

RESEARCH ARTICLE

BLOOD RESPIRATORY FUNCTIONS IN STREPTOZOTOCIN-INDUCED DIABETIC MALE ALBINO RATS TREATED WITH *CUCUMIS MELO* VAR. *FLEXUOSUS* LEAVES EXTRACT

Aziza A. M. El-Shafey; Marwa A. E. Abd El-Maksoud* ; Doaa S. Ibrahim

Zoology Department, Faculty of Science, Benha University, Qalyubia, Egypt

ABSTRACT

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***Correspondence:**

Marwa Abd El-Maksoud

Zoology Department, Faculty

of Science, Benha University

Qalyubia, Egypt

E-mail:

[marwa.abdelmaksoud@fsc.](mailto:marwa.abdelmaksoud@fsc.bu.edu.eg)

[bu.edu.eg](mailto:marwa.abdelmaksoud@fsc.bu.edu.eg)

Cucumis melo var. *flexuosus* (L.), snake melon or faqqous, is an ancient plant crop in many different parts of the world. The present study was designed to evaluate the efficacy of faqqous leaves extract on some blood respiratory functions in streptozotocin (STZ)-induced diabetic rats. Thirty five male albino rats were used in the current study and randomly divided into five groups: non-diabetic control group, diabetic control group (received a single intraperitoneal injection of 60 mg STZ/kg body weight), and other three diabetic groups treated orally with different doses of faqqous leaves extract (30, 60 or 120 mg/kg body weight) for 30 consecutive days. Induction of diabetes by STZ caused a significant decrease ($P < 0.05$) in most venous blood parameters including hemoglobin (Hb) content, hematocrit (Hct) value, oxygen partial pressure (PO_2), percentage of O_2 saturation (% O_2), carbon dioxide partial pressure (PCO_2), and the concentrations of bicarbonate (HCO_3^-), total CO_2 , and base excess (BE^-), as well as the logarithm of hydrogen ion (pH) of the arterial blood. Faqqous leaves extract (especially at 120 mg/kg body weight) modulated significantly almost all these changes in the blood of diabetic rats. Oxygen dissociation curve (ODC) shifted to the left in the diabetic control group compared with the non-diabetic control one; but shifted to the right in the diabetic groups treated with faqqous leaves extract. In conclusion, dietary supplementation of faqqous leaves extract improved significantly the blood respiratory functions of diabetic rats.

INTRODUCTION

Diabetes mellitus (DM) is a widespread disease associated with metabolic and endocrine disorders. It resulted from a decrease in insulin secretion and/or a reduction in the cellular insulin sensitivity and characterized by chronic hyperglycemia that led to long-term damage of the

different organs and a disturbance in the respiratory functions of blood^[1]. DM-induced also an excessive increase in the production of free radicals and reactive oxygen species in both human and experimental animals^[2]. Streptozotocin (STZ) is usually used to induce diabetes in the experimental animals. It causes oxidative

destruction of the beta cells of Langerhans islets, which are responsible for insulin secretion^[3].

A diet containing natural antioxidants helps in protecting humans from many diseases. Many plants are used nowadays in green medicine because they are usually safe and do not induce side effects. Quantity and quality of chemical constituents of different plants determine the importance of using such plants in medicine^[4]. It was reported that natural plants, especially those rich in flavonoids and alkaloids, can improve pulmonary circulation and protect from cardiovascular disorders^[5]. Also, Ebrahimzadeh *et al.*^[6] suggested that the phenolic compounds extracted from some natural plants such as *Crataegus pentaegyina* and *Crataegus microphylla* showed antihypoxic activity in the experimental animals. *Cucumis melo* var. *flexuosus* (snake melon or faqqous) is a non-sweet vegetable, like a cucumber. It belongs to the genus *Cucumis*, which contains many herbal plants used as antioxidant agents^[7]. Faqqous contains many bioactive phytochemical compounds such as phenolic compounds, alkaloids, saponins, tannins, and flavonoids that are responsible for its antioxidant, antidiabetic, and anti-inflammatory properties^[8]. Our pervious study concluded that the faqqous leaves extract showed potent antioxidant and antidiabetic activities, which resulted from its flavonoids and phenolic compounds^[9]. Therefore, the present study aimed to evaluate the effect of different doses of faqqous leaves extract on the blood respiratory functions of diabetic rats.

MATERIAL AND METHODS

Preparation of faqqous leaves extract

Fresh faqqous leaves were collected from healthy agricultural fields in Benha (Qalyubia, Egypt) and authenticated by our colleagues in the Faculty of Agriculture, Benha University. The leaves were dried in dark area at room temperature and extracted with ethanol using a Soxhlet apparatus as previously

described by Ibrahim and Abd El-Maksoud^[9]. The extract was evaporated to dryness and stored at 4°C.

