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Antidiabetic, hematoprotective and nephroprotective effects of the aqueous extract of *Falcaria vulgaris* in diabetic male mice

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Abstract: *Falcaria vulgaris* has been used in medicine as an antimicrobial agent. The aims of this study were to evaluate the hypoglycemic and nephroprotective activities of the aqueous extract of *F. vulgaris* in diabetic mice. Diabetes was experimentally induced by intraperitoneal injection of streptozotocin (STZ). The mice were divided randomly into 6 groups as follows: nondiabetic, untreated diabetic, a group that received orally 0.5 mg/kg glibenclamide, and three groups that were given orally 200, 600 and 1800 μg/kg of *F. vulgaris*, respectively, for 20 days. On the 20th day, the mice were dissected, and blood and kidney samples were collected for analysis of hematological, biochemical and stereological parameters. *F. vulgaris* at all doses, particularly 1800 μg/kg, significantly ($p \leq 0.05$) reduced the levels of urea, creatinine, WBC, eosinophils, basophiles, platelet and increased RBC, Hb, PCV, MCV, MCH, MCHC, lymphocytes and monocytes that were raised after diabetes induction, compared to the untreated diabetic group. Multiple doses of *F. vulgaris*, particularly1800 μg/kg, significantly ($p \leq 0.05$) decreased the total volumes of the kidney cortex, medulla, vessels and renal tubules, as well as the lengths of the vessels and renal tubules, compared to the untreated diabetic group. Also, *F. vulgaris* at all doses significantly prevented glomerular hypertrophy and reduction of glomeruli numbers in comparison with the untreated diabetic group. In conclusion, *F. vulgaris* possesses antidiabetic, hematoprotective and nephroprotective properties and can improve renal structural and blood biomarkers in STZ-induced nephrotoxicity in mice.

Key words: *Falcaria vulgaris*; aqueous extract; hypoglycemic activity; nephroprotective activity; streptozotocin

Abbreviations and acronyms: collecting ducts (CD); diabetic mice treated with 200, 600, 1800 μg/kg of *F. vulgaris* aqueous extract (FV200, FV600, FV1800, respectively); fasting blood glucose (FBG); glomeruli (G); hemoglobin (Hb); interstitial tissue (IT); loop of Henle (LH); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); mean corpuscular volume (MCV); packed cell volume (PCV); proximal and distal convoluted tubules (PCT, DCT, respectively); red blood cells (RBC); streptozotocin (STZ); vessels (V); white blood cells (WBC)

INTRODUCTION

Diabetes mellitus is a disease characterized by a disordered metabolism, resulting from either low insulin levels or insulin resistance in many body cells. Diabetes is the most important cause of renal failure and legal blindness and is one of the major risk factors of cardiovascular diseases [1]. Diabetic patients are five times more likely than nondiabetic patients to develop severe chronic leg ischemia, leading to foot ulceration and amputation [1].

The kidney is one of the organs affected by diabetes. However, the exact pathogenesis of nephropathy in diabetic patients is not understood, a decrease in proximal and distal cell capacity as well as oxidative and inflammatory changes being reported as the main causes [2]. Renal hypertrophy and glomerular hyperfiltration are two known complications that occur in the initial stages of diabetes mellitus [3]. Some studies have revealed that in early diabetes, glomerular hyperfiltration and renal hypertrophy can be reversed by insulin treatment [4,5]. However, in chronic diabetes,

glomerular hyperfiltration can be ameliorated by the strict control of blood glucose concentration; however, renal hypertrophy is irreversible [6]. Although renal hypertrophy and glomerular hyperfiltration play a pivotal role in increasing diabetic nephropathy, the relationship between them is still unclear [3].

Hematological parameters such as erythrocyte aggregation, erythrocyte deformability, hematocrit and plasma proteins in diabetes mellitus are often disturbed [7]. The resultant disturbance may be a risk factor for the progression of retinal failure in diabetic retinopathy, and renal failure in diabetic nephropathy [7].

The enormous costs of modern medicines indicate that other strategies are needed for better management of diabetes and its related problems [8]. Some plants have high contents of alkaloids, flavonoids, naphthoquinone, saponins, tannins and triterpenes, and they can decrease the rate of diabetes development [9,10]. The World Health Organization (WHO) has suggested that there should be further studies of the antidiabetic effects of medicinal plants [11].

