

EFFECTS OF AQUEOUS EXTRACT OF WATER CRESS ON GLUCOSE AND LIPID PLASMA IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Background: For treating diabetic patients, different nutrients are being used in some areas of Kerman province, *Nasturtium officinale* (NF) is one of them. In current research work, effects of NF on plasma lipid and glucose levels have been assessed in diabetic rats. **Methods:** In this study, 60 male rats were used. All rats randomly divided into six groups, consisting of one intact non-diabetic group, and remaining 5 groups were injected subcutaneously of 55 mg/Kg of streptozotocin to make them experimentally diabetic. Three groups of diabetic animals were eaten orally (via gavage) of low (25 mg/Kg), and high (75 mg/Kg) doses of aqueous extract of NF in a volume of 1.5 ml for short period (4 weeks) and long period (8-weeks) respectively. One group of diabetic animals was given 2–4 U of NPH insulin intraperitoneally (IP). The last remaining group of five diabetics was given nothing at the end of each experiment. In all groups, blood glucose and lipid levels were measured. **Results:** There was significant reduction of plasma glucose in treatment groups compared to diabetic group. The greatest decrease (96%) was observed by the high dose (long term group for NF extract) that was significantly greater than the insulin group (49%) ($p < 0.001$). There wasn't any change in diabetic animals' total cholesterol, and triglyceride levels of plasma. Both low and high doses of extracts increased LDL-cholesterol levels in diabetic animals ($p < 0.001$). In diabetic animals, plasma HDL-cholesterol levels (33 ± 2.2) decreased by long term dose of extract. **Conclusion:** Both doses decreased plasma glucose in diabetic animals, whereas they have no effect on plasma lipids or have negative effect. Therefore it is suggested that NF extract is useful for control of blood glucose.

Keywords: Diabetes mellitus, Glucose, Herbal drugs, Lipids, *Nasturtium officinale*

INTRODUCTION

Diabetes mellitus is the most prevalent chronic endocrine disease in the community and there is a high incidence of mortality and morbidity in patients with diabetes. It is known that diabetes mellitus, characterized by hypoglycaemia, is a genetically and clinically heterogeneous group of disorders with common feature of glucose intolerance.¹ Based on WHO recommendation, diabetes mellitus is classified into three major subtypes: type I (insulin dependent diabetes mellitus, IDDM), type II (non-insulin dependent diabetes mellitus, NIDDM) and malnutrition-related diabetes mellitus. IDDM or Juvenile-onset diabetes results from a cellular mediated autoimmune destruction of the β -cells of the pancreas.^{2,3} However, NIDDM or adult onset diabetes results from the development of insulin resistance and the affected individuals usually have insulin deficiency.⁴ Diabetes is a major cause of disability and hospitalization. It can result in a range of complications that occur primarily in the arteries and capillaries. Diabetes patients, particularly those with type II diabetes are at considerable risk of excessive morbidity and mortality from cardiovascular, cerebrovascular, and peripheral vascular diseases leading to myocardial infarction, strokes, and amputations.⁵

The use of medicinal plants has been among the earliest treatments of diabetes mellitus.⁶⁻⁸

Nowadays, more than 1200 species of the organisms (from 725 genera belonging to 183 families) are used to treat symptoms of diabetes mellitus. Half of these species have been used traditionally for curing diabetes mellitus. The hypoglycaemic property of almost fifty percent of these traditionally consumed medicines has been experimentally tested.⁹ One of these traditional hypoglycaemic herbs is water cress,^{10,11} which belongs to *Nasturtium* family. The aqueous extract of the dried aerial parts of water cress is used traditionally to treat diabetes in Southern Iran.¹²

Watercress is a perennial plant which thrives in clear, cold water and is found in ditches and streams everywhere. Watercress is cultivated for its leaves, which are principally used as salad greens or garnishes. Connected to a creeping rootstock, the hollow, branching stem, 1–2 feet in length, generally extends with its leaves above the water. The smooth, somewhat fleshy, dark green leaves are odd-pinnate with 1–4 pairs of small, oblong or roundish leaflets. Watercress is a valuable source of vitamins and a good detoxifying herb. Its high content of vitamin C and minerals makes it a remedy that is particularly valuable for chronic illnesses. The plant is thought to stimulate the appetite and relieve indigestion, to help in cases of chronic bronchitis, to be generally stimulating, and to act as a powerful diuretic.¹³ Watercress contains vitamins A, B₁, B₂, C, and E, gluconasturtine and minerals (especially

iodine, iron, and phosphorus).^{13,14} In the other hand, watercress can inhibit tumour genesis in rodents by modulating the metabolism of carcinogens.¹⁵ Histamine release inhibitors in watercress (*Nasturtium officinale*) also were isolated using a monitoring system with antigen-stimulated RBL-2H3 cells.¹⁶

The objective of this investigation was to examine effects of watercress on the glucose and lipid plasma in a Streptozotocin (STZ)-induced diabetes rats.

