

Article:

Impact of watercress (*nasturtium officinale*) aqueous extract on biochemical markers of diabetic rats

Sara R. Thabet¹, Nagwa S. Ahmed², Ahmed S. Osman¹.

¹Department of Biochemistry, Faculty of Veterinary Medicine, Sohag University, Sohag.

²Department of Medical Biochemistry, Faculty of Medicine, Sohag University, Sohag.

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Abstract

Diabetes mellitus is a common chronic metabolic disease that seriously harms human health. Therefore, there is a need to develop recent co-treatment strategies of plant origin which might have no side effects and are cost-effective. Watercress (*Nasturtium Officinale*) has anti-diabetic, antioxidant, and anti-inflammatory effects as documented previously. This study was conducted on 60 healthy male albino rats to investigate fasting blood glucose, insulin hormone, blood lipid profile (triglycerides, total cholesterol, LDL, and HDL), kidney functions (creatinine and urea), liver functions (ALT and AST) and cumulative blood sugar (HbA1c). The study was designed by dividing the rats into 4 groups: Group 1 (Negative Control Group), Group 2 (Diabetes positive Control injected with (45mg/kg body weight) Streptozotocin intraperitoneally), Group 3 (100 mg watercress /Kg body weight for 8 weeks, daily) and Group 4 (200 mg watercress /Kg body weight for 8 weeks, daily). Blood was collected after scarification on weeks 2, 4, 6, and 8 of the experiment for serum separation, and blood was collected on EDTA tubes for HbA1c at week 8 only. The results of this study indicated that the treated group exhibited a significant and progressive decrease in fasting blood glucose, triglycerides, total cholesterol, LDL, HDL, creatinine, urea, ALT, and AST and a significant and progressive increase in insulin hormone at all experiment times compared to the controls. Shifting to HbA1c, the 3rd and 4th groups showed a significant decrease compared with the controls at 8 weeks of the experiment. We concluded that administration of watercress aqueous extract at 200 mg watercress /kg body weight has hypoglycemic and hypolipidemic activity.

Keywords: Diabetes, Watercress (*Nasturtium officinale*), hypoglycemic activity, hypolipidemic activity.

Introduction

Diabetes mellitus (DM) is one of the most common metabolic disorders that is increasing at an alarming rate all over the world. Every year, a major portion of the annual health budget is spent on diabetes and related illnesses. In 2013 it was estimated that over 382 million people throughout the world had diabetes. The WHO estimates that diabetes will be the 7th primary cause of fatality by 2030 (Alam et al., 2021).

Diabetes mellitus is a complex metabolic disease correlated with a series of metabolic diseases characterized by

hyperglycemia caused by insulin insufficiency, insulin resistance, or both (AlSaeedi et al., 2021). There are four main common types of DM. Type 1 DM, Type 2 DM, gestational diabetes mellitus (GDM) and Monogenic diabetes (Alam et al., 2021). Long-term blood glucose increase is linked to macro-and microvascular complications, which can result in heart disease, stroke, blindness, and renal disease. Other factors such as hyperlipidemia and oxidative stress, in addition to hyperglycemia, play a key part in diabetic pathogenesis, raising the risk of complications (Kangralkar et al., 2010).

Streptozotocin was shown to be capable of generating peripheral insulin resistance or reducing insulin release from these cells, in addition to its capacity to produce insulin-dependent Diabetes mellitus (type 1) by total loss or destruction of pancreatic β cells. STZ can cause mild to severe hyperglycemia in animals, depending on the dose, strain, and age of the animals, nutritional state, and route of administration, among other conditions (Hayashi et al., 2006).

Nowadays, medicinal plants are resources of novel drugs, and many modernized medicines are originated from plants (Shakya, 2016). Watercress leaves are traditionally used as stomachic, depurative, diuretic, expectorant, hypoglycemic, odontalgic, and stimulant. Meanwhile, it has been used to treat jaundice, asthma, bronchitis, scurvy, tuberculosis, urinary tract infections, and calculi. *N. officinale* is rich in glucosinolates, carotenoids, and polyphenols, as well as Vitamin C, Vitamin A, and α -tocopherol. It is the main source of iron, calcium, iodine, and folic acid (Chaudhary et al., 2018).

Watercress (*Nasturtium officinale*), a member of the Brassicaceae, is a perennial aquatic or semi-aquatic, high-value, wild herb used for culinary purposes by people almost around the world, native to Western Asia, India, Europe, and Africa. However, it is now distributed almost globally. It has attractive dark green leaves, a strong flavor, and is rich in vitamins (Yamuna Pandey et al., 2018).