In Iranian traditional medicine, herbal medicines have been used for prevention and treatment of diabetic complications [12-17]. A list of medicinal plants in Iran that are consumed for their antidiabetic properties includes *Vaccinium arctostaphylos*, *Trigonella foenum*, *Thea sinensis*, *Silybum marianum*, *Securigera securidaca*, *Satureja khuzistanica*, *Plantago ovate*, *Opuntia streptacantha*, *Ocimum sanctum*, *Ipomoea batatas*, *Ginkgo biloba*, *Cuminum cyminum*, *Citrullus colocynthis* and *Allium sativum* [18].

One of the most important herbal medicines that is widely consumed in Iranian traditional medicine for the treatment of diabetes is *Falcaria vulgaris*, from the order Apiales, family Apiaceae [19]. The consumption and cooking of parts of *F. vulgaris* is because of the variety of flavors and textures of the species. *F. vulgaris* has been cultivated from the earliest times and it is economically important as a garden vegetable. *F. vulgaris* is one of the edible plants that has generated considerable interest throughout human history as a medicinal plant. Several extracts of this plant have traditionally been used to treat gastric ulcers and parasitic, viral, fungal and bacterial diseases [20]. One study demonstrated that a diet enriched with 5% *F. vulgaris* improved wound healing in the rat, and 10% *F. vulgaris* had an effect on skin tensile strength [21]. In phytochemical studies, spathulenol and carvacrol were identified as effective substances of *F. vulgaris* in the treatment of disease [22]. To the best of our knowledge, there are very little data about the antidiabetic, hematoprotective and nephroprotective effects of the aqueous extract of *F. vulgaris*, collected from Kermanshah province in western Iran. The present study was conducted to assess the antidiabetic, hematoprotective and nephroprotective effects of the aqueous extract of *F. vulgaris* in diabetic mice.

MATERIALS AND METHODS

Plant collection

Mature *F. vulgaris* was collected around Kermanshah city during April 2017. The plant was identified for the first time, and a voucher specimen was deposited at the Herbarium of the research center of the Faculty of Agriculture, Razi University, Kermanshah, Iran.

Plant extraction

The leaves of the plant were shade-dried for one week. The dried aerial parts of the plant were ground, and about 150 g of the obtained powder was extracted with 450 mL distilled water for 2 h at 40°C by continuous shaking. The extract was left for 24 h at room temperature. It was then filtered through Whatman paper no. 2. The extract was concentrated and lyophilized using a rotary evaporator (Panchun Scientific Co., Kaohsiung, Taiwan).

Animals

Male BALB/c mice weighing between 38-40 g were procured from the animal center of Kermanshah University of Medical Sciences. The animals were housed in an airconditioned room (22±2°C) with a 12 h light/dark cycle, and free access to standard pellet and water. All procedures were performed in accordance with the Institutional Animal Ethics Committee.

Induction of diabetes

Diabetes was induced by a single intraperitoneal (i.p.) administration of STZ (60 mg/kg.bw). FBG was as-

sessed every day with an Easy Gluco glucometer (Ames, Korea). After 3 days, mice with a blood glucose concentration above 350 mg/dL were considered diabetic.

Experimental design

The mice were divided into six groups (n=10) as follows: the control group received 200 µL saline orally for 20 days; untreated-diabetic mice received 200 µL saline orally for 20 days; a group of STZ-induced diabetic mice treated with glibenclamide received 600 µg/kg glibenclamide for 20 days; and three groups, FV200, FV600 and FV1800, of the STZ-induced diabetic mice received 200, 600 and 1800 µg/kg aqueous extract of *F. vulgaris* for 20 days, respectively.

Blood sampling and determination of biochemical parameters

Blood samples were taken on days 0, 4, 7, 10, 13, 16 and 20 from the tail vein to measure the blood glucose concentrations. At the end of day 20 of treatment, the animals of all groups were euthanized by xylazine (5 mg/kg) and ketamine HCl (40 mg/kg). Blood samples were drawn immediately from the animals' hearts. To separate the serum, the samples were centrifuged at 2000 x *g* for 15 min. Creatinine and urea concentrations were measured in the serum [16,17].

Determination of hematological parameters

Blood samples collected in EDTA bottles were analyzed for hematological parameters using a Hematology Analyzer (Mindray Auto, BC-5200, USA) following the manufacturer's instructions. The parameters analyzed included Hb, MCV, MCH, MCHC, PCV, RBC, WBC, as well as the differentials and platelets.