Induction of experimental diabetic

Diabetes was induced in experimental animals by a single intraperitoneal injection (60 mg/kg body weight) of freshly prepared solution of STZ (Sigma Co., St. Louis, MO, USA) dissolved in 0.1 M citrate buffer (pH 4.5) according to El-Shafey *et al.*^[10]. To avoid hyperglycemic coma, animals were given 5% glucose solution in drinking water for 2 days. After 48 hours, blood samples were withdrawn from the lateral tail vein to measure glucose concentration and rats having glucose ≥ 200 mg/dL were included in the study.

Animals and experimental design

The present study was performed on male Wistar albino rats (*Rattus norvegicus*), weighing 160 ± 10 g, which obtained from the Helwan Farm of Egyptian Organization for Vaccine and Biological Preparations (Cairo, Egypt). Rats were acclimatized in the laboratory under standard environmental conditions ($25 \pm 2^\circ\text{C}$, 12 hours light/dark cycle, and given food and water *ad libitum*) for 10 days before the beginning of the experiment. Animals were humanely treated according to the ethical guidelines of the Faculty of Science, Benha University. Rats were divided randomly into five groups of seven rats in each group as follows:

- Group I: non-diabetic control rats that received water, as vehicle, orally by gavage for 30 consecutive days.
- Group II: diabetic control rats injected intraperitoneally with a single dose of STZ (60 mg/kg body weight) and housed at the same experimental condition for 30 days.
- Groups III, IV, and V: diabetic rats treated orally with 30, 60 or 120 mg of faqqous leaves extract/kg body weight, respectively, dissolved in 1.0 mL distilled water for 30 consecutive days post-STZ-diabetic induction.

Sample preparation and assays

At the end of the experimental period, the overnight fasting animals were anesthetized by inhaling light diethyl ether. The blood samples were drawn from the dorsal aorta and posterior vena cava into 1.0 mL syringes containing heparin for estimating the blood respiratory functions: blood gases and acid-base status in both arterial (a) and venous (v) blood, and oxygen dissociation curve (ODC), as previously described by El-Shafey and Seliem^[11]. Determination of hemoglobin (Hb) content, hematocrit (Hct) value, blood gases (oxygen and carbon dioxide partial pressures “PO₂ and PCO₂, respectively, in mmHg” and the percentage of blood O₂ saturation “% O₂”), and blood acid-base status parameters (the concentrations of bicarbonate "HCO₃⁻", total CO₂, and base excess "BE⁻" in mmol/L, as well as the logarithm of hydrogen ion “pH”) were carried out by using Medica Easy Stat Analyzer (Medica Products for Health, Bedford, MA, USA).

Statistical analysis

Data are presented as means ± standard deviations of seven readings. Statistical analyses were achieved by the one-way analysis of variance (ANOVA) using the Statistical Package for Social Science (SPSS) computer program (version 20.00, IBM Software Inc., Chicago, IL, USA). The post

hoc Duncan’s multiple range test was used to determine individual probability values for comparison between groups^[12]. Differences were considered significant at $P < 0.05$. The ODC Figure was drawn using Sigma Plot (version 10) program produced by Systat Software Inc. (Chicago, IL, USA).

RESULTS

Data in Table “1” showed that the Hct value and Hb content of the diabetic control rats (group II) decreased significantly ($P < 0.05$) in the venous blood only, compared with the non-diabetic control rats (group I). On the other hand, treatment of diabetic rats with the faqqous leaves extract (at all used doses) caused a significant increase ($P < 0.05$) in the Hct value and Hb content of both arterial and venous blood compared with the diabetic control rats (Table 1).

Diabetic control rats showed a significant decrease ($P < 0.05$) in the PO₂, % O₂ saturation, and PCO₂ of venous blood only, compared with the non-diabetic control rats (Table 2). Only the higher dose of faqqous leaves extract (120 mg/kg body weight) was able to revert back all changes in the venous blood PO₂, % O₂ saturation, and PCO₂ of diabetic rats to the values of the non-diabetic control rats (Table 2).

Table 1: Effect of oral administration of faqqous leaves extract on blood hematocrit (Hct) value and hemoglobin (Hb) content of streptozotocin (STZ)-diabetic rats.

