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Anti-diabetic Effect of *Cichorium intybus* Leaves and *Plantago ovate* Seeds in High Fat Diet-streptozotocin Induced Diabetic Rats

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Abstract The current study was carried out to evaluate the effect of chicory leaves (*Cichorium intybus*) and psyllium seeds (*Plantago ovate*) on diabetic rats. Ten rats (control normal) were fed on balanced diet. Forty rats were received high fat diet for one month then injected with a single intraperitoneal low dose (30 mg/Kg) of streptozotocin to induce diabetes. Diabetic rats were classified into 4 groups (n=10) and received either high fat diet (diabetes/HFD), balanced diet (diabetes/balanced), balanced diet containing 20% of chicory leaves powder or balanced diet containing 20% of psyllium seeds powder for a month. Administration of chicory leaves or psyllium seeds to diabetic rats produced significant reduction in blood glucose levels and significant reduction in cholesterol, triglyceride and low density lipoprotein cholesterol (LDL-C) levels. The elevated activities of alanine transaminase, aspartate transaminase and lactate dehydrogenase enzymes of diabetic rats were reversed by the feeding on the balanced diets containing either chicory leaves or psyllium seeds. Administration of chicory leaves or psyllium seeds to diabetic rats kept the activities of both superoxide dismutase and catalase as well as the values of malondialdehyde near to those of normal rats. According to the results it could be concluded that the intake of chicory leaves or psyllium seeds may be useful for controlling blood glucose level and combating the metabolic disorders and complications that accompanied with diabetes.

Keywords: *type 2 diabetes mellitus, chicory leaves, psyllium seeds, hyperglycemia, lipid profile*

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1. Introduction

Type 2 diabetes mellitus is considered a metabolic disorder which is characterized by β-cell dysfunction and high blood glucose level in the context of the relative insulin insufficiency [1]. Oxidative stress (excess in free radicals and defect in internal antioxidant defense system), impaired lipid and lipoprotein metabolism, hypertension, vascular endothelial dysfunction and sub-clinical inflammation among the metabolic disorders that accompanied by type 2 diabetes [2]. Microvascular and macrovascular complications were induced through the metabolic disorders [3]. According to Modi [4] the lifestyle alteration of diet and exercise, replacement therapy of insulin, insulin secretagogues, sensitizers of insulin and inhibitors of alpha-glucosidase among the major forms of therapy for type 2 diabetes. Patients have to consume multiple drug combinations to control the glucose level and prevent diabetes complications. However, these drugs may cause some side effects among them hypoglycemia, gastrointestinal upset and lactic acid intoxication. Therefore it is better for patients to use the medicinal plant which useful for controlling the diabetes symptoms [5]. Chicory (*Cichorium intybus* L) is belonging to the family Asteraceae. Leaves, flowers and roots of chicory can be used, since the leaves and flowers can be added to the salad also the roots can be used in the coffee blends. The slightly bitter taste of chicory leave may be attributed to its content of a bitter glycoside named cichorine [6]. Chicory leaves are considered important medicinal herb which contain important constituents such as caffeic acid derivatives, flavonoids and polyphenol [7]. Roots and leaves of chicory contain inulin and fructo-oligosaccharides prebiotic which possess a lot of benefits for the human health [8]. *Plantago ovata* (psyllium) is a member of the family Plantaginaceae. The seeds and husk of the plant are of great medicinal and commercial importance. psyllium seeds have various medicinal characteristics as traditional medicine [9]. Psyllium is a characteristic fiber got from psyllium seed. It is an exceptionally fanned arabinoxylan polysaccharide which has a high water holding and gelling limit. Gel-framing portion of the soluble base extractable polysaccharides of psyllium is made out of arabinose, xylose and hints of different sugars [10]. The objective of the current study is the evaluation of the anti-diabetic effect of chicory leaves and psyllium seeds in high fat diet-streptozotocin induced diabetic rats.

2. Materials and Methods

2.1. Materials

Plant materials: Chicory leaves and psyllium seeds were purchased from local market. Botanical identification of specimens of each plant was carried out in the Orman Botanical Garden, Giza, Egypt.