STEREOLOGICAL STUDY

Volume density

Nephrons and cells are functional units in a kidney. The number, size, and distribution of nephrons, cells, and other components contain important information about the function and organization of the kidney. Therefore, it is important that the various structural components are measured correctly. Quantification of these components is also important when examining how kidneys react to trauma, chemicals, and disease [23,24]. To achieve these objectives, the stereology method was used. To perform the stereology method, the left kidney was removed and weighed and fixed in 10% neutral buffered formalin solution for one week. The immersion method was then used to determine the primary volume of the kidney. To estimate the final volume of the organs, the amount of tissue shrinkage must be specified [23,24]. Isotropic uniform random (IUR) sections must be obtained to estimate tissue shrinkage and tubular lengths [24,25]. In total, 7-10 slabs were obtained from each kidney using the orientator method. A circular piece was sampled from a kidney slab and the area of this piece was calculated. The slabs and circular pieces were processed, sectioned (5 µm thickness) and stained by the periodic acid-Schiff (PAS) method. The area of the circular piece was calculated again, and tissue shrinkage was estimated by the following relation [26]:

$$\text{Volume shrinkage} := 1 - \left(\frac{AA}{AB}\right)^{1.5}$$

where AA and AB are the areas of the circular piece after and before tissue processing. The total volume of the organ was then estimated using:

$$V_{final} := V_{primary} \times (1 - \text{volume shrinkage})$$

Tissue sections were examined using a video microscopy system composed of a microscope (Olympus CX2, Japan) connected to a video camera (Dinocapture ver.5, dino-lit.com 30.5 mm) and a P4 PC, and the stereological parameters were estimated. The fractional volume of the renal structures was estimated using a point probe (with an area of 100 cm^2 and containing 25 points), and the following formula (Fig. S1A):

$$Vv := \frac{P_{structure}}{P_{reference}}$$

with $P_{structure}$ = the sum of points hitting the interested structures, and $P_{reference}$ = the sum of points hitting the reference space.

Length density

The length density of the tubules and vessels was estimated using an unbiased counting probe (740×740 μm). The tubule structures were considered in such a manner so that they lay completely or partly inside the counting probe and did not touch the down and left lines. Otherwise, they were not considered (Fig. S1B). The length density was estimated by the following formula:

$$Lv := 2 \times \frac{\Sigma Q}{a(\text{frame}) \times \Sigma \text{ frame}}$$

ΣQ = the sum of the tubules counted, *a* (frame)= probe area, 547600 μm^2, Σ_{frame} = total number of the counted frames.

Numerical density

The physical dissector procedure was applied to estimate the numerical density of the glomeruli. Two parallel sections with 20 μm distance (1th and 5th sections) were prepared; the first section served as the reference plane and the fifth section as the look-up plane. Two counting probes with an area of 547 600 μm^2 were attached to the monitor at a final magnification of 135×. The counting rules of the physical dissector were applied. Thus, a glomerulus was considered if it was found in the reference plane and not in the look-up plane, and if it did not hit the down and left lines of the probe. The numerical density of the glomeruli was estimated using:

$$N_v := \frac{\Sigma Q^-}{a(\text{frame}) \times h \times \Sigma P}$$

ΣQ$^-$= the sum of the counted glomeruli, *a* (frame)= probe area, ΣP= total number of the examined fields and *h*= dissector height. The absolute value of each parameter was calculated by multiplying its density by the reference space [26].

Statistical analysis

All data were expressed as mean and standard deviation. Statistical comparison between group means was done by one-way ANOVA followed by Duncan's post-hoc test, with $p \leq 0.05$ considered as significant.

RESULTS

Effect of *F. vulgaris* on fasting blood glucose concentration

The effect of *F. vulgaris* intake on FBG in the diabetic mice is presented in Table 1. There was no significant change in the blood glucose concentration of control mice throughout the study. The blood glucose concentration of untreated diabetic mice increased by about 550% ($p \leq 0.05$) in a time-dependent manner. Treatment of STZ-induced diabetic mice with *F. vulgaris* at all doses significantly ($p \leq 0.05$) decreased the blood glucose concentration similar to the glibenclamide-treated mice at days 16 and 20 of the experiment. *F. vulgaris* exerted its maximum effect on day 20 of the experiment.

Effect of *F. vulgaris* on the biochemical parameters of kidneys

The values of the kidney biochemical parameters are presented in Table 2. STZ-induced diabetes increased the concentrations of urea and creatinine significantly ($p \leq 0.05$) as compared to the control group. Different

Table 1. Effects of *F. vulgaris* aqueous extract on blood glucose of diabetic mice.

