the polysaccharide inulin, potentially useful for diabetics.

Inulin is a plant-derived carbohydrate with the benefits of soluble dietary fibers which are not digested or absorbed in the small intestine, but fermented in the colon by beneficial bacteria. Functioning as a prebiotic, inulin has been associated with enhancing the gastrointestinal system and immunity system. In addition, it has been shown that it increases the absorption of calcium and magnesium, influences the formation of blood glucose and reduces the level of cholesterol, serum lipoproteins [6, 7].

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Cieslik and Filipiak-Florkiewicz [8] mentioned that JAT can be used as functional food. Emphasis is given to inulin and its derivative fructo-oligosaccharides from these tubers. Shalaby [9] found that JAT powder contain 85.78% total carbohydrate, 7.4% crude protein, 0.72% ether extract, 9.82% crude fiber and 5.53% ash. Kaur and Gupta [10] and Daubioul *et al.* [11] indicated that inulin-type fructans such as oligofructose (OFS) in the diet decreased triacylglycerol accumulation in the liver tissue and decreased significantly serum aminotransferase and aspartate aminotransferase after 3weeks.

El-Hofi [2] found that Jerusalem artichoke powder (JAP) had higher content of crude proteins, crude fibers and ash. It contained 8.26% crudes protein, 5.42% crude fibers, 6.82% ash, 0.11% crude fats and 73.50% inulin as percentage of dry basis. Also, Cieslik *et al.* [12] determined the level of selected ingredients in (JAF). The flour of Jerusalem artichoke contains protein 7.4, sucrose 15, dietary fiber 15.4 ash 7.2 and fructose 44.1 g/100 g.

The present study aims to investigate the biological effects of Jerusalem artichoke tubers on glycemic responses in diabetic rats.

MATERIALS AND METHODS

Materials: Jerusalem artichoke tubers (JAT) were purchased from the Faculty of Agriculture, Benha University, washed and sliced individually to thickness of approximately 1 mm. The slices were then soaked in acidic solution to inhibit polyphenol oxidase activity [13]. Thereafter were subjected to dryness process by solar energy, blended, packed and kept in deep freezer.

Biological Experiment: Twenty five male albino rats each weighting 150±10 g were obtained from the Agricultural Research Center, Dokki Cairo (ARC). These rats were allowed to be acclimatized to laboratory condition for one week prior to the experiment and fed on basal diet as control according to NRC [14].

After the adaptation period, the rats were injected by alloxan solution at a rat of 150 mg/kg body weight of recrystallized alloxan [15] to induce hyperglycemia. Rats were fed on basal diet for 72 h during which hyperglycemia was developed. To ensure occurrence of diabetes in rats, blood samples were withdrawn 72 h after alloxan injection. The diabetic rats were divided into five groups (G1, G2, G3, G4 and G5), 5 rats each. Each group was fed on experimental diet as shown in Table 1. During the whole experiment, rats were kept separate in well aerated cages; diet and water were supplied *ad-lib*. Each rat was weighed every week and food intake was recorded daily.

At the end of experimental period (5 weeks) animals were fasted over night before sacrificed, the blood samples collected in test tubers from each rat and centrifuged at 3000 rpm for 20 min. The serum was kept frozen (-20°C) until analysis. Organs (liver, spleen, kidney and heart) were separated, weighed, then kept in formalin solution (15%) until histopathological examination.

Determination of Biological Parameters: Body weight gain and food efficiency ratio were calculated.

Biochemical analysis of serum:

- Blood glucose was analyzed by the method of Tietz [16].
- Creatinine was determined by the method reported by Henry [17].
- Urea in serum was determined according to the method the described by Patton and Crouch [18].
- Uric acid in serum was analyzed by the method of Fassati [19].
- Enzyme activities of alanine amino transferase (ALT) and aspartate amino transferees (AST) were determined calorimetrically according to the method of Retiman and Frankel [20].
- Total protein was determined in serum according to the method described by Tietz [16].
- Cholesterol was analyzed by the method of Allain *et al.* [21].
- Triglycides was determined reported by Fossati and Principe [22].
- Serum high density lipoprotein (HDL) was determined using the method by Castell [23].
- Serum low density lipoprotein (LDL) was calculated according to Fossati and Prencipe [22].

$$\text{Very low density lipoprotein VLDS (mg/dl)} = \frac{\text{Triglycrides}}{5}$$

Low density lipoprotein (mg/dl) = Total cholesterol - (very low density lipoprotein + high density lipoprotein)

Statistical Analysis: The data of the present study was statistically analyzed according to SAS [24].

