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Original Article

The Effects of Chicory Leaf Aqueous Extract on Body Weight, Serum Glucose and Lipid Levels in Streptozotocin Induced Diabetic Rats

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ABSTRACT

Background and Objectives: Diabetes mellitus is a common chronic disease worldwide. Although it is treated with a number of methods including diet therapy, hypoglycemic agents, insulin and some herbs, its' prevalence and complications are rapidly increasing. *Chicory intybus* has been used as a traditional diabetes treatment in Iran, Egypt, and other countries but there is a lack of convincing evidence on its effect. This study was designed to evaluate the effects of aqueous extract of chicory leaf on body weight, serum insulin, glucose and lipids in diabetic male Wistar rats.

Materials and Methods: Forty male Wistar rats were divided into five groups. Four groups were given streptozotocin intraperitoneally to induce diabetes. Three of the diabetic groups received varying concentrations of chicory aqueous extract for three weeks (12, 25 and 50 mg /kg body weight). The forth diabetic group and the non-diabetic group received distilled water (diabetic and healthy control groups respectively). Fasting blood glucose, serum triglyceride (TG), total cholesterol (TC), LDL and HDL levels were measured after 3 weeks of treatment.

Results: The results did not show any significant effects on fasting blood glucose (173.5±126, 154.5±100, 247.7±46, 170.7±150.8 mg/dL) and serum lipids; LDL(19.5±7.7, 26.4±6.7, 24.2±7.7, 24.7±6.8 mg/dL), HDL(41±22.8, 41.2±12, 44.7±12.1, 40±4.6 mg/dL), TG (103.5±35, 80.4±53.8, 111±70, 94±8.9 mg/dL) and TC (76.2±27.3, 80.4±53.8, 77±18.4, 72.7±15 mg/dl) in Streptozotocin induced diabetic groups receiving 12, 25 and 50 mg/kg aqueous chicory extract and diabetic control groups respectively.

Conclusions: Chicory leaf aqueous extract had no significant effects on serum glucose and lipids of streptozotocin-induced diabetic rats.

Keywords: Diabetes, Chicory extracts, Blood glucose, Cholesterol, Triglyceride, Rat

Introduction

Diabetes mellitus is a very common health problem of the world, in both developed and developing countries and is one of the leading causes of death worldwide (1) . It is estimated that the number of world diabetics will exceed to over 400 million by 2030 (2). Recent studies indicate that the prevalence of diabetes in Iran, and in other developed and developing countries is increasing (3-6). Diabetes results in severe complications including hyperlipidemia, neuropathy, nephropathy, retinopathy and cardiovascular disorders and therefore impose great economic costs to the families and countries (7, 8). Diet therapy, exercise, pharmacotherapy and herbal medicines are strategies to treat diabetes mellitus. Herbs have been used for diabetes mellitus treatment in ancient Iran, China, Egypt and other countries (9, 10). Chicory intybus (*Cichorium intybus L.)*, is a traditional herbal medicine, which belongs to the *Asteraceae* family with six species mostly in Europe and Asia (11) also is known as *Kasni* in

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Iran(12). This herb is said to have a number of beneficial and therapeutic properties such as its use in the treatment of wound healing(13), depression(14), hypertension(15) jaundice (16), hiccups (17) and diabetes (18). The effects of herbs on diabetes treatment is currently the subject of vast number of studies (9, 19-26). Alkaloids, inulin, sesquiterpene lactones, coumarins, unsaturated sterols, flavonoids, saponins, tannins and other medicinal constituents, potentially effective in diabetes treatment, are detected in the chicory roots and leaves (27). The effects of ethanol extract of whole plant *Cichorium intybus* on diabetes was studied in STZ diabetic rats and were reported to reduce blood glucose, blood triglyceride and blood cholesterol (28). Ziamajidi *et al*. also reported hepatic protective effects for chicory extract in hepatic steatosis in rats (*in vivo*), and in HepG2 cells (*in vitro*) (29). The components of chicory root are different from leaves and so their effects on diabetes treatment may be different. The effects of chicory whole plant, chicory seed (29) and chicory root on blood glucose and lipids have been reported previously (28-30). However, the effects of aqueous extract of chicory leaf on weight, blood glucose and lipids need to be examined. This study was performed to evaluate the effects of chicory leaf aqueous extract on blood glucose and lipids in Streptozotocin (STZ) induced diabetic rats.