Animals: Male albino rats of 102.64±2.88g as Mean ± SD (6 weeks age) were used in the present study. Rats were obtained from the animal house of the National Research Centre, Cairo, Egypt. The rats were individually kept in stainless steel cages at room temperature. Water and food were presented *ad-libitum*.

2.2. Methods

Preparation of plant materials: Chicory leaves were washed with water, cut into thin slices then dried in an air-circulated oven at 40°C to complete dryness. Psyllium seeds were re-dried. Dried chicory leaves and psyllium seeds were reduced separately into powder form as far as possible and sieved through 100-mesh sieve then stored in plastic bags in a refrigerator at 4°C. The proximate analysis (moisture, crude protein, crude fiber, ash and crude fat) of the plants was performed using standard AOAC [11] procedure. Available carbohydrates were calculated on dry weight basis as follows:

$$\textit{Available carbohydrates} = 100 \square (\textit{proteins} + \textit{fat} + \textit{crude fiber} + \textit{ash})$$

The experimental diets: Diets prepared as in Table 1. The high fat diet was prepared according to Zulet *et al.* [12] but with a modification by adding lard instead of coconut oil as a source of saturated fat. The composition of salt mixture and vitamin mixtures were according to Briggs and Williams [13] and Morcos [14] in succession. A dose of 0.1 ml of oil soluble vitamins was given orally for each ratper week**.**

Table 1. Composition of the experimental diets (%)

Ingredients	Diets			
	Balanced	High fat	Chicory leaves	Psyllium seeds
Casein	12*	12	12	12
Corn oil	15	-	15	15
Lard	-	20	-	-
Sucrose	10	10	10	10
Starch	55.5	48.19	35.5	35.5
Salt mix.	3.50	3.50	3.50	3.50
Vitamin Mix.	1.00	1.00	1.00	1.00
Fiber	3	3	3	3
cholesterol	-	2	-	-
Cholic acid	-	0.06	-	-
Bile salt	-	0.25	-	-
Chicory leaves	-	-	20	-
Psyllium seeds	-	-	-	20
Total Calories (kilocalorie)	437.00	452.76	425.30	426.63

* 12g casein has been estimated to contain 10g protein.

The design of the animal study: Fifty rats were classified into two dietary groups. The first group (10 rats) administrated balanced diet (control normal). To induce rat models of diabetes upon the protocol of Srinivasan *et al*. [15] forty rats (the second groups) were fed on a high-fat diet and after one month cholesterol was determined. Onset of hypercholesterolemia was identified by blood total cholesterol levels >200 mg/dl. All rats were weighed and the body weight gain as well as feed efficiency ratio were calculated then rats were fasted overnight and injected intraperitonealy with 30 mg/kg body weight (w/w) of streptozotocin (STZ) (from Sigma chemical Co) in citrate buffer, pH 4.5 then given glucose solution (5%) for 48 h after the injection to prevent hypoglycemia. To ensure occurrence of diabetes in rats, blood samples were withdrawn 72 h after streptozotocin injection then the blood glucose levels were determined. Rats with blood glucose higher than 200 mg/dl were considered diabetic and used in the following procedures. After the development of diabetes the diabetic rats were classified into 4 sub-groups of ten rats each. Rats in the first sub-group fed on the same high fat diet (diabetes/HFD). The second sub-group (diabetes/balanced) of rats fed on balanced diet. The remaining two diabetic sub-groups of rats received balanced diet containing 20% of chicory leaves or psyllium seeds in powder form for a month. At the end of the animal study body weight gain and feed efficiency ratio were calculated. Rats were fasted overnight and the blood samples were withdrawn for the determination of blood glucose levels depending on Trinder [16], total cholesterol according to Watson [17], high-density lipoprotein cholesterol (HDL-C) using the method of Burstein *et al*. [18], low-density lipoprotein cholesterol (LDL-C) in accordance with Schriewer *et al*. [19] and triglycerides according to the method of Megraw *et al*. [20]. Cholesterol /HDL-C ratio was calculated. The levels of creatinine and urea were determined depending on Larsen [21] and Fawcett and Scott [22] in succession as indicators of kidneys function. The activities of aspartate transaminase (AST) and alanine transaminase (ALT) were determined according to Reitman and Frankel [23] also the activity of lactate dehydrogenase (LDH) was determined according to King [24] as indicators of liver functions. Malondialdehyde (MDA) as well as catalase and superoxide dismutase (SOD) activities were assayed according to the methods of Ohkawa *et al.* [25], Beers and Sizer [26] and Nishikimi *et al.* [27] respectively. After blood sampling rats were sacrificed and the entire liver, kidneys, pancreas and heart were removed from each animal and weighed.