		Group I	Group II	Group III	Group IV	Group V
Hct (%)	a	43.00±1.63 ^b	42.50±2.88 ^b	50.50±5.06 ^a	52.75±3.30 ^a	49.00±2.94 ^a
	v	35.75±4.78 ^b	29.25±2.36 ^c	42.25±2.98 ^a	47.75±1.70 ^a	43.50±5.91 ^a
Hb (g/dL)	a	13.32±2.08 ^b	13.45±1.60 ^b	16.8±1.67 ^a	17.15±0.81 ^a	16.47±1.88 ^a
	v	13.75±1.36 ^a	9.90±1.15 ^b	13.97±1.37 ^a	14.92±0.93 ^a	15.52±0.88 ^a

Data are presented as means ± standard deviations (n = 7). Group I: non-diabetic control rats, group II: STZ-diabetic control rats, groups III, IV, and V: diabetic rats received 30, 60 or 120 mg/kg body weight of faqqous leaves extract, respectively. a: arterial blood; v: venous blood. Values with different letters in the same row were significantly different ($P < 0.05$).

Table 2: Effect of oral administration of faqqous leaves extract on blood oxygen partial pressure (PO₂), percentage of O₂ saturation (% O₂), the partial pressure of O₂ at which blood is 50% saturated (P₅₀), and carbon dioxide partial pressure (PCO₂) of streptozotocin (STZ)-diabetic rats.

		Group I	Group II	Group III	Group IV	Group V
PO ₂ (mm Hg)	a	109.75±7.41 ^a	103.22±3.20 ^{ab}	101.00±6.97 ^b	103.25±3.40 ^{ab}	103.5±2.64 ^{ab}
	v	49.42±2.48 ^a	35.5±3.69 ^c	45.00±5.35 ^{ab}	43.00±2.16 ^b	44.5±3.69 ^{ab}
% O ₂	a	98.22±0.63 ^a	97.8±0.41 ^{ab}	97.00±0.97 ^{ab}	96.95±0.51 ^b	97.47±0.88 ^{ab}
	v	75.20±3.19 ^a	63.85±4.10 ^b	60.95±3.16 ^b	65.63±2.58 ^b	71.52±3.48 ^a
P ₅₀ (mmHg)		29.70	23.60	30.40	33.50	33.70
PCO ₂ (mm Hg)	a	35.30±2.99 ^b	35.45±1.47 ^b	44.40±2.02 ^a	42.10±1.70 ^a	37.35±2.80 ^b
	v	48.20±1.39 ^c	40.02±1.17 ^d	54.17±1.69 ^a	51.25±0.89 ^b	53.02±2.10 ^{ab}

Data are presented as means ± standard deviations (n = 7). Group I: non-diabetic control rats, group II: STZ-diabetic control rats, groups III, IV, and V: diabetic rats received 30, 60 or 120 mg/kg body weight of faqqous leaves extract, respectively. a: arterial blood; v: venous blood. Values with different letters in the same row were significantly different ($P < 0.05$).

On the other hand, diabetic control rats showed a decrease in the partial pressure of O₂ at which blood is 50% saturated (P₅₀) as shown in Table “2”. ODC in the diabetic control rats shifted to the left, while the ODC in the diabetic rats treated with faqqous leaves extract (especially at the higher high dose, 120 mg/kg body weight) shifted to the right, compared with that of the non-diabetic control rats (Figure 1).

Arterial blood pH and the concentrations of HCO₃⁻, total CO₂, and BE⁻ of both arterial and venous blood of the diabetic control rats decreased significantly ($P < 0.05$) compared with the non-diabetic control rats (Table 3). The higher dose of faqqous leaves extract (120 mg/kg body weight) modulated significantly or completely the changes in the arterial blood pH, the concentration of arterial blood HCO₃⁻, and the concentration of both arterial and venous blood total CO₂ of diabetic rats. Only the lower dose (30 mg/kg body weight) of faqqous leaves extract was able to revert back the value of the venous blood HCO₃⁻ concentration to that of the non-diabetic control rats. While, faqqous leaves extract (at all used doses)

caused a significant increase ($P < 0.05$) in the concentration of both arterial and venous blood BE⁻ of diabetic rats compared with the non-diabetic control rats (Table 3).