MATERIALS AND METHODS

Animals: Male Mari Albino rats (n=60), with a weight of 250–300 g, were used in this study. Animals were kept in the animal room of the Kerman University of Medical Sciences at a temperature of 22 ± 2 °C with a 12 h–12 h dark/light cycle and were allowed free access to food and water *ad libitum*.¹⁷ For better results, Animals were deprived of food since 18 hours before the start of the experiment. However, they had access to water.¹⁸

Experimental groups: All animals were randomly divided into the eight groups (n=10):

- I. Control healthy rats
- II. Control Streptozotocin (STZ)-induced diabetic rats
- III. Experimental group: received 1 dose of 75 mg/Kg watercress (for 4 weeks, daily)
- IV. Experimental group: received 1 dose of 75 mg/Kg water cress (for 8 weeks, daily)
- V. Experimental group: received 1 dose of 25 mg/Kg watercress (for 4 weeks, daily)
- VI. Experimental group: received 2–4 IU of NPH insulin intraperitoneally (IP).

Induction of experimental diabetes: STZ (55 mg/Kg body weight) was dissolved in 0.01 M sodium acetate buffer at pH 4.6 just before use¹¹, and injected subcutaneously (SC) between two ears of animal. Three days after STZ administration, the diabetic rats with blood glucose concentration upper 300 mg/dl were selected and concentrated as diabetic animal.¹⁷

Plant material and total extract: Aerial parts of watercress were collected during the flowering period in the region of Kerman borders (East Iran). Samples of the plant were identified by a botanist from the Division of Pharmacognosy, Kerman University of Medical Sciences, Iran. The plant was air dried, powdered and extracted by percolation method using ethanol (80%). Then, the required doses were prepared.¹⁹

Oral administration of the plant extract: The plant extract was administrated by gavage (i.g.) to rats of group III, IV and V daily in a dose of 1.5 ml/rat (equivalent to 25 and 75 mg/kg body weight) for 4 consecutive weeks.²⁰ The control healthy rats (group I, n=10) and the control diabetic rats (group II, n=10) received the same volume of distilled water (i.g.), and administrated 2–4 IU NPH insulin/Kg IP to insulin group.²¹

Biochemical parameters assay: The hypoglycaemic activity of the plant extracts was evaluated as follows: Blood samples were collected in several stages as before the onset of experiment, 3 days after the experiment and 2, 4 and 8 weeks after experiment from rat tail tip. Blood samples were obtained by tail vein puncture of both the normal and STZ-induced diabetic rats. Blood glucose levels were determined using an auto analyser machine (Kone Specific, Fenland). The serum lipids that contain of total cholesterol, triglyceride and HDL-cholesterol (H-C) were measured in four stages with auto analyzer that include of before the experiment and 2, 4, 8 weeks later the experiment. LDL cholesterol was calculated from the above measurement by using Friedwald formula.

The data were recorded as Mean \pm SEM, and for the analysis and comparison of the data, one-way ANOVA and unpaired *t*-test were used. A *p*-value <0.05 was considered significant.

RESULTS

Prior to the extract administration, there was significant difference ($p<0.001$) between blood glucose level of the two diabetic and control groups (I and II) (149 ± 12.8 and 528 ± 30 mg/dl, respectively), (Figure-1).

Blood glucose level of all treated rats was significantly lower than the diabetic group ($p<0.001$), (Figure-2). The most reduction was seen in the group IV (96%) that was significantly greater than the insulin group (49%) ($p<0.001$). In contrast, the blood glucose level of the healthy (group I) remained unchanged during the course of the investigation. In addition, the blood glucose level of treated diabetic rats was significantly reduced compared to the insulin rats.

The changes in level of serum lipids in control and experimental rats are illustrated in Table-1. The results revealed that serum total cholesterol (Cho) was significantly increased in groups IV (66.8 ± 6.2 mg/dl) ($p<0.001$) and V (67.2 ± 2.4 mg/dl) ($p<0.01$) after watercress administration compared to the control group (48 ± 1.5), (Table-1).

Comparison of serum TG in all groups did not show any significant changes. But serum HDL-cholesterol level of treated diabetic rats with 75 mg/Kg dosage (8 weeks) of watercress was significantly reduced (17.4 ± 2 mg/dl) compared to other groups ($p<0.01$), (Table-1).