Groups (*n* =10)	Blood glucose concentration (mg/dL)						
	0 days	4 days	7 days	10 days	13 days	16 days	20 days
C	78.9±4.2^a	82.1±3.8^a	80.5±6.4^a	79.6±3.5^a	78.2±6^a	83.5±5.5^a	80.8±4.5^a
UD	420.2±16^b	412.5±13.7^b	421.7±22.5^c	410.8±16.8^c	412.9±20.2^c	408.6±14.9^c	407.3±15.1^c
G	417.5±14.2^b	374.9±16.4^b	343.9±12.8^{bc}	311.2±17.1^b	294.7±17.3^b	263.7±11.2^b	217.3±9.4^b
FV200	423.2±13.2^b	401.7±12.9^b	372.7±18.9^c	339.8±14.5^{bc}	329.1±22.2^{bc}	296.5±11.7^b	250.6±12.7^b
FV600	421.7±11.8^b	396.9±14.7^b	352.1±18.6^{bc}	309.7±20.1^b	287.4±13.2^b	262.8±18.6^b	223.7±10.2^b
FV1800	425.0±17.3^b	361.3±15.2^b	320.4±19.5^b	287.5±13.2^b	257.1±18.1^b	227.4±14.1^b	179.5±7.4^{ab}

C – Control, UD – untreated diabetic, G – glibenclamide-treated
Non-identical letters indicate a significant difference between the groups ($p \leq 0.05$)

Table 2. Effect of *F. vulgaris* aqueous extract on urea and creatinine serum concentrations of diabetic mice

Groups (n =10)	Urea (mg/dL)	Creatinine (mg/dL)
C	13.92±1.16a	1.18±0.04a
UD	39.76±1.04d	2.67±0.12d
G	28.87±1.21c	1.86±0.08c
FV200	32.13±1.12c	2.19±0.06c
FV600	28.89±0.85c	1.91±0.06c
FV1800	21.65±0.72b	1.47±0.04b

C – Control, UD – untreated diabetic, G – glibenclamide-treated
Non-identical letters indicate a significant difference between the groups (p≤0.05)

doses of *F. vulgaris* significantly (p≤0.05) ameliorated the above values. There were no significant differences in the concentrations of urea and creatinine (p≤0.05) among the FV200, FV600 and glibenclamide groups.

Effect of *F. vulgaris* on hematological parameters

The number of WBC and platelets, and the percentages of eosinophils and basophils significantly (p≤0.05) increased in untreated diabetic mice. Also, the percentages of lymphocytes and monocytes, the concentration of Hb and the levels of MCV, MCH, MCHC, PCV and RBC were significantly (p≤0.05) reduced in the untreated diabetic group. There were no significant differences in hematological parameters (p≤0.05) among the glibenclamide and FV200 and FV600 groups, with the treatment with *F. vulgaris* significantly (p≤0.05) ameliorating the above parameters. The percentages of eosinophils and basophils were significantly (p≤0.05) decreased in the glibenclamide and FV200, FV600 and FV1800 groups, and were similar to the control values. Also, the concentration of Hb was significantly (p≤0.05) increased in the FV1800 group and was similar to the control group. No significant difference (p≤0.05) was found among all groups in the percentage of neutrophils (Tables 3 and 4).

Effect of *F. vulgaris* on stereological parameters

The body weight and mean absolute weight and volumes of the kidney and its components in treated and untreated groups are presented in Tables 5-7. The body weight decreased significantly (p≤0.05) in untreated diabetic mice compared to the controls. Intake of *F. vulgaris* at all doses, similar to the glibenclamide and control groups significantly (p≤0.05) increased the body weight in comparison with the untreated group.

Table 3. Effects of *F. vulgaris* aqueous extract on WBC parameters of diabetic mice.