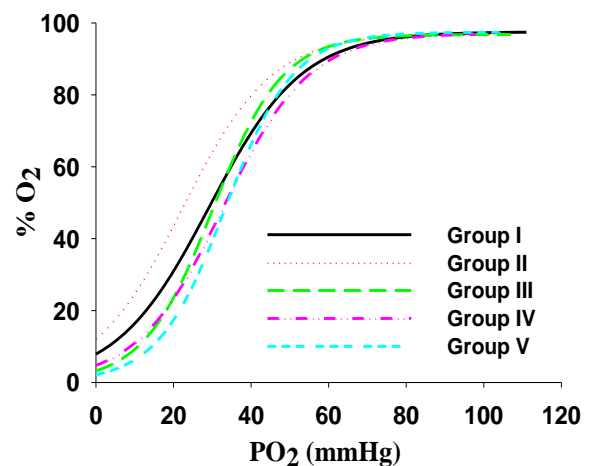


Figure 1: Blood oxygen dissociation curve in control rats (group I), diabetic rats that received vehicle (group II), and diabetic rats treated with 30, 60 or 120 mg of faqqous leaves extract/kg body weight (groups III, IV, and V, respectively). % O₂: percentage of blood O₂ saturation; PO₂: blood oxygen partial pressure.

Table 3: Effect of oral administration of faqqous leaves extract on blood logarithm of hydrogen ion (pH) and the concentrations of blood bicarbonate (HCO_3^-), total carbon dioxide (CO_2), and base excess (BE^-) of streptozotocin (STZ)-diabetic rats.

		Group I	Group II	Group III	Group IV	Group V
pH	a	7.37±0.03 ^a	7.24±0.02 ^c	7.32±0.05 ^b	7.35±0.02 ^{ab}	7.35±0.02 ^{ab}
	v	7.28±0.03 ^a	7.27±0.02 ^a	7.26±0.02 ^a	7.25±0.06 ^a	7.28±0.02 ^a
HCO_3^- (mmol/L)	a	25.00±2.29 ^a	18.37±1.72 ^c	18.67±0.68 ^c	18.50±0.87 ^c	20.95±1.37 ^b
	v	27.75±2.90 ^a	24.00±1.01 ^b	25.50±1.87 ^{ab}	24.47±2.01 ^b	24.05±1.19 ^b
Total CO_2 (mmol/L)	a	25.87±1.51 ^a	17.17±0.74 ^c	23.22±1.72 ^b	22.20±1.16 ^b	24.10±1.62 ^{ab}
	v	30.92±1.34 ^a	22.10±1.44 ^c	28.57±1.21 ^b	30.35±0.90 ^{ab}	30.97±1.18 ^a
BE^- (mmol/L)	a	-6.37±0.25 ^a	-9.38±0.16 ^e	-8.62±0.29 ^d	-7.80±0.14 ^c	-7.10±0.08 ^b
	v	-1.42±0.22 ^c	-3.96±0.14 ^d	1.00±0.11 ^b	1.35±0.13 ^a	1.45±0.24 ^a

Data are presented as means ± standard deviations (n = 7). Group I: non-diabetic control rats, group II: STZ-diabetic control rats, groups III, IV, and V: diabetic rats received 30, 60 or 120 mg/kg body weight of faqqous leaves extract, respectively. a: arterial blood; v: venous blood. Values with different letters in the same row were significantly different ($P < 0.05$).

DISCUSSION

DM is one of the most chronic diseases, which correlated with anemia^[13]. In the present study, the diabetic control group that received vehicle had a significant reduction in the venous blood Hct value and Hb content. This may be resulted from the decrease in the erythrocyte membrane fluidity and the increase in the RBCs fragility induced by enhancing non-enzymatic glycosylation of RBCs membrane proteins in diabetic rats^[13]. Also, hyperglycemia increased the production of free radicals that enhanced lipid peroxidation leading to RBCs hemolysis and a reduction in the erythrocyte life span^[14]. In the current study, treatment of diabetic rats with faqqous leaves extract modulated the resulted anemia. This may be attributed to the antioxidant components of the faqqous leaves extract, especially phenolic compounds and flavonoid, which can inhibit lipid peroxidation and protect RBCs from damage^[9].

DM was associated with hypoxia that led to oxidative stress^[15,16]. This was clear in the present study by a reduction obtained in

the venous blood PO_2 and O_2 saturation after STZ treatment. In addition, there was a decrease in the arterial blood pH, HCO_3^- , total CO_2 , and BE^- levels that attributed to blood ketoacidosis caused by diabetes as a result of a lack of insulin^[17,18]. Diabetic rats showed metabolic acidosis that followed by respiratory compensation to maintain acid-base balance^[19,20]; this was clear here by a significant decrease shown in the PCO_2 especially in the venous blood. The decrease in the blood pH, HCO_3^- , and BE^- of diabetic rats may be attributed to the renal dysfunction and the inhibition of carbonic anhydrase, a critical enzyme responsible for reabsorption of bicarbonate that plays a key role in acid-base balance throughout the nephron^[21,22]. Shapero *et al.*^[23] also reported that diabetes was associated with excessive production of ketones, which cause metabolic acidosis.