This work aims to investigate the biochemical effects of watercress aqueous extract for streptozotocin-induced diabetic rats including fasting blood glucose, insulin hormone, triglycerides, total cholesterol, low-density lipoproteins (LDL), high-density lipoproteins (HDL), creatinine, urea, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and Cumulative blood sugar: Glycosylated hemoglobin (HbA1c)

Materials and Methods

Ethical Considerations: Animal handling and rights have been approved by the Veterinary Medical Research Ethics Committee, Faculty of Veterinary Medicine, Sohag University, Sohag, Egypt (Approval number: Soh.un.vet /00029R).

Materials:

Animals: 60 Healthy Male Albino Rats, 3 months old, 320 \pm 20-gram body weight, were purchased from the Faculty of Science, Sohag University, and were housed in Animal Laboratory in Sohag Faculty of Science, at a temperature of 22 \pm 2 °C with a 12 h–12 h dark/light cycle and were allowed free access to food and water ad libitum. Rats were housed

in groups in 8 metal cages (75 \times 50 \times 35cm). Animals were deprived of food for 18 hours before the start of the experiment. Rats were put under observation for one week before the experiment.

Chemicals: Streptozotocin, Trisodium citrate dihydrate, and Citric acid monohydrate were purchased from Sigma-Aldrich Company (Sigma-Aldrich St. Louis, MO, USA).

Analytical kits:

a) Blood glucose test strips (on call plus, Acon laboratories, Inc.) were used for the estimation of blood glucose level.

b) ELISA kits: Insulin kit (Catalog No:201-11-0708) Purchased from SUNRED Company, China.

c) Kits of triglycerides, total cholesterol, HDL, LDL, creatinine, urea, ALT, and AST were purchased from Chema Diagnostica company (Via Campania, Monsano, Italy) and Spectrum company (21, Misr El Taameer Bldgs Zone one Masaken Elshraton 11361, Cairo, Egypt).

Experimental design: The experiment was designed in 4 groups (n=15) to finalize the aims of this study:

Group 1: (Negative Control group): Control healthy rats. This group of rats received standard rat ration and drinking water.

Group 2: (Diabetes Positive Control): Streptozotocin (STZ)-induced diabetic rats injected with (45mg/kg body weight) Streptozotocin intraperitoneally.

Group 3: STZ-induced diabetic rats received 1 dose of 100 mg watercress /Kg body weight (for 8 weeks, daily).

Group 4: STZ-induced diabetic rats received 1 dose of 200 mg watercress /Kg body weight (for 8 weeks, daily).

Methods

Induction of diabetes: The overnight fasted rats were rendered diabetic by a single intraperitoneal injection of 45 mg/kg streptozotocin (Sigma- Aldrich, USA) freshly dissolved in 0.1 M. citrate buffer (pH 4.5) (Salah-Eldin et al., 2015). Diabetes was identified by polydipsia and polyuria and by measuring blood glucose levels 48 h after injection of STZ in a blood sample obtained by tail prick, using a glucometer (On call plus, ACON Laboratories, Germany). Only STZ-injected rats with blood glucose levels of 250 mg/dl or more were selected as diabetics for the following experiment. Rats were given free access to standard ration and water after receiving STZ, as well as a 15% glucose solution added to the drinking water to prevent hypoglycemic shock The day on which hyperglycemia had been confirmed was designated as day zero (0).

Plant material and aqueous extract: Aerial parts of the watercress (*Nasturtium officinale*) were obtained from an accredited supplier, in Akhmim city (Sohag, Egypt). Samples of the plant were identified by a botanist from the Division of Botany, Faculty of Science, Sohag University, Egypt. The plant was dried in shadow (green watercress contains 10% dry matter and each 1 gram dry matter contains 0.375 gram active principle) then boiled in distilled water, filtered and the filtrated solution was the extract.

Oral administration of the plant extract: The plant extract was administrated by gavage (i.g.) to rats of groups 3 and 4 daily in a dose of 1 ml/rat (equivalent to 100 and 200 mg/kg body weight) for 8 weeks (Shahrokhi et al., 2009). The control healthy rats (group 1, n=15) and the control diabetic rats (group 2, n=15) received the same volume of distilled water (i.g.).