Histopathological Examination: Rats internal organs were subjected to histological examination according to the method of Drury and Wallington [25].

Table 1: Food composition contents of experimental diets (g/100 g)

	Contents						
Rat group	Casein	Corn	Vitamin mix	Mineral mix	Cellulose	JAT	Starch
Control-	12	10	1	4	5	--	68
Control +	12	10	1	4	5	--	68
JAT 5%	12	10	1	4	5	5	63
JAT 10%	12	10	1	4	5	10	58
JAT 15%	12	10	1	4	5	15	53

** JAT: Jerusalem artichoke tubers dried and powdered.

RESULTS AND DISCUSSION

Effect of Experimental Diets on Food Intake, Body Weight Gain (B.W.G) and Feed Efficiency Ratio (F.E.R) of Diabetic Rats: Data in Table 2 showed, significant increases in body weight gain (BWG) for all groups (diabetic rats) fed on different levels (5, 10 and 15%) of Jerusalem artichoke tubers (JAT) comparing with the positive control. The highest value of percentage body weight gain % was (25.43±2.18) in found for the negative control, while the lowest value was (12.65±3.31) recorded for the positive control. Moreover, significant increase in food intake for all groups (Diabetic rats) fed on different levels (5, 10 and 15%) of JAT comparing with the positive control took place. The highest value was (13.42±0.67) in group fed with 10% JAT, while the lowest value (10.76±0.13) was in the positive control. In addition, significant increase revealed in food efficiency ratio (FER) for all groups (diabetic rats) fed on different levels (5, 10 and 15%) of JAT comparing with the positive control. The highest value was (0.086±0.007) in the negative control group, while the lowest value was (0.045±0.001) in group fed with 15% JAT. From the above results, it could be observed that addition of Jerusalem artichoke tubers to the diet at different levels (5, 10 and 15%) improved the total body weight and feed efficiency ratio relative to hyperglycemic rats. Such results are in agreement with those reported by El-Hofi [2].

Effect of Experimental Diets on Organs Weights of Diabetic Rats: The weights of liver, kidney, heart and spleen of experimental rats fed JAT are presented in Table 3. From the obtained results, it could be observed that, the liver and spleen of hyperglycemic rats had high mean values than that of rats fed basal diet (control). Moreover, the rats fed JAT at the concentration 5, 10 and 15% had the similar level. These results are in agreement with those reported by El-Hofi [2].

Effect of JAT on Glucose Levels in Diabetic Rats: Data in Table 4 showed, significant decreases of serum glucose for all groups (diabetic rats) fed on different levels (5, 10 and 15%) of JAT compared with the control positive.The mean values of serum glucose content were 66.25±2.98, 150.25±7.80, 112.87±19.58, 103.57±4.97 and 109.40±4.73 mg/dl serum for rats fed basal diet,hyperglycemic rats and rats fed different levels (5, 10 and 15%) of JAT respectively. From the above results, it could be observed

Table 2: Effect of experimental diets on food intake (F.I.), body weight gain (B.W.G) and feed efficiency ratio (F.E.R)

Rat groups	Initial weight (g)	Final weight (g)	B.W.G (g)	B.W.G (%)	Food intake (g/day)	F.E.R
Control (-)	152.50 *a* ±9.46	191.28 *a* ±11.69	38.78 *a* ±3.53	25.43 *a* ±2.18	12.98 a ±0.64	0.086 ±0.007a
Control (+)	147.62 *a* ±16.90	166.30 *a* ±14.02	18.68 *b* ±3.06	12.65 *b* ±3.31	10.76 b ±139	0.049 b ±0.004
JAT 5%	145.00 *a* ±10.00	172.*80 a* ±16.30	27.80 *ab* ±7.71	19.17 *ab* ±4.59	12.87 a ±0.45	0.062 ab ±0.01
JAT 10%	145.00 *a* ±12.90	185.20 *a* ±22.58	40.20 *a* ±14.72	27.*72 a* ±9.65	13.42 a ±0.67	0.086 ab ±0.03
JAT 15%	145.00 *a* ±15.81	163.37 *a* ±13.74	18.37 *b* ±2.03	12.67 *b* ±2.69	11.65 ab ±1.24	0.054 b ±0.001

**Values with same letters non significant difference (p=0.01) and vice versa; **JAT: Jerusalem artichoke tubers see table 1 for group designations.