Groups (n =10)	Parameters					
	WBC (×10⁹/L)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Eosinophils (%)	Basophils (%)
C	7.92±0.24^{a}	52.96±0.75^{a}	11.35±0.33^{a}	34.54±0.79^{a}	0.57±0.02^{a}	0.42±0.02^{a}
UD	15.87±0.43^{d}	48.23±0.83^{d}	8.89±0.25^{c}	34.12±1.01^{a}	2.95±0.05^{a}	1.43±0.06^{b}
G	11.24±0.18^{c}	50.86±0.64^{c}	10.79±0.28^{b}	34.19±0.89^{a}	0.34±0.03^{b}	0.28±0.01^{a}
FV200	11.99±0.33^{c}	49.99±0.71^{c}	10.05±0.17^{b}	34.32±0.62^{a}	0.75±0.04^{a}	0.51±0.04^{a}
FV600	11.16±0.24^{c}	50.67±0.63^{c}	10.67±0.2^{b}	34.63±0.74^{a}	0.31±0.02^{a}	0.26±0.02^{a}
FV1800	9.68±0.23^{b}	51.73±0.55^{b}	11.19±0.19^{ab}	34.56±0.82^{a}	0.22±0.03^{a}	0.19±0.01^{a}

C – Control, UD – untreated diabetic, G – glibenclamide-treated; non-identical letters indicate a significant difference between the groups (p≤0.05).

Table 4. Effects of *F. vulgaris* aqueous extract on platelet and RBC parameters of diabetic mice.

Groups (n =10)	Parameters						
	Platelets (×10⁹/L)	RBC (×10¹²/L)	PCV (%)	MCV (fL)	Hb (g/dL)	MCH (pg)	MCHC (g/dL)
C	206.78±7.21^{a}	8.26±0.12^{a}	50.13±0.98^{a}	71.98±1.13^{a}	15.12±0.52^{a}	23.82±0.86^{a}	37.12±0.46^{a}
UD	679.34±9.34^{d}	3.72±0.23^{d}	22.13±0.47^{d}	46.34±1.86^{c}	9.42±0.21^{c}	17.44±0.72^{d}	21.54±0.74^{d}
G	399.56±9.1^{c}	6.02±0.36^{c}	41.57±0.78^{bc}	60.12±1.1^{b}	13.24±0.33^{b}	20.76±0.65^{c}	28.44±0.62^{c}
FV200	456.13±7.62^{c}	5.35±0.24^{c}	37.87±0.83^{c}	57.04±1.32^{b}	12.46±0.18^{b}	19.94±0.51^{c}	25.62±0.58^{c}
FV600	388.72±11.25^{c}	5.89±0.27^{c}	41.04±0.72^{bc}	59.78±1.05^{b}	13.11±0.27^{b}	20.66±0.73^{c}	28.33±0.73^{c}
FV1800	307.62±8.42^{b}	6.89±0.21^{b}	44.76±0.68^{ab}	63.89±1.04^{b}	14.64±0.3^{a}	22.14±0.92^{b}	32.18±0.39^{b}

C – Control, UD – untreated diabetic, G – glibenclamide-treated; non-identical letters indicate a significant difference between the groups (p≤0.05).-treated; non-identical letters indicate a significant difference between the groups (p≤0.05).

Table 5. Effects of *F. vulgaris* aqueous extract on body weight, kidney weight, absolute volumes of the kidney, cortex and medulla (mean±SD).

Groups (n =10)	Parameters				
	Body weight (g)	Kidney weight (mg)	Kidney volume (mm³)	Cortex volume (mm³)	Medulla volume (mm³)
C	39.97±0.22[a]	141.6±3.92[a]	113.6±4.35[a]	76.0±1.82[a]	33.7±1.7[a]
UD	32.66±0.3[b]	216.6±7.29[d]	180.4±4.78[d]	135.4±2.22[d]	46.1±1.28[c]
G	37.38±0.45[a]	169.8±3.76[b]	142.3±3.12[b]	107.8±2.2[b]	34.0±2.7[a]
FV200	36.25±0.56[a]	191.9±3.03[c]	165.8±4.07[c]	124.6±2.11[c]	40.6±1.34[b]
FV600	37.41±0.4[a]	171.7±3.26[b]	143.7±3.19[b]	109.1±1.59[b]	35.1±1.52[a]
FV1800	38.26±0.38[a]	157.4±3.62[b]	130.5±2.67[b]	100.0±2.26[b]	34.7±1.76[a]

C – Control, UD – untreated diabetic, G – glibenclamide-treated; non-identical letters indicate a significant difference between the groups (p≤0.05).

Table 6. Effects of *F. vulgaris* aqueous extract on the absolute volumes of different kidney structures (proximal and distal convoluted tubules, collecting ducts, loop of Henle, interstitial tissues and vessels (mean±SD)).