Samples collection:

A. Blood samples used for determination of fasting blood sugar: During the experiment, the animal's fasting blood glucose levels were measured by sterilizing their tails with 10% alcohol, taking a blood sample via tail prick, and allowing the blood to touch a test strip that was inserted into a calibrated glucose meter (On-Call Plus Glucometer, ACON Laboratories, Germany). After 5 seconds, a direct reading in mg/dL was obtained (Airaodion et al., 2019).

B. Blood samples used for serum separation: Blood samples were obtained by scarification of both the normal and STZ-induced diabetic rats into three clean, dry, sterile, and labeled centrifuged tubes from three rats of each group in the indicating time: at 2, 4, 6, and 8 weeks. The tubes without anticoagulant where 3 ml of blood were allowed to flow smoothly into labeled centrifuge tubes, left to clot and the serum was obtained by centrifugation at 3000 r.p.m for 10 minutes. The clear supernatant serum was collected into dry, sterile, and labeled Eppendorf tubes. The serum was subsequently stored at -20°C until further biochemical analysis and hormonal assay.

C. Whole blood samples: Rats were sacrificed at the end of the experiment (at 8 weeks), and individual blood samples from each group were collected in dry and clean tubes containing EDTA (Ethylenediamine-tetra acetic acid) as an anticoagulant for assessment of glycosylated hemoglobin.

Biochemical analysis:

A. Determination of fasting blood glucose level: Fasting blood glucose level was measured continuously during the experiment at 2, 4, 6, and 8 weeks.

B. Estimation of serum insulin: By the Iflash 1800 chemiluminescence immunoassay analyzer (Shenzhen Yhlo Biotech Co. China), the level of serum insulin was determined (Goodman, 1996) (Chevenne et al., 1999).

C. Estimation of lipogram, kidney function tests, and liver function tests: By T80 UV spectrophotometer (PG Instruments company, UK), the levels of triglycerides, total cholesterol, LDL, and HDL (Naghsh and Kazemi, 2014) creatinine and urea (Tietz, 1994), as well as activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) (Tietz, 1994), were determined.

D. Determination of cumulative blood sugar: By Arkary Automatic Glycohemoglobin Analyzer (ADAMS A1c HA-8190V, ADAMS A1c, HA-8190V) is a fully automated Glycohemoglobin. (HbA1c) analyzer based on HPLC (High-Performance Liquid Chromatography). HA-8190V detects and separates variant hemoglobin automatically (Weykamp et al., 2011).

Statistical analysis:

Data were analyzed by using GraphPad Prism 9 (GraphPad, Inc., San Diego, CA). One-way ANOVA (Tukey's test) was used for multiple comparisons to detect differences between groups. All data were expressed as mean \pm standard deviation of the mean (SD). Statistical significance was considered at a level of $P < 0.05$.

Results

The results obtained in this study were statistically analyzed, and the mean and standard deviation values of the biochemical parameters (Fasting blood glucose, insulin hormone, triglycerides, total cholesterol, LDL, HDL, creatinine, urea, ALT, AST, and HbA1c) of the Streptozotocin-induced diabetic rats in the treated and control groups were presented in tables 1 to 11.

Fasting blood glucose levels significantly and progressively decreased ($p < 0.05$) from 2 weeks to the experimental end (8 weeks) in 3rd and 4th groups compared to the control groups while Insulin hormone levels non significantly increased ($p < 0.05$) from 2 weeks and significantly increased from 4 weeks to the experimental end (8 weeks) in the 3rd and 4th groups compared to the control groups as in Tables (1) and (2).

Table 1: Effects of watercress aqueous extract on fasting blood glucose levels (mg/dl) in experimental rats:

| Experimental periods | Groups | | | | P value |
|----------------------|--------------------------------|-------------------------------|-------------------------------|-------------------------------|---------|
| | Group 1 | Group 2 | Group 3 | Group 4 | |
| 2 weeks | 100.33 ± 11.68 ^a | 465 ± 16.7 ^c | 363 ± 54.02 ^b | 377.3 ± 38.07 ^b | 0.001 |
| 4 weeks | 102.7 ± 14.01 ^a | 449.3 ± 43.14 ^c | 354 ± 9.64 ^b | 316.6 ± 45.36 ^b | 0.001 |
| 6 weeks | 102.6 ± 6.65 ^a | 475 ± 40.28 ^d | 317 ± 15.71 ^c | 215 ± 7.21 ^b | 0.001 |
| 8 weeks | 10367 ± 6.03 ^a | 496 ± 18.68 ^d | 286.3 ± 17.09 ^c | 189.7 ± 11.5 ^b | 0.000 |

A, b, c, d in the same row means that there were significant differences between the collected samples at $p < 0.05$.