Table 3: Effect of experimental diets on organs / body weight (BW) ratio

Rat groups	Liver/B.W (%)	Kidney/B.W. (%)	Heart/B.W. (%)	Spleen/B.W. (%)
Control (-)	1.93±0.11 a	0.42±0.05 a	0.21±0.02 a	0.21±0.06 a
Control (+)	2.17±0.47 a	0.47±0.07 a	0.12±0.12 a	0.29±0.04 a
JAT 5%	1.83±0.13 a	0.47±0.07 a	0.18±0.19 a	0.24±0.36 a
JAT 10%	1.93±0.32 a	0.49±0.05 a	0.20±0.02 a	0.23±0.05 a
JAT 15%	2.07±0.40 a	0.48±0.02 a	0.22±0.02 a	0.29 ±0.06 a

** Values with same letters nonsignificant difference (p=0.01) and vice versa.

** JAT: Jerusalem artichoke tubers see footnotes of table 1 for treatment designations of groups.

Table 4: Effect of JAT on glucose content (mg/dl) in diabetic rats

Groups	Control(-)	Control(+)	JAT (5%)	JAT (10%)	JAT (15%)
Glucose (mg/dl)	66.25±2.98 c	150.25±7.80 a	112.87±19.58 b	103.57±4.97 b	109.40±4.73 b

**Values with same letters non significant difference (p=0.01) and vice versa.

**JAT: Jerusalem artichoke tubers see footnotes of table 1 for treatment designations of groups.

Table 5: Effect of JAT on Kidney function in diabetic rats

Rat groups	Urea (mg/dl)	Uric Acid (mg/dl)	Creatinine (mg/dl)
Control (-)	32.75±2.05 ab	2.80±0.18 a	0.85±0.17 b
Control (+)	28.17±6.08 b	2.09±0.16 a	1.28±0.28 a
JAT 5%	35.07±1.08 a	2.63±0.26 a	0.90±0.08 b
JAT 10%	32.17±2.48 ab	2.62±0.02 a	0.85±0.12 b
JAT 15%	29.75±1.98 ab	2.39±0.06 a	0.83±0.13 b

Table 6: Effect of JAT on the liver function and total protein in diabetic rats

Rat groups	ALT (U/L)	AST (U/L)	Total protein (g/dl)
Control (-)	7.55±0.78 abc	21.00±1.41 a	4.72±1.44 a
Control (+)	9.58±1.15 a	25.30±4.36 a	4.61±2.27 a
JAT 5%	5.66±0.82 c	24.00±4.20 a	3.63±0.13 a
JAT 10%	8.34±1.22 ab	25.75±1.32 a	3.78±0.47 a
JAT 15%	7.11±1.68 bc	26.74±1.84 a	3.55±0.22 a

** Values with same letters non significant difference (p=0.01) and vice versa; ** JAT: Jerusalem artichoke tubers see footnotes of table 1 for treatment designations of groups.

that the percent decrease in serum glucose levels were 24.88, 31.07 and 27.19% for rats fed at the different levels (5, 10 and 15%) of JAT respectively when compared with control (+) rats. These observations may be due to the dietary fibers both soluble (Fructo-oligosaccharides, FOS) and soluble in (Fructo-oligosaccharides, FOS) as suggested by Kopec and Cieslik [26].These results are in agreement with those reported by Alegria and Vivanco [5].

Effect of JAT on Serum Urea, Uric Acid and Creatinine: Data in Table 5 showed significant increases in serum urea for all groups (diabetic rats) fed on different levels (5, 10 and 15%) of JAT compared with control positive. The mean values of serum urea contents were 32.75±2.05, 28.17± 6.08, 35.07±1.08, 32.17±2.48 and 29.75±1.98 mg/dl for rats fed basal diet, hyperglycemic rats and rats fed different levels (5, 10 and 15%) of JAT,respectively. Moreover, nonsignificant increase observed in serum uric acid for all groups (diabetic rats) fed on different levels (5, 10 and 15%) of JAT compared with the control positive. In addition, a significant decrease in serum creatinine for groups fed on different levels (5, 10 and 15%) of JAT compared with the control positive were recorded. The highest value was 1.28±0.28 mg/dl in the positive control, while the lowest value was 0.83±0.13 mg/dl in group fed on (5%) of JAT. The obtained data are in agreement with those reported by El-Hofi [2].

Effect of JAT on Serum ALT, AST and Total Protein in Diabetic Rats: Data in Table 6 revealed, significant decreases in serum ALT for all groups (Diabetic rats) fed on different levels (5, 10 and 15%) of JAT compared with the positive control. The highest value was 9.58±1.15 u/l in the positive control, while the lowest value was 5.66±0.82 u/l in group fed on (5%) of JAT. Moreover non significant increase in serum AST recorded for all groups fed on different levels (5, 10 and 15%) of JAT compared with the positive control.