Groups (n =10)	Structures					
	PCT	DCT	CD	LH	V	IT
C	63.27±1.84[a]	15.50±0.49[a]	21.04±3.01[a]	1.23±0.01[a]	8.85±0.19[a]	11.21±0.15[a]
UD	120.68±2.95[d]	26.91±0.55[d]	29.88±0.59[c]	1.83±0.04[b]	17.17±0.31[c]	18.83±0.27[c]
G	89.14±3.09[bc]	20.58±0.32[bc]	24.79±0.28[b]	1.45±0.02[a]	12.43±0.21[b]	14.55±0.14[b]
FV200	99.90±2.86[c]	22.11±0.3[c]	25.46±0.53[b]	1.51±0.02[a]	13.22±0.29[b]	15.17±0.24[b]
FV600	87.85±3.15[b]	20.74±0.26[bc]	24.42±0.29[b]	1.44±0.02[a]	12.50±0.13[b]	14.63±0.21[b]
FV1800	79.18±2.36[b]	18.89±0.42[b]	22.4±0.33[a]	1.35±0.01[a]	10.00±0.28[a]	13.47±0.22[b]

C – Control, UD – untreated diabetic, G – glibenclamide-treated; non-identical letters indicate a significant difference between the groups (p≤0.05).

Kidney weight and volume were increased by 53% and 58% (p≤0.05), respectively, in untreated diabetic mice compared to the control group; also, cortical and medullary volumes increased by 79% and 23% (p≤0.05), respectively. The body weight increased by 23% (p≤0.05) in untreated mice, compared to the controls. Intake of *F. vulgaris* significantly ($p \leq 0.05$) ameliorated the renal, cortical and medullary volumes in comparison to the untreated group. In addition, there were no significant differences in body weight and medullary volumes among the glibenclamide and FV600 and FV1800 groups (p≤0.05).

The volumes of the CD, DCT, IT, PCT, LH and V were significantly increased (p≤0.05) in untreated mice as compared to the control (Table 6, Fig. 1). Intake of *F. vulgaris* by mice at all doses significantly (p≤0.05) decreased the volumes of the above structures. In the FV1800 group, the volumes of CD and V were significantly (p≤0.05) reduced and were similar to values obtained in the control group. There were no significant differences in the LH among the glibenclamide, FV200, FV600, FV1800 and control groups (p≤0.05).

The GV increased significantly (p≤0.05) following diabetes induction, and treatment with all doses of *F. vulgaris* and glibenclamide significantly (p≤0.05) improved glomerular hypertrophy. The GV was significantly (p≤0.05) reduced in the FV1800 group and was similar to the control group (Table 7).

Table 7. Effects of *F. vulgaris* aqueous extract on the absolute volume and number of glomeruli (Mean ± SD).

Groups(n =10)	Volume (mm³)	Number of glomeruli
C	0.00207±0.00008[a]	28906.64±59.34[a]
UD	0.00324±0.00008[d]	2145.62±72.26[c]
G	0.00242±0.0001[b]	25090.51±119.63[b]
FV200	0.00267±0.00006[c]	2411.85±100.9[b]
FV600	0.00241±0.00007[b]	25107.63±119.65[b]
FV1800	0.00220±0.00006[a]	26769.70±318[b]

C – Control, UD – untreated diabetic, G – glibenclamide-treated; non-identical letters indicate a significant difference between the groups (p≤0.05).

The number of glomeruli in untreated diabetic mice was reduced significantly (p≤0.05) in comparison with the control. The decline in the number of glomeruli was prevented significantly (p≤0.05) by *F. vulgaris* at all doses. There was no significant difference between the glibenclamide and *F. vulgaris* groups (p≤0.05) (Table 7).

Table 8. Effects of *F. vulgaris* aqueous extract on the absolute lengths of kidney structures (proximal and distal convoluted tubules, collecting ducts, loop of Henle and vessels (mean±SD)).

Groups (n =10)	Structures (m)				
	PCT	DCT	CD	LH	V
C	31.78±0.72[a]	22.79±0.47[a]	39.88±0.49[a]	19.32±0.57[a]	51.20±0.64[a]
UD	60.75±0.75[d]	45.85±0.51[c]	55.45±0.54[c]	30.94±0.63[c]	79.24±0.79[c]
G	41.53±0.64[c]	31.97±0.47[b]	45.97±0.67[b]	23.25±0.42[b]	56.93±0.42[a]
FV200	44.92±0.66[c]	35.20±0.71[b]	47.44±0.93[b]	24.89±0.56[b]	61.53±0.66[b]
FV600	41.47±0.8[c]	31.63±0.39[b]	45.67±0.48[b]	22.53±0.42[b]	57.56±0.62[ab]
FV1800	37.28±0.35[b]	26.98±0.49[a]	42.90±0.58[ab]	19.47±0.55[a]	52.50±0.36[a]

C – Control, UD – untreated diabetic, G – glibenclamide-treated; non-identical letters indicate a significant difference between the groups (p≤0.05).