Table 2 Effects of watercress aqueous extract on insulin hormone (μ IU/ml) in experimental rats:

| Experimental periods | Groups | | | | P value |
|----------------------|-----------------------------|-----------------------------|------------------------------|------------------------------|---------|
| | Group 1 | Group 2 | Group 3 | Group 4 | |
| 2 weeks | 1.62 ± 0.02 ^b | 1.05 ± 0.04 ^a | 1.24 ± 0.18 ^a | 1.21 ± 0.16 ^a | 0.003 |
| 4 weeks | 1.6 ± 0.03 ^b | 1.09 ± 0.19 ^a | 1.26 ± 0.19 ^{ab} | 1.3 ± 0.09 ^{ab} | 0.015 |
| 6 weeks | 1.61 ± 0.03 ^c | 0.94 ± 0.13 ^a | 1.26 ± 0.05 ^b | 1.41 ± 0.14 ^{bc} | 0.001 |
| 8 weeks | 1.61 ± 0.05 ^c | 1 ± 0.03 ^a | 1.3 ± 0.12 ^b | 1.52 ± 0.08 ^c | 0.001 |

A, b, c in the same row means that there were significant differences between the collected samples at $p < 0.05$.

Serum triglycerides and total cholesterol levels significantly and progressively decreased ($p < 0.05$) from 4 weeks to the experimental end (8 weeks) in the 3rd and 4th groups compared to the controls as in Tables (3) and (4) respectively.

LDL levels significantly and progressively decreased ($p < 0.05$) from 4 weeks while HDL levels significantly and progressively decreased ($p < 0.05$) from 2 weeks to the experimental end (8 weeks) in 3rd and 4th groups compared to the controls as shown in Tables (5) and (6) respectively.

Creatinine and urea of the investigated rats significantly and progressively decreased ($p < 0.05$) from 4 weeks to the end of the experiment (8 weeks) in the 3rd and 4th groups compared to the controls as in Tables (7) and (8).

Activities of ALT and AST significantly and progressively decreased ($p < 0.05$) from 2 weeks to the end of the experiment (8 weeks) in the 3rd and 4th groups compared to the controls as shown in Tables (9) and (10).

Administration of watercress significantly reduced HbA1c levels in diabetic rats at 8 weeks as in Table (11).

Table 3: Effects of watercress aqueous extract on serum triglycerides (mg/dl) in experimental rats:

| Experimental periods | Groups | | | | P value |
|----------------------|------------------------------|------------------------------|------------------------------|------------------------------|---------|
| | Group 1 | Group 2 | Group 3 | Group 4 | |
| 2 weeks | 61.67 ± 1.17 ^a | 82.63 ± 2.53 ^b | 82.5 ± 1.45 ^b | 80.66 ± 1.24 ^b | 0.001 |
| 4 weeks | 61.9 ± 1.05 ^a | 85.13 ± 1.6 ^c | 82.6 ± 1.77 ^{ab} | 80.08 ± 0.8 ^b | 0.001 |
| 6 weeks | 61.36 ± 1.09 ^a | 83.9 ± 1.66 ^d | 79.46 ± 0.85 ^c | 76.7 ± 1.46 ^b | 0.001 |
| 8 weeks | 61.4 ± 0.55 ^a | 84.7 ± 1.57 ^d | 76.72 ± 1.54 ^c | 73.4 ± 1.55 ^b | 0.000 |

A, b, c, d in the same row means that there were significant differences between the collected samples at $p < 0.05$.

Table 4: Effects of watercress aqueous extract on serum total cholesterol (mg/dl) in experimental rats:

| Experimental periods | Groups | | | | P value |
|----------------------|------------------------------|------------------------------|------------------------------|------------------------------|---------|
| | Group 1 | Group 2 | Group 3 | Group 4 | |
| 2 weeks | 51.63 ± 1.36 ^a | 62.9 ± 4.04 ^b | 60.6 ± 1.55 ^b | 59.11 ± 1.03 ^b | 0.002 |
| 4 weeks | 50.9 ± 1.6 ^a | 63.7 ± 2.4 ^c | 58.03 ± 1.5 ^b | 56.23 ± 0.7 ^b | 0.001 |
| 6 weeks | 51.16 ± 1.46 ^a | 62.2 ± 2.45 ^c | 55.86 ± 1.24 ^b | 53.3 ± 0.61 ^{ab} | 0.001 |
| 8 weeks | 50.63 ± 0.7 ^a | 61.63 ± 1.83 ^b | 53.06 ± 1.2 ^a | 51.63 ± 1.46 ^a | 0.001 |