Serum LDL-cholesterol level in treated group with 25 mg/Kg dosage was $32/1\pm 1/66$ that its level is higher than the other groups, in exception group IV ($p<0.01$). In addition, the blood LDL-cholesterol levels of the treated diabetic rats with 75 mg/Kg of watercress for 8 weeks were significantly increased (42.4 ± 4.13) compared to the all other groups, in exception group V.

Table-1: Comparison of total cholesterol, triglycerides, HDL- and LDL-cholesterol in different groups

Parameter \ Group	Control	Diabetic	25 (mg/dl)	75(1) (mg/dl)	75(2) (mg/dl)	Insulin
Total cholesterol (mg/dl)	48±1.5	55±3.9	67.2±2.4 ^a	52.8±3.54	66.8±6.2 ^b	56±2.7
Triglyceride (mg/dl)	23.8±1.57	17±3.36	18.7±4.6	21.8±2.3	36±13.1	34.5±2
HDL-cholesterol (mg/dl)	28±1.3	33±2.17	31.4±1.87	31±1.84	17.4±2 ^c	32.1±1.27
LDL-cholesterol (mg/dl)	15.3±1.64	18.2±2	32.1±1.66 ^d	16.7±2.76	42.4±4.13 ^e	16.8±2.1

n=10 in each group; Data are expressed as Mean±SEM. 25 mg/dl: dose of 25 mg/ dl of watercress for 4 weeks; 75(1) mg/dl: dose of 75 mg/dl of watercress for 4 weeks; 75(2) mg/dl: dose of 75 mg/dl of watercress for 8 weeks. a=($p<0.01$) vs control group, b=($p<0.01$) vs control group, c=($p<0.01$) vs other groups, d=($p<0.01$) vs control, diabetic, 75(1) and insulin groups, e=($p<0.01$) vs. control, diabetic, 75(1) and insulin groups.

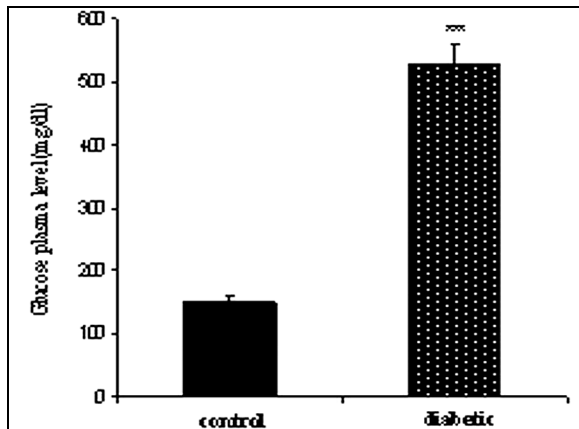


Figure-1: Comparison of plasma glucose level in control and diabetic groups, n=10 in each group.
(Data are expressed as Mean±SEM. *** $p<0.005$ vs control group)

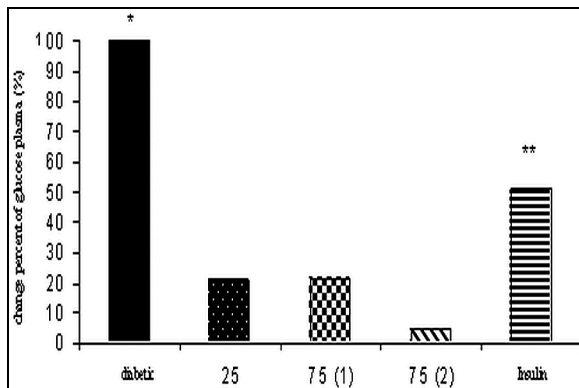


Figure-2: Comparison of change percent of plasma glucose (%) in treated and diabetic groups. n=10 in each group (Mean±SEM)

25 mg/dl= dose of 25 mg/ dl of watercress for 4 weeks; 75(1) mg/dl= dose of 75 mg/ dl of watercress for 4 weeks; 75(2) mg/dl= dose of 75 mg/ dl of watercress for 8 weeks. * $p<0.005$ v s other groups. ** $p<0.005$ vs other groups.