Statistical analysis: The data of the experiment were presented as the mean ± SE. Results of nutritional parameters after one month of the animal study were statistically analyzed via student's t-test but one-way analysis of variance ANOVA followed by Duncan's test was used in the statistical analysis of other data. p<0.05 was used as the criterion of statistical significance in all cases.

3. Results

Regarding the chemical composition of chicory leaves and psyllium seeds results in Figure 1 disclose that chicory leaves and psyllium seeds contain low contents of crude fat (0.44 and 0.91% on dry weight basis, in succession) and high contents of crude fiber (8.88 and 7.8 % on dry weight basis, in succession) which makes them good for health.

The data of nutritional parameters of rats received balanced diet and rats given high fat diet after a month of the animal study are tabulated in Table 2. The results indicated that high fat diet elevated the body weight gain since the final body weight and the body weight gain of rats fed on high fat diet significantly increased compared to rat fed on balanced diet although there was no significant change in total food intake. The total intake of high fat diet (452.76g) provided total calories 1962 kilocalories while the total intake of balanced diet (437g) provided total calories 1940 kilocalorie.

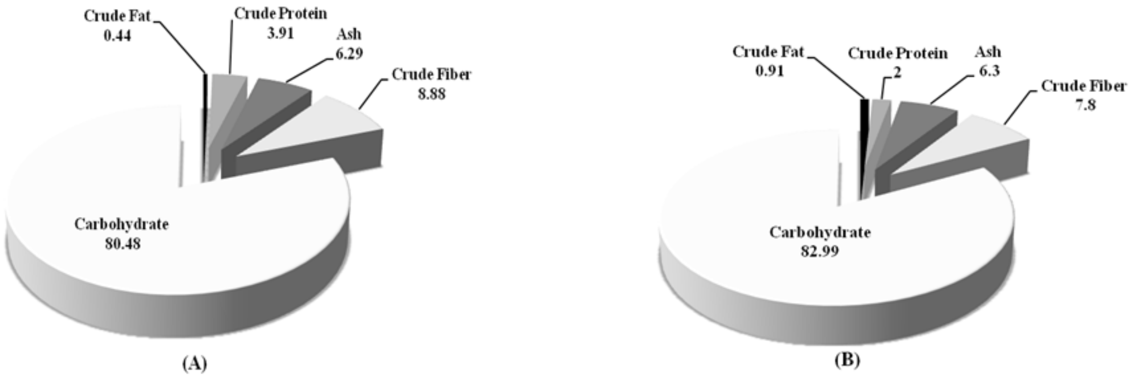

Figure 1. Chemical composition of chicory leaves (A) and psyllium seeds (B) (g/100g dry sample)

Table 2. Nutritional parameters of normal rats and rats fed on high fat diet before STZ injection (Mean ± SE)

Parameters	Control normal	Control HFD
Body weight (initial) (g)	102.20 ± 0.93	102.75 ± 0.46
Body weight (final) (g)	146.00 ± 2.54	155.75* ± 1.48
Body weight gain (g)	43.80 ± 2.29	53.00 * ± 1.35
Body weight gain (%)	30	34
Total Food Intake (g)	443.82 ± 5.15	433.43 ± 3.96
Feed Efficiency Ratio	0.10 ± 0.005	0.12 ± 0.004

* The significance of control HDF vs. control normal at 0.05 probability.