The ODC determined the uptake and release of oxygen in the lungs and tissues, respectively^[24]. This curve shifted to the right or left in response to changes in PCO_2 , pH, and 2,3-diphosphoglycerate (2,3-DPG). The 2,3-DPG reduces the Hb- O_2 affinity

and enhances the release of oxygen to the tissues^[25]. A shift to the left corresponds to an increase in the oxygen affinity and a decrease in the release of oxygen to the tissues^[24]. ODC in the diabetic control group shifted to the left of that in the non-diabetic control group, and its P₅₀ decreased, in the current study. This may be due to the hyperglycemia that caused a fall in the 2,3-DPG content of the RBCs and an elevation in the percentage of glycated Hb, which showed higher oxygen affinity than normal Hb^[25,26]. This, in turn, decreased offloading of oxygen to the tissues and caused tissue hypoxia that may be compensated by increasing the cardiac output (to raise blood flow), Hb content (to raise the blood-oxygen carrying capacity) or the concentration of 2,3-DPG (to counterbalance the formation of glycated Hb)^[25,26].

Faqqous leaves extract (especially at 120 mg/kg body weight) ameliorated significantly most disturbances in blood gases and pH, which resulted from diabetes, and returned the blood respiratory functions near to normal values. It shifted the ODC in diabetic rats to the right of that in the non-diabetic control rats and increased their P₅₀ values. All of these observations indicated the efficacy of faqqous leaves extract to compensate the metabolic acidosis in diabetic rats. In addition, our previous studies proved the antioxidant, antidiabetic, renoprotective, and neuroprotective activities of faqqous leaves extract in STZ-diabetic rats, which may also be attributed to its phenolic compounds and flavonoids^[9,27,28]. In conclusion, our study proved that the faqqous leaves extract was propitious in improving the blood respiratory functions of diabetic rats, probably due to its antioxidant and antidiabetic activities.

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وظائف الدم التنفسية في ذكور الجرذان المُهقّ المحدث بها داء السكري بمادة الإستربتوزوتوسين وعولت بمستخلص أوراق نبات القثاء

عزيزة عبدالصمد محمد الشافعي، مروة عاطف عليوة عبدالمقصود، دعاء صبري إبراهيم

قسم علم الحيوان، كلية العلوم، جامعه بنها، القليوبية، جمهورية مصر العربية

يُعتبر نبات القثاء من المحاصيل القديمة في مختلف أنحاء العالم. وصممت هذه الدراسة لتقييم تأثير تناول مستخلص أوراق نبات القثاء علي وظائف الدم التنفسية في ذكور الجرذان المُهقّ المحدث بها داء السكري بمادة الإستربتوزوتوسين. وفي هذه الدراسة تم استخدام عدد "35" من ذكور الجرذان المُهقّ قسمت عشوائيا إلي خمس مجموعات كالتالي: المجموعة الضابطة، مجموعة السكري (المعاملة بالإستربتوزوتوسين عن طريق الحقن داخل الصفاق بجرعة واحدة مقدارها 60 مليجرام /كجم من وزن الجسم)، وثلاث مجموعات أخرى محدث بها داء السكري وتمت معاملتها بجرعات مختلفة من مستخلص أوراق نبات القثاء (30 أو 60 أو 120 مليجرام /كجم من وزن الجسم، علي التوالي). وسببت المعاملة بالإستربتوزوتوسين نقصا ذو دلالة إحصائية في معظم قياسات الدم الوريدي مثل المحتوى من الهيموجلوبين، وقيمة الهيماتوكريت، والضغط الجزئي للأكسجين، ونسبة التشبع بالأكسجين، والضغط الجزئي لثاني أكسيد الكربون، وتركيز كل من البيكربونات وثاني أكسيد الكربون الكلي والزيادة القاعدية، وأيضا الأس الهيدروجيني في الدم الشرياني. كما عدل مستخلص أوراق نبات القثاء (خاصة عند استخدام الجرعة 120 مليجرام /كجم من وزن الجسم) معظم هذه المعايير في الجرذان المحدث بها داء السكري إلي المعدلات السوية. وقد تحول منحنى تفكك الأكسجين إلي اليسار في مجموعة السكري غير المعاملة بمستخلص أوراق نبات القثاء مقارنة بالمجموعه الضابطة غير المصابة بداء السكري، ولكنه تحول إلي اليمين في مجموعات السكري المعاملة بمستخلص أوراق نبات القثاء. والخلاصة أن المعاملة بمستخلص أوراق نبات القثاء أظهرت تحسنا ملحوظا في وظائف الدم التنفسية للجرذان المصابة بداء السكري.