A, b, c, d in the same row means that there were significant differences between the collected samples at $p < 0.05$

Table 5: Effects of watercress aqueous extract on serum LDL (mg/dl) in experimental rats:

| Experimental periods | Groups | | | | P value |
|----------------------|------------------------------|------------------------------|-------------------------------|------------------------------|---------|
| | Group 1 | Group 2 | Group 3 | Group 4 | |
| 2 weeks | 12.7 ± 1.53 ^a | 20.7 ± 2.77 ^b | 20.04 ± 1.24 ^b | 18.03 ± 0.15 ^b | 0.002 |
| 4 weeks | 12.76 ± 1.07 ^a | 20.16 ± 0.97 ^c | 18.46 ± 0.72 ^{bc} | 17.47 ± 0.65 ^b | 0.001 |
| 6 weeks | 12.73 ± 0.64 ^a | 20.26 ± 0.46 ^c | 17.53 ± 0.5 ^b | 17.06 ± 0.21 ^b | 0.000 |
| 8 weeks | 12.7 ± 1.14 ^a | 20 ± 0.95 ^c | 17.07 ± 1.01 ^b | 16.3 ± 0.61 ^b | 0.001 |

A, b, c, d in the same row means that there were significant differences between the collected samples at $p < 0.05$.

Table 6: Effects of watercress aqueous extract on serum HDL (mg/dl) in experimental rats:

| Experimental periods | Groups | | | | P value |
|----------------------|-------------------|-------------------|-------------------|-------------------|---------|
| | Group 1 | Group 2 | Group 3 | Group 4 | |
| 2 weeks | 29.2 ± 2.08 a | 40.2 ± 1.63 c | 34.43 ± 2.28 b | 33.5 ± 1.9 b | 0.001 |
| 4 weeks | 28.9 ± 1.15 a | 38.7 ± 0.75 c | 33.6 ± 1.93 b | 32.88 ± 1.66 b | 0.003 |
| 6 weeks | 29.13 ± 1.2 a | 39.2 ± 0.92 c | 31.9 ± 1.65 b | 31.97 ± 1.01 b | 0.004 |
| 8 weeks | 29.53 ± 1.55 a | 39.13 ± 1.72 b | 31.8 ± 1.74 a | 31.56 ± 2.02 a | 0.001 |

A, b, c in the same row means that there were significant differences between the collected samples at $p < 0.05$.

Table 7: Effects of watercress aqueous extract on serum creatinine (mg/dl) in experimental rats:

| Experimental periods | Groups | | | | P value |
|----------------------|------------------|------------------|------------------|-------------------|---------|
| | Group 1 | Group 2 | Group 3 | Group 4 | |
| 2 weeks | 0.63 ± 0.06 a | 0.94 ± 0.13 b | 0.84 ± 0.09 b | 0.79 ± 0.07 ab | 0.02 |
| 4 weeks | 0.58 ± 0.03 a | 0.98 ± 0.16 c | 0.8 ± 0.14 bc | 0.74 ± 0.05 ab | 0.01 |
| 6 weeks | 0.6 ± 0.04 a | 0.98 ± 0.15 b | 0.74 ± 0.06 a | 0.65 ± 0.05 a | 0.009 |
| 8 weeks | 0.6 ± 0.02 a | 0.91 ± 0.14 b | 0.67 ± 0.07 a | 0.62 ± 0.04 a | 0.006 |

A, b, c in the same row means that there were significant differences between the collected samples at $p < 0.05$.

Table 8: Effects of watercress aqueous extract on serum urea (mg/dl) in experimental rats:

| Experimental periods | Groups | | | | P value |
|----------------------|-------------------|-------------------|-------------------|-------------------|---------|
| | Group 1 | Group 2 | Group 3 | Group 4 | |
| 2 weeks | 54 ± 2.64 a | 82.35 ± 4.19 b | 82.26 ± 2.08 b | 79.11 ± 1.15 b | 0.001 |
| 4 weeks | 54.76 ± 4.21 a | 86.6 ± 2.62 c | 79.8 ± 2.9 bc | 75.1 ± 4.47 b | 0.001 |
| 6 weeks | 55.4 ± 2.65 a | 85.8 ± 2.58 d | 79.2 ± 2.21 c | 73.3 ± 2.14 b | 0.000 |
| 8 weeks | 53 ± 3.1 a | 84.2 ± 3.3 d | 74.3 ± 3.01 c | 68.1 ± 1.9 b | 0.000 |

A, b, c, d in the same row means that there were significant differences between the collected samples at $p < 0.05$.