The nutritional parameters of diverse experimental groups at the end of the animal study are tabulated in Table 3. Body weight gain of diabetic rats which continued on the high fat diet was the lowest (17.94g) compared with the different experimental groups. Feeding diabetic rats on diet containing chicory leaves lead to significant improvement in the body weight gain and feed efficiency ratio compared with diabetes/HFD group.

Table 4 declares the mean organs (liver, kidneys, pancreas and heart) weight and the ratio of these organs weight to body weight in different experimental groups. It could be seen that both liver and kidneys weight of diabetic rats continued feeding on HFD increased compared with normal rats. liver and kidneys weight were less in chicory leaves or psyllium seeds groups than diabetes/HFD and diabetes/balanced groups. Non-significant change in the heart weight between the different groups.

Table 3. Nutritional parameters of different experimental groups (Mean ± SE).

Groups	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Body weight gain (%)	Total food intake (g)	Feed efficiency Ratio
Control normal	$146.00^{a}\pm2.54$	$187.60^{bc}\pm2.00$	$41.60^{d}\pm1.54$	22.17	$435.50^{cd}\pm7.60$	$0.095^{d}\pm0.003$
Diabetes/HFD	$155.80^{b}\pm3.34$	$173.74^{a}\pm3.48$	$17.94^{a}\pm1.32$	10.32	$401.10^{a}\pm5.10$	$0.044^{a}\pm0.003$
Diabetes/balanced	$155.7^{b}\pm3.78$	$179.38^{ab}\pm3.90$	$23.68^{b}\pm1.40$	13.20	$417.50^{ab}\pm7.11$	$0.056^{b}\pm0.003$
Chicory leaves	$155.90^{b}\pm2.16$	$188.14^{c}\pm1.94$	$32.24^{c}\pm1.93$	17.14	$444.60^{d}\pm4.57$	$0.072^{c}\pm0.005$
Psyllium seeds	$155.60^{b}\pm2.82$	$180.09^{abc}\pm2.01$	$24.49^{b}\pm1.93$	13.60	$420.04^{bc}\pm3.72$	$0.058^{b}\pm0.004$

The same letters in each column mean non-significant difference; distinct letter means the significance in the experimental groups at 0.05 probability

Table 4. Organs weight and organs/body weight ratio of different experimental groups (Mean ± SE).

Groups	Control normal	Diabetes/HFD	Diabetes/balanced	Chicory leaves	Psyllium seeds
Liver weight (g)	$6.80^{ab}\pm0.27$	$9.79^{c}\pm0.29$	$7.56^{b}\pm0.37$	$6.72^{ab}\pm0.22$	$6.48^{a}\pm0.16$
Liver weight (%)	3.63	5.66	4.23	3.57	3.62
Kidney weight (g)	$1.36^{a}\pm0.08$	$1.82^{b}\pm0.04$	$1.69^{ab}\pm0.18$	$1.43^{a}\pm0.90$	$1.45^{a}\pm0.14$
Kidney weight (%)	0.72	1.05	0.94	0.75	0.81
Pancreas weight (g)	$0.54^{c}\pm0.03$	$0.34^{a}\pm0.02$	$0.41^{ab}\pm0.02$	$0.50^{bc}\pm0.04$	$0.43^{ab}\pm0.04$
Pancreas weight (%)	0.29	0.20	0.23	0.27	0.24
Heart weight (g)	$0.95^{a}\pm0.08$	$0.98^{a}\pm0.11$	$0.85^{a}\pm0.06$	$0.86^{a}\pm0.04$	$0.82^{a}\pm0.05$
Heart weight (%)	0.50	0.56	0.48	0.46	0.46

The same letters in each column mean non-significant difference; distinct letter means the significance in the experimental groups at 0.05 probability.

As shown in Figure 2 it is observed that the development of diabetes caused significant increase in the glucose levels of diabetic rats compared with control normal group. After two months of the experiment the highest blood glucose value (248.02 mg/dl) was significantly observed in the group of rats that fed on high fat diet for a month then injected with streptozotocin then fed on high fat diet for another one when compared with the other investigated groups. The increased levels of glucose on induction of diabetes were significantly reversed in feeding on diets containing either chicory leaves (85.32 mg/dl) or psyllium seeds (140.92 mg/dl).