Materials and Methods

Forty adult male Wistar rats, 12 weeks of age, have randomly divided into five groups of eight. Four groups of rats were induced diabetes by intra-peritoneal injection of 50 mg/kg of STZ. The fifth group of rats did not receive STZ (healthy control). A diabetic group (diabetic control) received distilled water through gavage during the study. The third, fourth and fifth groups of diabetic rats received chicory leaf extract through the gavage (12.5, 25 and 50 milligrams/kilogram body weight, respectively).

The rats were kept in 20 ± 2°C room temperature, 12 h light/dark cycle and had free access to standard rat diet and water.

Diabetes induction: Diabetes was induced by intraperitoneal injection of 50 mg/kg body weight STZ. Blood glucose was measured using a glucometer (high precision German IME-DC) at intervals of 24 hours during the first week, and if blood glucose was above 200 mg/dl , rats were considered as diabetic (31).

Extraction: The *Chicory intybus* was collected from the margins of dry lands farming in Boirahmad, Iran. Plant leaves were detached from roots, washed gently, dried under the shadow, and powdered using a kitchen mill. One kilogram of powdered leaves was soaked in 5 liters of distilled water and was stored at room temperature for 72 hours. The soaked mixture was stirred and filtered using Whatman filter paper No 1. The dissociated liquid filtrate was concentrated using rotary apparatus under the conditions of vacuumed atmosphere and dried at 50 °C temperature. The solutions were made using dried extract according to the required concentrations.

Blood sampling and laboratory tests: After diabetes induction, chicory extract solutions were given to the rats through daily gavage for three weeks. Blood glucose of all rats were measured at the baseline (day 1), two weeks (day 14) after receiving chicory extract and at the end of the study on the last day of third week (day 21), using a high precision German IME-DC glucometer in rat tail blood.

Finally, after an overnight fasting, rats were anesthetized with *ether du petrol* and one milliliter blood was drawn from each rat's heart. Blood samples were centrifuged by 3000 RPM, the sera were separated and sent to the laboratory to measure blood glucose, serum cholesterol, LDL-C, HDL-C and triglyceride. The weight of the rats were measured at the 1st and last day of the intervention.

Data Analysis: Findings were analyzed using the SPSS version 19. Statistical significance was detected at *p* value less than 0.05. Differences between groups were tested using one way analysis of variance followed by *Scheffe post hoc* test to determine significant difference. Repeated measure ANOVA were used to test the fasting blood glucose changes in three consecutive measures. Before-after groups' mean weight were compared with paired samples *t* test.

Results

The mean weight of all groups at the baseline measurements were not significantly different. The animals final body weights (day 21) showed a significant reduction from the initial weights in all diabetic groups but the healthy control group did not show any significant weight change. The final body

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weight between the diabetic groups were not significantly different (Table 1).

Increasing fasting serum glucose in diabetic groups receiving the STZ compared with the healthy control group showed that the rats were indeed diabetic (F=7.74; p<0.001). Serum fasting glucose levels in diabetic groups were not significantly different after two weeks (F= 0.08; p=0.96), and after three weeks of extract gavage (F=0.48; p =0.7), as shown in table 2. Table 3 shows serum lipid levels of all groups. As shown, there is a clear, although non-significant difference between the lipid levels of healthy control group with diabetic groups. Serum levels of triglyceride are increased in diabetic groups but cholesterol, HDL-C, and LDL-C levels are decreased compared to the healthy control.

Table 1. Effects of chicory extract on body weight levels (gr).

Time Group	Initial	Final	Weight change	Paired t test sig
Healthy control	409.1±48	410±48.6$^{\psi}$	0.83±7.3$^{\psi}$	0.79
Diabetic control	335±75.9	247±36.8*	-88±40*	0.022
Diabetic +12.5 mg/kg extract	365.7±45.1	294.3±26.4*	-71.4±30.8*	0.001
Diabetic +25 mg/kg extract	346.7±58.6	265±25.3*	-81.7±42.5*	0.001
Diabetic +50 mg/kg extract	390±40.8	288.7±14.4*	-101.2±27.8*	0.005
ANOVA, Sig	F=1.6 p=0.2	F=18.5 P =0.0001	F=9.7 P=0.0001	

The values are mean±SD for 8 rats in each group. Means with different superscript (*, ψ) within a column are significantly different from each other at P <0.05 as determined by one way ANOVA and Scheffe post hoc test.