The mean absolute length of kidney subcomponents in treated and untreated groups are shown in Table 8 and Fig. 1. The lengths of the CD, DCT, PCT, LH and V were significantly (p≤0.05) increased in untreated mice as compared to the controls, while *F. vulgaris* at all doses significantly (p≤0.05) decreased the lengths of the CD, DCT, PCT, LH and V when compared to the untreated group (p≤0.05). No significant difference (p≤0.05) was found between FV1800 and the control group in the lengths of the CD, DCT and LH. Moreover, there were no significant differences (p≤0.05) in the lengths of V in the glibenclamide, FV600, FV1800 and controls.

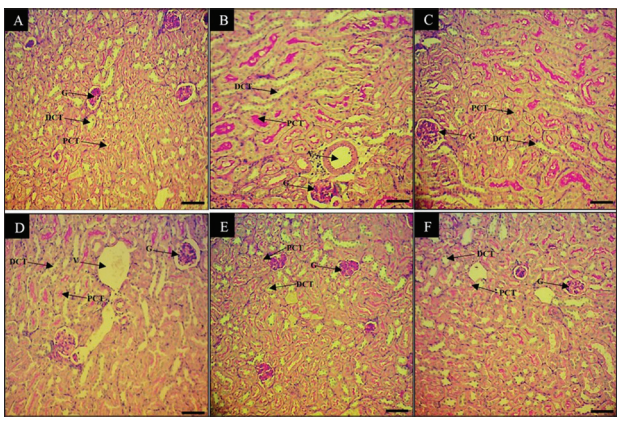

Fig. 1. Longitudinal sections of mice kidney (scale bar=150 µm) after PAS staining. **A** – Control group; **B** – untreated diabetic group; **C** – glibenclamide-treated group; **D**, **E** and **F** – diabetic mice treated with 200, 600 and 1800 µg/kg of *F. vulgaris*, respectively. The lengths and volumes of the G, PCT, DCT and IT increased in the untreated diabetic group (**B**) when compared with the control (**A**). Administration of glibenclamide (**C**) and *F. vulgaris* (**D**, **E**, **F**) decreased the lengths and volumes of the above structures in comparison with the untreated diabetic group (**B**).

DISCUSSION

Diabetes is the most prevalent endocrine disorder, affecting more than 285 million people worldwide. By 2030, this number is expected to rise to 438 million or 7.8% of the adult population [27]. This disease is accompanied by many risk factors such as hyperglycemia, dyslipidemia, hypertension, atherosclerosis, decreased fibrinolytic activity, poor wound healing, hematological disorders such as enhanced platelet aggregation, as well as renal hypertrophy [27].

Ethnomedicinal herbs are used to prevent and treat various diseases, including diabetes [16-18, 28-32]. In this study, we examined the nephroprotective effect of the aqueous extract of *F. vulgaris* in STZ-induced mice. The problems in estimating structural parameters such as size, number and distribution in the three-dimensional kidney from two-dimensional sections or projections of the kidney were overcome by the use of stereological methods. These methods are practical tools based on sound mathematical and statistical principles [33, 34].

Hypoglycemic effect of *F. vulgaris*

In our study, diabetes was induced by a single intraperitoneal injection of STZ, a naturally occurring chemical that is particularly toxic to insulin-producing β-cells of the pancreas [35,36]. STZ as an alkylating agent of nitrosourea class causes DNA inconvenience and cell death [37,38]. The kidney is susceptible to STZ toxicity, and it is difficult to differentiate between diabetic nephropathy-related events and direct effects of STZ [39,40]. In this regard, a single high dose of STZ possesses a nonspecific cytotoxic property that causes renal failure in rodents such as mice [41-43]. The results of serum glucose concentrations revealed that *F. vulgaris* at all doses on days 16 and 20 caused