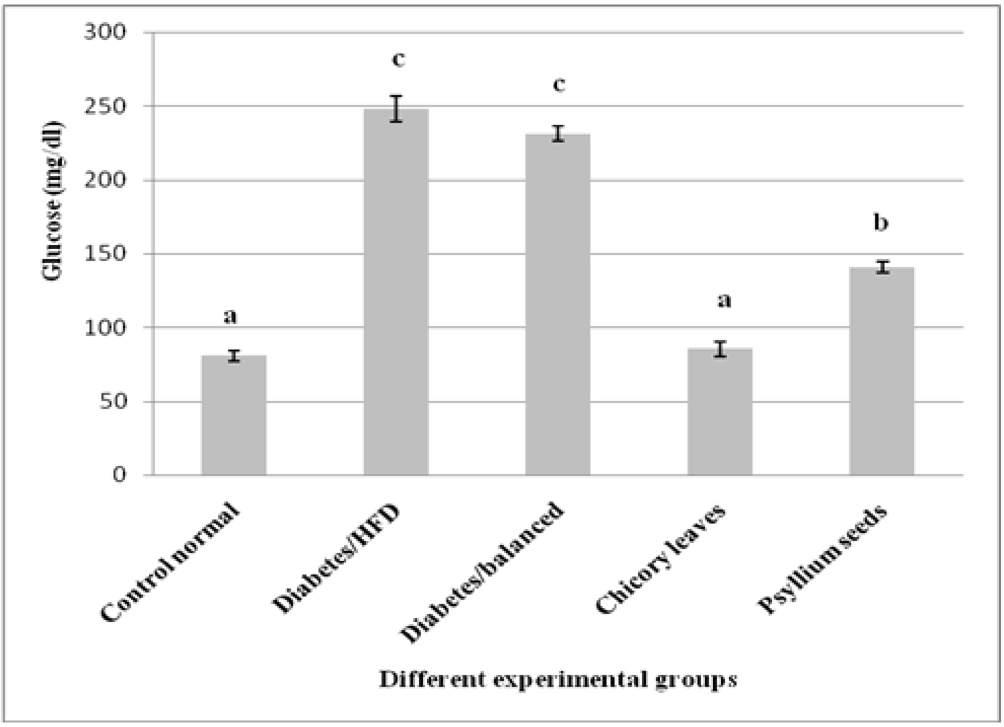

Figure 2. Glucose levels of different experimental groups at the end of the study (Mean ± SE).

The same letters mean non-significant difference; distinct letter means the significance in the experimental groups at 0.05 probability.

Results in Table 5 declare that continuous feeding of diabetic rats on high fat diet for another month was associated with a significant increase in their serum total cholesterol, low density lipoprotein cholesterol as well as triglycerides levels. The increase in total cholesterol was associated with increase in cholesterol/HDL-C ratio. Feeding diabetic rats on balanced diet alone didn't show improvement in the lipid profile while feeding diabetic rats on balanced diet containing 20% of either chicory leaves or psyllium seeds produced significant decrease in total cholesterol, cholesterol/HDL-C ratio, LDL-C as well as triglyceride levels near to control normal group, while HDL-C level significantly increased. However, chicory leaves showed the best improvement in LDL-C and triglycerides levels.

Table 5. lipid profile of different experimental groups (Mean ± SE).

Groups	Total Cholesterol (mg/dl)	HDL- Cholesterol (mg/dl)	Cholesterol/ HDL Ratio	LDL- Cholesterol (mg/dl)	Triglycerides (mg/dl)
Control normal	$108.11^{a}\pm8.48$	$41.33^{c}\pm2.82$	$2.76^{a}\pm0.32$	$32.26^{a}\pm1.49$	$76.91^{a}\pm3.36$
Diabetes/HFD	$360.96^{c}\pm16.29$	$21.91^{a}\pm0.93$	$16.66^{c}\pm0.84$	$103.92^{c}\pm3.41$	$130.74^{c}\pm2.08$
Diabetes/balanced	$315.16^{b}\pm11.94$	$22.73^{a}\pm1.34$	$14.36^{b}\pm1.17$	$99.07^{c}\pm7.67$	$108.62^{b}\pm3.13$
Chicory leaves	$123.00^{a}\pm8.15$	$40.04^{bc}\pm2.52$	$3.14^{a}\pm0.23$	$42.49^{a}\pm2.12$	$78.31^{a}\pm3.45$
Psyllium seeds	$142.62^{a}\pm17.13$	$34.52^{b}\pm1.71$	$4.14^{a}\pm0.44$	$57.90^{b}\pm4.61$	$101.16^{b}\pm5.09$