Table 2. Mean and standard deviation of blood glucose (mg/dl) in all groups on the baseline, two weeks and three weeks of study

Time Group	Baseline	Day 14	Day 21	P Value
Healthy control	62.6±9.3ψ			
Diabetic control	308.7±133.8*	168.7±91	173.5±126	F=2.8 , P=0.09 ANOVA Repeated measure
Diabetic +12.5 mg/kg extract	202±71*	175.8±87	154.5±100	
Diabetic +25 mg/kg extract	259.6±76*	204.3±129	247.7±46	
Diabetic +50 mg/kg extract	190.2±150*	176.2±104.7	170.7±150.8	
F ANOVA P Value	F=7.74 p<0.001	F= 0.08 P=0.96	F=0.48 p =0.7	

The values are mean±SD for 8 rats in each group. Means with different superscript (*, ψ) within a column are significantly different from each other at P <0.05 as determined by Scheffe post hoc test

Table 3. Mean and standard deviation of serum lipids levels (mg/dl) in groups of rats at the end of the experiment

Factors Group	TC	HDL	LDL	TG
Healthy control	90.8±18.4	57.7±12.6	30±6.7	53±10.7
Diabetic control	76.2±27.3	41±22.8	19.5±7.7	103.5±35
Diabetic +12.5 mg/kg extract	80.4±53.8	41.2±12	26.4±6.7	80.4±53.8
Diabetic +25 mg/kg extract	77±18.4	44.7±12.1	24.2±7.7	111±70
Diabetic +50 mg/kg extract	72.7±15	40±4.6	24.7±6.8	94±8.9
P Value	F=0.59 P=0.63	F=1.3 p=0.3	F=1.47 p=0.25	F=1.06 p=0.4

The values are mean±SD for 8 rats in each group.

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Discussion

In this study, we evaluated the effects of chicory leaf aqueous extract on blood glucose and lipids level of STZ induced diabetic rats. Our findings did not show any significant effects of chicory leaf aqueous extract on serum glucose and lipids in STZ induced diabetic rats. The outcome of the current study is contrary to Pushparaj *et al*, (28) that in their study on the effects of alcoholic extract of chicory, a 20 percent reduction in serum glucose, 91 percent in triglycerides and 16 percent reduction in the serum cholesterol of STZ-induced diabetic rats was reported. Pushparaj and colleagues used whole plant including leaves, roots and stems for alcoholic extraction and also a higher concentrations of extraction than ours (120 mg/kg vs. 12.5, 25 and 50 mg/kg in our study) that may explain the different findings in two studies. The root and stems has different compounds than leaves, especially soluble fibers. Inulin a major component of chicory root is a fructan and soluble fiber and has hypolipidemic effects through the growth of bifidobacteriaand reducing fat absorption(32).

The STZ diabetes induced rats showed a significant weight reduction compared to the control group, but Chicory leaf extract did not affect the weight of the rats or did not prevent weight reductions. Ghamarian *et al* showed that chicory seed extract prevents weight reduction in both STZ diabetic rats and Niacinamid-STZ diabetic rats (33). Ghamarian *et al* used the seed extracts injection intraperitoneal, but we used leave extract by gavage. The differences in plant part (leave against seeds) and application route (gavage against intraperitoneal injection) may explain the inconsistencies shown between the two studies.

Kim *et al* (32) had examined antilipidemic properties of aqueous extract of chicory root and showed that healthy rats receiving chicory extract had higher Serum HDL cholesterol concentrations, but reported no significant effect on cholesterol concentrations.

Khaksari and colleagues studied the effects of chicory containing diet on the blood glucose in diabetic rats. It was shown that blood glucose was decreased significantly in rats in a dose-dependent manner(30). Toush and colleagues examined the effect of chicuric acid, a component in chicory plant, on blood glucose of diabetic rats. Their findings showed that chicuric acid has the potential anti-diabetic and insulin secretagogue effect (34).

Previous studies used whole chicory plant including the leaf, root and stem (28) or the root extracts (32) or used the whole plant mixed with the rats diet(30), but we used the aqueous extract of chicory leaves. Our findings on chicory leaf extract is not similar to other studies, which may be due to the different application route and compounds in different parts of chicory. Chicory has abundant amounts of inulin in the root(35) and contain anthocyanins, phenols (36) as well as sesquiterpenes (37). Inulin is a water-soluble fiber and possesses hypolipidemic effects (38). Based on our findings chicory leaf aqueous extract is not effective in reducing blood glucose or blood lipids.

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Financial disclosure

All experimental procedures including treating the rats used in this study were conducted based on Ethical regulation of medical research on laboratory animals in Iran. The study was approved by ethic committee of Yasuj University of Medical Sciences.

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