Table 9: Effects of watercress aqueous extract on serum ALT (U/L) in experimental rats:

| Experimental periods | Groups | | | | P value |
|----------------------|------------------|-------------------|-------------------|-------------------|---------|
| | Group 1 | Group 2 | Group 3 | Group 4 | |
| 2 weeks | 86.6 ± 3.51 a | 223 ± 6.24 d | 203.3 ± 10.2 c | 190.2 ± 4.04 b | 0.001 |
| 4 weeks | 86.3 ± 4.5 a | 223 ± 7.93 d | 188.3 ± 2.89 c | 171.6 ± 5.68 b | 0.001 |
| 6 weeks | 87 ± 2.64 a | 226 ± 7.54 d | 178 ± 4.35 c | 165 ± 5.56 b | 0.000 |
| 8 weeks | 86.6 ± 4.04 a | 229.3 ± 6.11 c | 161.3 ± 4.16 b | 149.3 ± 9.01 b | 0.000 |

A, b, c, d in the same row means that there were significant differences between the collected samples at $p < 0.05$.

Table 10: Effects of watercress aqueous extract on serum AST (U/L) in experimental rats:

| Experimental periods | Groups | | | | P value |
|----------------------|-------------------|-------------------|-------------------|-------------------|---------|
| | Group 1 | Group 2 | Group 3 | Group 4 | |
| 2 weeks | 218.6 ± 4.04 a | 313 ± 7.5 c | 299.7 ± 8.73 b | 288.3 ± 4.93 b | 0.001 |
| 4 weeks | 220.3 ± 6.11 a | 314 ± 5.56 d | 285 ± 4.58 c | 271 ± 3 b | 0.001 |
| 6 weeks | 217.5 ± 4.16 a | 312.6 ± 9.8 d | 270.3 ± 8.32 c | 253.6 ± 5.68 b | 0.000 |
| 8 weeks | 218.6 ± 4.5 a | 314.2 ± 7.93 c | 258.6 ± 7.37 b | 245.7 ± 8.32 b | 0.001 |

A, b, c, d in the same row means that there were significant differences between the collected samples at $p < 0.05$

Table 11: Effects of watercress aqueous extract on HbA1c (%) in experimental rats (at 8 weeks):

| Experimental periods | Groups | | | | P value |
|----------------------|------------------|-------------------|------------------|------------------|---------|
| | Group 1 | Group 2 | Group 3 | Group 4 | |
| 8 weeks | 4.19 ± 0.49 a | 11.25 ± 1.55 d | 9.32 ± 0.81 c | 7.28 ± 0.76 b | 0.001 |

A, b, c, d in the same row means that there were significant differences between the collected samples at $p < 0.05$.

Discussion

This work was carried out to evaluate the biochemical parameters including fasting blood glucose, insulin hormone, triglycerides, total cholesterol, low-density lipoproteins (LDL), high-density lipoproteins (HDL), creatinine, urea, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and Cumulative blood sugar: Glycosylated hemoglobin (HbA1c) of streptozotocin-induced diabetic rats received watercress aqueous extract.

Fasting glucose measurement is used in clinics to diagnose diabetes mellitus. It evaluates the hepatic production of glucose (gluconeogenesis) from glycogen, lactate, or amino acids during fasting periods. Using medicinal plants and animal models, this test provides the means to measure the hypoglycemic activity of an extract by evaluating its effect on hepatic glucose metabolism, specifically in gluconeogenesis (Andrade-Cetto, 2012).

The results of this study indicate that the aqueous extract of watercress has a hypoglycemic effect. In our study, we observed that the fasting blood glucose levels significantly and progressively decreased ($p < 0.05$) from 2 weeks to the experimental end (8 weeks) in 3rd and 4th groups compared to the control groups. Insulin hormone levels non significantly increased ($p < 0.05$) from 2 weeks and significantly increased from 4 weeks to the experimental end (8 weeks) in the 3rd and 4th groups compared to the control groups. The possible mechanism by which watercress extract exerts its hypoglycemic action may be due to a stimulation of Langerhans islets, improving insulin secretion, an improvement of peripheral sensitivity to remnant insulin, and to the antioxidant properties of watercress (Hadjzadeh et al., 2015).

Also, watercress could probably affect blood glucose levels through these mechanisms:

1-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 (Ozkan et al., 2005) This increase may result from vitamin C deficiency. The deficiency may be replenished, after usage of watercress.