significant differences in treated diabetic groups when compared to the untreated diabetic group, but there was no significant difference between the experimental doses of *F. vulgaris* and the antidiabetic drug glibenclamide. It is generally accepted that glibenclamide, a sulfonylurea-family drug, causes a reduction in blood glucose predominantly via stimulation of insulin release from pancreatic β-cells, and that during long-term treatment, an insulin-independent blood glucose-decreasing mechanism may operate [44]. It can be assumed that a plant extract such as *F. vulgaris,* similarly to glibenclamide, produced hypoglycemic and antidiabetic effects by enhancing insulin release or insulin-like effects [16,17,44]. In agreement with the results of the present research, in a similar study [45], *F. vulgaris* (100 and 150 mg/kg) significantly reduced serum glucose concentrations ($p<0.01$) and ameliorated the diameter of islets ($p<0.05$) in diabetic rats when compared to the diabetic group.

Hematoprotective effect of *F. vulgaris*

Changes in different cellular components of the immune system and of hematological indices in chemically-induced nephrotoxicity have been reported [46-48]. One study revealed that the administration of toxic chemicals such as STZ produced pancytopenia, indicated by microcytic hypochromic anemia in the blood and by a reduction in RBC and Hb, as well as PCV, MCV, MCH and MCHC [45]. Also, diabetes-associated anemia is reported to be due to the increased nonenzymatic glycosylation of RBC membrane proteins, which correlates with hyperglycemia [46]. Oxidation of these membrane proteins in the presence of chronic hyperglycemia in uncontrolled diabetes mellitus increases the production of lipid peroxides, leading to hemolysis of the RBC and their reduced survival [45].

Acute stress associated with toxic chemicals has been widely shown to be associated with enhanced WBC and platelet counts, a significant increase in the neutrophil count and the neutrophil/lymphocyte ratio [46,47]. It was suggested that the higher platelet count may contribute to vascular events in patients with insulin resistance [46]. Raised platelet values are commonly seen in inflammatory and infectious diseases and are considered as part of the acute phase reaction [46,47].

In the present study, the number of WBC and percentage of eosinophils and basophiles increased, but the percentage of lymphocytes and monocytes and the levels of RBC, Hb, PCV, MCV, MCH and MCHC decreased significantly ($p\leq0.05$) in untreated diabetic mice. Treatment with *F. vulgaris* at all doses (especially FV1800) and with glibenclamide significantly ($p\leq0.05$) ameliorated the above parameters in comparison with untreated diabetic mice.

Nephroprotective effect of *F. vulgaris*

Renal damage is determined by measuring biochemical and histological parameters. The enhanced serum parameters such as urea and creatinine have been attributed to the damaged structural integrity of the kidney because these are released in the circulation after cellular damages [46-48]. In our study, we observed acute renal damage following STZ administration as an increase in urea and creatinine concentration as compared to control mice. But, *F. vulgaris* at all doses and glibenclamide could significantly ($p\leq0.05$) decrease urea and creatinine parameters.

Similar to the glibenclamide-administered group, in mice exposed to *F. vulgaris* intake, the renal structural changes at all doses, especially at 1800 dose, were improved. Untreated diabetic mice revealed some degree of renal hypertrophy, which was mainly due to the enlargement of the cortex, medullary and its subcomponents. These changes were ameliorated significantly with *F. vulgaris*. The pathogenesis of renal hypertrophy can be attributed to the overproduction of oxygen-free radicals following toxic chemical administration [48-51]. Thus, compounds with antioxidant activity can ameliorate these changes and prevent the progression of nephropathy [52]. *F. vulgaris* as a medicinal plant rich in antiinflammatory and antioxidant compounds such as spathulenol, carvacrol, alpha-pinene and limonene, and it has strong antiinflammatory and antioxidant properties [20,53,54]. Previous studies have indicated that ethnomedicinal plants rich in antiinflammatory and antioxidant compounds decrease weight, volumes and lengths of kidney and its subcomponents in STZ-induced nephrotoxicity in mice [55,56].

To conclude, our study demonstrates the antidiabetic, hematoprotective and nephroprotective activi-

ties of *F. vulgaris*, suggesting its possible use as a therapeutic supplement or drug. Additional clinical trials are needed to further evaluate the clinical potential of this plant in humans.

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Conflict of interest disclosure: The authors declare that there is no conflict of interest.

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Supplementary Data

Supplementary Fig. S1. Estimation of volume density using a point probe.
Available at: http://serbiosoc.org.rs/NewUploads/Uploads/Zangeneh et al_2686_Fig. S1.pdf