The same letters in each column mean non-significant difference; distinct letter means the significance in the experimental groups at 0.05 probability.

The levels of creatinine and urea as well as the activities of ALT, AST and LDH are presented in Table 6. ALT, AST and LDH levels were significantly ($P<0.05$) higher in diabetic rats compared to those of normal rats. Continuous feeding of diabetic rats on high fat diet for another month was associated with a significant increase in their LDH level (251.87 U/L). Significant decrease in creatinine and urea as well as AST, ALT and LDH activities was observed in the diabetic rats administrated balanced diet with 20% of either chicory leaves or psyllium seeds powder in comparison to diabetic rats which feed on either high fat diet or balanced diet.

Table 6. Kidneys and liver functions of different experimental groups (Mean ± SE)

Groups	Content of		Enzyme activity		
	Creatinine (mg/dl)	Urea (mg/dl)	AST (IU/l)	ALT (IU/l)	LDH (U/L)
Control normal	$0.45^{a}\pm0.02$	$32.55^{a}\pm1.08$	$72.90^{a}\pm2.50$	$29.10^{a}\pm1.46$	$162.86^{a}\pm8.60$
Diabetes/HFD	$0.65^{c}\pm0.01$	$47.50^{b}\pm1.09$	$91.40^{b}\pm2.97$	$51.70^{c}\pm1.43$	$251.87^{b}\pm10.33$
Diabetes/balanced	$0.62^{c}\pm0.02$	$44.58^{b}\pm0.96$	$90.20^{b}\pm2.36$	$47.90^{c}\pm1.66$	$238.71^{b}\pm9.56$
Chicory leaves	$0.49^{ab}\pm0.02$	$34.88^{a}\pm2.10$	$75.80^{a}\pm5.73$	$33.90^{b}\pm1.42$	$167.56^{a}\pm11.68$
Psyllium seeds	$0.53^{b}\pm0.02$	$36.61^{a}\pm0.83$	$81.50^{ab}\pm2.93$	$34.50^{b}\pm2.08$	$184.97^{a}\pm14.63$

The same letters in each column mean non-significant difference; distinct letter means the significance in the experimental groups at 0.05 probability.

Table 7 summarizes the activities of SOD, catalase (internal antioxidative enzymes) and serum levels of MDA of different experimental groups. It is observed that induction of diabetes resulted in reduction of the activities of both SOD and catalase enzymes, while the values of MDA decreased. The decreased activities of SOD and catalase of diabetic animals were significantly ($P < 0.05$) reversed by the feeding on diets containing either chicory leaves or psyllium seeds. Treatment of diabetic rats with chicory leaves or psyllium seeds kept their MDA values (2.88 and 3.01nmol/ml respectively) slightly different from that of normal rats.

Table 7. Malondialdehyde, superoxide dismutase and catalase of different experimental groups (Mean ± SE)

Groups	Content of	Antioxidative enzymes activity	
	Malondialdehyde (nmol/ml)	Superoxide dismutase (U/ml)	Catalase (U/ml)
Control normal	$2.47^{a} \pm 0.11$	$351.58^{d} \pm 5.31$	$8.04^{c} \pm 0.31$
Diabetes/HFD	$5.03^{d} \pm 0.11$	$243.82^{a} \pm 9.61$	$4.45^{a} \pm 0.18$
Diabetes/balanced	$4.53^{c} \pm 0.14$	$273.18^{b} \pm 8.24$	$4.74^{a} \pm 0.29$
Chicory leaves	$2.88^{b} \pm 0.05$	$341.40^{d} \pm 6.79$	$6.70^{b} \pm 0.21$
Psyllium seeds	$3.01^{b} \pm 0.18$	$309.11^{c} \pm 7.09$	$6.20^{b} \pm 0.40$

The same letters in each column mean non-significant difference; distinct letter means the significance in the experimental groups at 0.05 probability.