2- Cu^{+2} is the other major compound of watercress (Palaniswamy et al., 2003) and there are some reports that Cu^{+2} deficiency may cause hyperglycemia (Nazari and Nourmohammadi, 1998).

3- Mn^{+2} is 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 (Lewis and Petry, 2001).

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 (Naziroglu et al., 2004), reduction of oxidative stress and elimination of glutathione (Ozkan et al., 2005), reduction of glycosylated insulin in pancreas that is followed by reduction of resistance to insulin by vitamin C (Yasser et al., 2002), improvement of β -cell function by vitamin C and increase in insulin plasma level (Naziroglu et al., 2004), reduction of resistance to insulin by Cu^{+2} , insulin-like effects of Cu^{+2} and Mn^{+2} (Moshtaghi and Ani, 2001), hypersensitivity to insulin in response to vitamin D, enhancement of insulin secretion in response to glucose by Fe^{+2} (Wang and Tuohimaa, 2006), and increase of NO production in response to calcium in β -cells that is followed by increment in insulin secretion (Barceloux, 1999).

These current results were confirmed by the findings of Fenton-Navarro (2018) who showed that the hypoglycemic effect of aqueous watercress extract during acute mode was 76.6% more than that of insulin, and once aqueous watercress was used in the chronic period, the levels of glucose reached the normal range during the third week to the eighth week of the experiment (Hadjzadeh et al., 2015).

Similar to the present study, Syamsinah and Anggraini (2016) demonstrated that a raw food (the mixture of 1 portion of WC and 1 portion of black rice bran) for 4 weeks was able to lower the blood sugar content of the DM experimental animals and Hadjzadeh et al., (2015) showed that the treatment of diabetic rats with hydroalcoholic extract of WC (200 mg/kg) leaves over four weeks outstandingly mitigated serum glucose in comparison to diabetic untreated rats.

These findings agree with those results reported by Hosseini et al. (2009), who showed that ethyl acetate extract of aerial parts of watercress at a dose of 100 mg/kg

decreased the blood glucose levels in diabetic animals after 2 months of treatment. However, their results also showed that the aqueous and methanolic extract of aerial parts of watercress at doses of 800 and 1000 mg/kg had no effect on blood glucose levels after 1 week of treatment. This discrepancy between our results and their study could be due to the type of extract and duration of the treatment.

Confirming our results, Engelen et al., (2006) reported that watercress comprised glucosinolates (gluconasturtiin), which has been traditionally used for the treatment of diabetes, an endocrinal chronic disease caused by altered carbohydrate metabolism and characterized by elevated serum glucose levels.

Our results indicated that the serum triglycerides and total cholesterol levels significantly and progressively decreased ($p < 0.05$) from 4 weeks to the experimental end (8 weeks) in the 3rd and 4th groups compared to the controls. Serum LDL levels significantly and progressively decreased ($p < 0.05$) from 4 weeks while serum HDL levels significantly and progressively decreased ($p < 0.05$) from 2 weeks to the experimental end (8 weeks) in 3rd and 4th groups compared to the controls.

The reduction of triglycerides after the administration of watercress might be attributed to decreased triglycerides absorption and higher excretion of triglycerides via feces (Shukla et al., 2004). The decrease in the level of total cholesterol after receiving watercress may be due to increased excretion of bile acids, lower absorption of total cholesterol from the intestine, binding of watercress with bile acids in the intestine (Kokhdan et al., 2021), reduction of cholesterol biosynthesis (Sharma et al., 2003), and increasing of LDL receptors (Hadjzadeh et al., 2015). Furthermore, other studies suggested that the reduction of total cholesterol after treatment with watercress may be related to valued polyphenolic combinations such as total phenolics and flavonoids in this extract (Yazdanparast et al., 2008).

In agreement with our results Hadjzadeh et al., (2015) reported that the hydroalcoholic extract of watercress demonstrated a significant decrease in serum total cholesterol and low-density lipoprotein (LDL) cholesterol levels on repeated oral administration in streptozotocin (STZ) diabetic rats. They claimed that treatment with watercress extract at a dose of 200 mg/kg for 4 weeks significantly declined the levels of total cholesterol and LDL cholesterol compared to diabetic animals. They represented that the plant flavonoids,

phenolic compounds, and glycosides were responsible for hypocholesterolemia and hypolipidemic activities.