In addition the data shows that nonsignificant decrease in total protein for all groups (Diabetic rats) fed on difference levels of JAT (5, 10 and 15%) compared with the positive control. The highest value was 4.61±2.27 g/dl in the positive control, while the lowest value was 3.55±0.22 g/dl in group fed on (15%) JAT. From the obtained results, it could be observed that activities of (ALT) and (AST) had the highest values for rats of control (+) group comparing that of rats fed on basal diet and rats fed at different levels (5, 10 and 15%) of JAT, which was not so for (AST) with groups fed on 10 and 15% of JAT. These results are in agreement with those reported with Kaur and Gupta [10] and Daubioul *et al.* [11].

Effect of JAT on Serum Total Cholesterol, Triglycerides, HDL, LDL and VLDL in Diabetic Rats: Data in Table 7 show that significant decrease in serum total cholesterol, triglycerides, LDL and VLDL cholesterol for all groups (diabetic rats) fed on different level (5, 10 and 15%) of JAT compared with control positive took place.The highest values were in positive control, while the lowest values were in group fed on JAT (10%). In addition the data showed that nonsignificant changes of serum HDL for all groups (diabetic rats) fed on (5, 10 and 15%) JAT compared with the control positive.

The obtained results indicted that JAT reduced the levels of total cholesterol, triglycerides, HDL, LDL and VLDL-cholesterol in the serum of diabetic rats. The hypolipidaemic effect of JAT my be due to increasing fecal lipid excretion and decreasing lipid absorption, as reported by Cieslik *et al.*[8].These results are in agreement with those reported by Anderson and Hanna [4].

Table 7: Effect of JAT on cholesterol, triglycide, HDL, LDL and VLDL in diabetic rats

Rat groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control (-)	52.00±4.96 d	54.50±2.51 d	24.75±1.50 a	17.12±5.51 c	10.90±0.50 d
Control (+)	148.50±5.32 a	81.75±2.21 a	31.75±4.57 a	100.90±7.98 a	16.36±0.44 a
JAT 5%	83.45±5.63 b	73.55±10.32 ab	29.10±6.76 a	39.20±7.04 b	14.75±2.01 ab
JAT 10%	56.20±4.46 cd	61.67±4.60 cd	25.75±3.09 a	18.10±3.28 c	12.34±0.92 cd
JAT 15%	69.87±13.31 bc	68.77±4.54 bc	30.25±8.26 a	25.80±10.96 bc	13.74±0.92 bc

** Values with same letters means nonsignificant difference (p=0.01) and vice versa.

** JAT: Jerusalem artichoke tubers see footnotes of table 1 for treatment designations of groups.

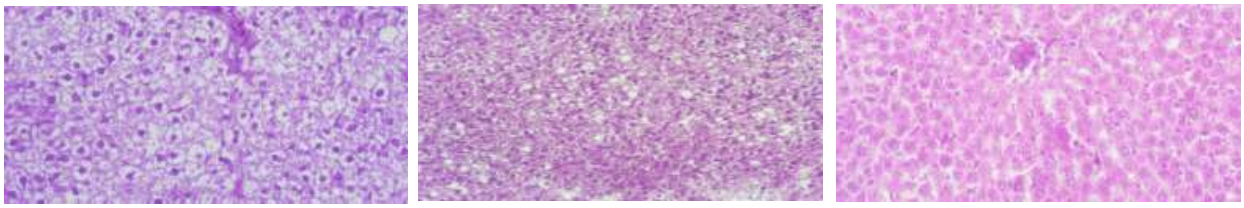

Photo. (1) liver control + Photo. (2) liver (5%) Photo. (3) liver (10%)

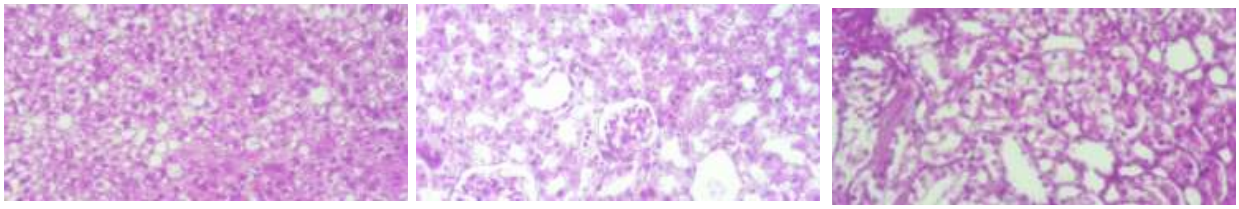

Photo. (4) liver (15%) Photo. (5) kidney control+ Photo. (6) kidney (5%)