DISCUSSION

The results of this study indicate that the aqueous extract of watercress has a hypoglycaemic effect. In our study we have observed that the blood glucose levels of the diabetic group were increased significantly compared to the control group and the both dosage of watercress were significantly reduced the blood glucose level by 80–90% which is higher than the insulin effect only (49%). The prolonged

consumption of watercress (8 weeks) has better effect than the short-term (4 weeks). It is probable that watercress could effect on the blood glucose levels by these mechanisms:

The major compound of watercress is vitamin C. On the other hand, in diabetes mellitus, vitamin C metabolism is abnormal, and subjects have been shown to have low vitamin C and high dehydro-L-ascorbic acid concentrations in plasma. It has been shown in various studies that diabetes mellitus is associated with increased formation of free radicals, and with heavy oxidative stress.^{22–24} This increase may result from vitamin C deficiency. The deficiency may be replenished, after usage of watercress.

Cu⁺² is the other major compound of watercress²⁵ and there are some reports that the Cu⁺² deficiency may cause hyperglycemia.²⁶

Mn⁺² is the another element in the watercress which is also necessary for carbohydrate metabolism. Fe⁺² is also present in the watercress. The reduction in plasma glucose level by watercress consumption may be caused by Fe⁺² because glucose tolerance was better in the offspring of iron-restricted dams compared with controls.²⁷ In addition, Vitamin D deficiency is the other cause of diabetic mellitus type II because in individuals with impaired glucose tolerance, such as type 2 diabetes, lower serum 25 (OH) D levels have been documented.²⁸ Vitamin D may compensate this deficiency. The increment of Ca⁺² concentration by this plant may improve the glucose metabolism because Ca⁺² pump activity is altered in diabetic patients.²⁸

Additionally, Isosulfocianid benzene is also present in the watercress but, there is no change in the plasma glucose level after its usage. Probably, this compound doesn't have effect on the reduction of plasma glucose level.²⁹

The mechanism of reduction in blood glucose level by the components of watercress is as follows:

Induction of glucose consumption of insulin by vitamin C,³⁰ reduction of oxidative stress and elimination of glutathione,²⁴ reduction of glycosylated insulin in pancreas that is followed by reduction of resistance to insulin by vitamin C,²³

improvement of β -cell function by vitamin C and increase in insulin plasma level,³⁰ reduction of resistance to insulin by Cu^{+2} ,³¹ insulin-like effects of Cu^{+2} and Mn^{+2} ,³² hypersensitivity to insulin in response to vitamin D,³³ enhancement of insulin secretion in response to glucose by Fe^{+2} ,³⁴ and increase of NO production in response to calcium in β -cells that is followed by increment in insulin secretion.³⁵

Reports of other authors confirm our above observations. Borcea *et al* have demonstrated that vitamin C supplementation improves the consumption of glucose in diabetic patients.³⁶ Additionally, it has been proposed that vitamin C therapy improves β -cell function and tissues insulin resistance increases plasma insulin and can lower blood glucose and glycated haemoglobin levels. The vitamin C supplementation may strengthen the antioxidant defence due to reducing blood glucose.^{22,30} The reduction of plasma glucose level by Mn^{+2} ,²⁶ vitamin D,²⁸ and Fe^{+2} ,²⁷ is also reported.

In our study, a significant reduction in plasma HDL-cholesterol levels was observed after long-term consumption, but no changes were found in plasma total cholesterol, triglycerides levels. In contrast, the LDL-cholesterol level was enhanced with low and high doses of watercress.

Okolize *et al* demonstrated a significant enhancement in the total cholesterol, triglyceride plasma levels with fenil isotiosianate.²⁹ In addition, Paul *et al*, also reported that there are no overall effect of vitamin C supplementation on plasma concentration of HDL and LDL or total cholesterol.³¹ Moshtaghi *et al* found that the plasma TG level was enhanced after Mn^{+2} consumption.³² Pajohesh *et al* reported that vitamin D₃ was enhanced LDL, cholesterol plasma levels.³³ However, our finding differs from other studies in some aspects: LDL and triglyceride plasma levels reduction by vitamin C in type II diabetes mellitus,³⁶ reduction of plasma total cholesterol and TG levels after vitamin C therapy,³⁰ reduction of total cholesterol and LDL-cholesterol by vitamin D₃,³⁴ and TG reduction by Fe^{2+} .

CONCLUSION

The aqueous extract of the aerial parts of watercress showed anti-diabetic effect without any negative effect on the plasma lipids. Therefore, this plant may have some clinical benefits for diabetic disorders. However, further studies are needed to determine possible mechanisms of action, establish safety profiles of the extract, and evaluate potential value of watercress for management of diabetic disorders.

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DISCLOSURE

An article based on this study with a different title has been published in part. Persian version of the same article has been printed in Journal of Rafsanjan University of Medical Sciences and Health Services Winter 2008;6(4(25)):245–54 by the same authors.

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