Our findings were in harmony with that detected by Yazdanparast et al., 2008 who mentioned that intragastric administration of WC (500 mg/kg BW per day for 10 and 30 days) to rats with hypercholesterolemia lowered their serum total cholesterol (TC) (by 34.2 and 37%), triglycerides (TG) (by 30.1 and 44%), and low-density lipoproteins cholesterol (LDL-C) (by 52.9 and 48%) although it raised the serum high-density lipoproteins cholesterol (HDL-C) level (by 27 and 16%). The atherogenic index (AI, determined in terms of LDL/HDL ratio) and the HMG CoA reductase activity showed a significant decrease in rats with hypercholesterolemia which were treated with WC in comparison to rats with a high-fat diet (Bahramikia and Yazdanparast, 2008).

Syamsianah and Anggraini (2016) observed that the ingestion of raw food (the mixture of 1 portion of WC and 1 portion of black rice bran) for four weeks outstandingly lowered total cholesterol and triglycerides levels and heightened HDL levels but supplementation did not have a significant effect on LDL levels in the experimental animals with diabetes mellitus (DM). Contrastingly, Gill et al., (2007) showed that daily consumption of 85 gram WC for 8 weeks did not have any effect on the plasma lipid profile (TG, TC, HDL-C, and LDL-C).

The serum Creatinine and urea of investigated rats significantly and progressively decreased ($p < 0.05$) from 4 weeks to the end of the experiment (8 weeks) in the 3rd and 4th groups compared to the controls.

Our findings were in harmony with that detected by Shahani et al., (2017) who demonstrated that administration of watercress extract (100 and 200 mg/kg) significantly reduced the ROS formation and serum level of blood urea nitrogen, and creatinine modulating the pathological changes in kidney tissue.

In agreement with our results Karami et al., (2017) revealed that after administration of watercress extract (500 mg/kg), MDA, creatinine, and uric acid levels in serum were significantly reduced. Karami et al., (2018) confirmed the traditional use of watercress as a treatment for urinary disorders as oral administration of *Nasturtium officinale* ethanol extract (250 and 500 mg kg⁻¹) and 500 mg kg⁻¹ vitamin E reversed the increase in serum creatinine levels due to vancomycin injection.

The activities of ALT and AST can be used to evaluate hepatic function. These enzymes were elevated in the

diabetic animals but significantly and progressively decreased ($p < 0.05$) from 2 weeks to the end of the experiment (8 weeks) in the 3rd and 4th groups compared to the controls. Protection of liver tissue is likely due to the presence of flavonoids, which have been reported to be hepatoprotective agents (Scalbert et al., 2005).

These results were confirmed by the findings of Azarmehr et al., (2019) who showed that administration of watercress extract (500 mg/kg) caused a marked decrease in AST activity in comparison to APAP-treated rats. Natanzi et al., (2010) revealed that total extract, alcohol-water (175 mg/kg), and aqueous extract (50 mg/kg) of the whole part of *N. officinale* significantly prevented acetaminophen-induced rise in serum levels of aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase.

These results are compatible with the findings obtained by Natanzi et al., (2009) who evaluated hepatoprotective effects of watercress by using a rat liver perfusion system. Adult male rats were selected to investigate the hepatoprotective effect of watercress against acetaminophen toxicity. Alcoholic extract of watercress significantly lowered the increased activity of both AST and ALT enzymes induced by acetaminophen.

In this study, at 8 weeks, the highest level of cumulative blood sugar was observed in the control positive group (group 2) which was significantly higher ($p < 0.05$) in comparison to the control negative group (group 1), it also reflected the diabetic status of rats in this group. The lowest level was seen in the control negative group which was significantly lowered from those of other groups. These data show that *N. officinale*'s hypoglycemic effect is due in part to its insulin mimic activity, which results in greater peripheral glucose consumption and diminished gluconeogenesis in the liver (Abdulrazaq et al., 2012). Administration of watercress significantly reduced HbA1c levels in diabetic rats. These findings are corroborated by Abd-elnoor, (2019) and Ojo et al., (2013).

Conclusion

Our analysis showed that watercress aqueous extract orally administered to diabetic rats had beneficial effects on reducing and maintaining normal glucose levels from the second week up to the eighth week of administration. This treatment provided additional hepatoprotective protection and improved lipid profile and kidney functions.

Authors' contribution

The work was equally distributed between authors S.R.T. Contributed to the sample collection, statistical analysis, and interpretation of data and wrote the paper. N.S.A. and A.S.O. designed the research study and contributed to the final editing, correction, and revision of the paper. All authors have read and approved the final version of the manuscript.

Conflict of interest

There is no conflict of interest.

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