4. Discussion

There is an increasing demand for the use of naturally available plants and herbs to help control of blood glucose level. The effects of chicory leaves and psyllium seeds on the concentration of blood glucose, lipids profile, kidneys and liver functions and body antioxidant status were investigated in rats supplied high fat diet then injected with low dose of streptozotocin in order to induce mild hyperglycemia and hypercholesterolemia in rat models that appear to mirror the type 2 diabetes patients as reported by Mansor *et al.* [28]. The improvement in the body weight gain, total food intake and feed efficiency ratio in rats fed on chicory leaves is in harmony with the results that stated by Urias-Silvas *et al.* [29] who disclosed that chicory inulin is useful for regulation of appetite. In the present study high-fat feeding followed by injection with STZ increased glucose level. Rats continued on the same high fat diet as well as rats received balanced diet showed elevation in the blood glucose levels while administration of chicory leaves or psyllium seeds decreased glucose level to near normal value. Pushparaj *et al.* [30] disclosed that ethanol extract of chicory could ameliorate a diabetic state by reducing the hepatic glucose-6-phosphatase activity. Ghamarian *et al.* [31] suggested that chicory has insulin-sensitizing activity. Psyllium seeds lower the glucose level via lowering both gastric emptying and small bowel motility and increasing the viscous contents of the small intestine which reduces the absorption of glucose. Cholesterol synthesis may be decreased via the action of chicory inulin that inhibits hydroxymethylglutaryl-CoA reductase [32]. Fermentation of inulin through the action of beneficial flora in the gut produces short-chain fatty acids which contribute in cholesterol synthesis alteration [33]. The hypocholesterolemic effect of psyllium seeds may be attributed to its content of arabinoxylans (highly branched polysaccharide with high water holding). Tong *et al.* [34] concluded that dietary arabinoxylans decrease the cholesterol and low density lipoprotein cholesterol concentrations since arabinoxylans stimulate the excretion of lipids in the stool, arabinoxylans also organize the activities of 3-hydroxy-3-methyl glutaryl-coenzyme A (HMG-CoA) reductase and cholesterol 7-α hydroxylase and elevate short-chain fatty acids in the colon. Results indicated that feeding on either chicory leaves or psyllium seeds may be able to manage the disturbances of kidneys and liver functions that accompanied with diabetes since AST, ALT and LDH enzymes are excreted in the blood duo to the injury of the liver [35]. Free radicals induce damage and cause the insulin resistance, dysfunction of beta cell, glucose intolerance and diabetes [36]. A high amount of superoxide which produced in the hyperglycaemia causes the cells damage also the free radicals activate several pathways which in turn cause dysfunction of the endothelial [37]. Superoxide dismutase (SOD) enzyme catalyzes the dismutation of superoxide anion to O_2 and H_2O_2 which in turn can be counteracted by catalase reactions thus the level of cellular damage is reduced [38]. The decreased activities of superoxide dismutase and catalase and increased malondialdehyde on induction of diabetes were reversed by the feeding on chicory leaves or psyllium seeds. This indicated an antioxidant effects that may be attributed to phenolic compounds in both plants [39,40]. Chicory leaves have radical scavenging feature and offer significant protection against protein oxidation and DNA damage [41].

5. Conclusion

It could be concluded that feeding on chicory leaveas or psyllium seeds causes decreasing in blood glucose levels, improvement in the lipid profile and management of the disturbance in kidneys and liver functions in diabetic rats. Feeding on chicory leaveas or psyllium seeds also showed increasing in the internal antioxidant enzymes. Thus, chicory leaveas or psyllium seeds have anti-diabetic effects and may be useful for combating the metabolic disorders and complications that accompanied with diabetes.

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