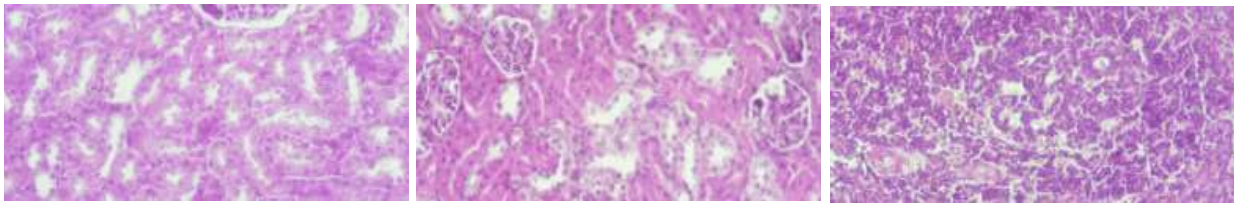

Photo. (7) kidney (10%) Photo. (8) kidney (15%) Photo. (9) spleen control+

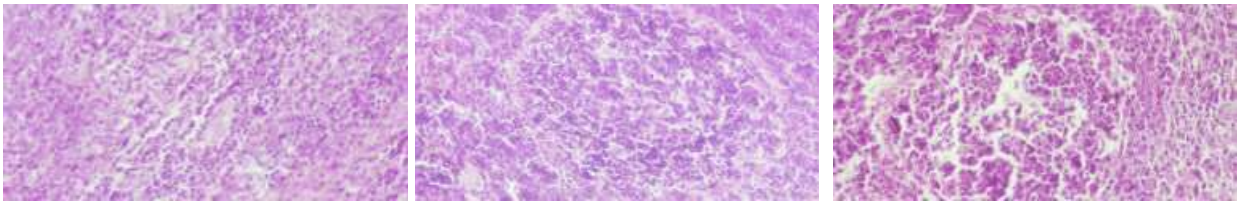

Photo. (10) spleen (5%) Photo. (11) spleen (10%) Photo. (12) spleen (15%)

Histopathological Examination:

Liver: Microscopic examination of liver from rats in control (+) revealed slight hydropic degeneration and vacuolations of hepatocytes (Photo 1). While, for liver from group (3) rats fed on (5% JAT) there was no hstopathological changes except vacuolar degeneration of hepatocytes (Photo 2). Examined sections from group (4) rats fed on (10%JAT) revealed no hstopathological changes except slight hydropic degeneration of hepatocytes (Photo 3). However, liver of rats from group (5) rats fed on (15% JAT) showed vacuolar degeneration of hepatocytes and dissociation of hepatic cords was observed in (Photo 4). Also very slight changes compared to control were seen being minute vacuoles in the cytoplasm of some hepatocytes a well as sinusoidal leucocytosis.

Kidney: Histopathological examination of kidney sections of rats from control (+) group revealed slight vacuolations of epithelial lining some renal tubules associated with perivascular edema (Photo 5). Moreover, kidney of group (3) (JAT 5%) showed vacuolations of epithelial lining of some renal tubules (Photo 6). However, small vacuoles were noticed in the cytoplasm of epithelial lining of some renal tubules (Photo 7). Meanwhile, examined sections from group (4) (10% JAT) and group (5) (15% JAT) revealed no histopathological changes (Photo 8).

Spleen: Histopathological examination of spleen sections of rats from control (+) group showed slight lymphocytic depletion and congestion of splenic arterioles (Photo 9). Meanwhile, spleen of rats of group (5% JAT) revealed no histopathological changes (Photo 10).). Moreover, spleen from rats fed (10% JAT) of group (4) showed no Histopathological changes except lymphocytic depletion (Photo 11). However, spleen of rats fed (15% JAT) of group (5) reveal no histopathological changes (Photo 12).

CONCLUSION

From the above-mentioned results, it could be concluded that the diet containing Jerusalem artichoke tubers reduced serum glucose levels, triglycerides, total cholesterol and LDL-cholesterol in the hyperglycemic rats. Also, the obtained results indicate that there is an improvement in liver and kidney functions as a result of these additives.

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