

Hasan V. GÜNEŞ¹
İrfan DEĞİRMENCI¹
Miriş AYDIN¹
Berrin BOZAN²
Erinç ARAL³
Zeynep TUNALIER²
Cengiz ÜSTÜNER¹
Murat ERÇAKIR³
K. Hüsnü C. BAŞER³
Ayşe BAŞARAN¹

The Effects of *Rumex patientia* L. and *Urtica dioica* L. on Some Blood and Urine Parameters, and Liver and Kidney Histology in Diabetic Rats

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¹Osmangazi University, Medical Faculty,
Department of Medical Biology.

²Anadolu University, Medicinal and Aromatic
Plant and Drug Research Center (TBAM).

³Osmangazi University, Medical Faculty,
Department of Histology. Eskişehir-Turkey

Abstract: The effects of *Rumex patientia* and *Urtica dioica* on levels of blood glucose, plasma amino acids and other parameters, urine excreta, and liver and kidney histology were examined in diabetic rats induced by streptozotocin. Streptozotocin increased blood glucose and changed the levels of amino acids and other parameters, and

caused degenerative changes in the liver and kidney. *Rumex patientia* had some protective effect on these parameters changed by streptozotocin, while *Urtica dioica* had no protective effects.

Key Words: Amino acids; Creatinine clearance; HPLC; *Rumex patientia*; Streptozotocin; Enzyme; *Urtica dioica*

Introduction

From the Ebers papyrus of about 1550 BC, it is known that certain plants were used to treat diabetes (1). After the introduction of insulin therapy, the use of traditional treatments for diabetes greatly declined in western countries. However, in some developing countries, in which conventional medicines are not readily available, traditional treatments for diabetes remain a common form of therapy (1, 2). In some areas of Turkey, plants are used still in diabetes therapy.

Streptozotocin (STZ), a N-nitroso-N-methylurea derivative of 2-deoxy-D-glucose, is a diabetogenic agent acting through the selective destruction of pancreatic islet β cells (3-8). It is known that insulin increases the transport of amino acid into the cell and increases the degradation of proteins. It causes changes in the levels of some amino acids. Thus, STZ has some effects on blood and urine amino acid levels (9-11).

STZ increases the levels of serum glutamic oxalocetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), bilirubin, blood urea nitrogen (BUN) and serum uric acid (12, 13).

In addition, STZ displays nephrotoxic and hepatotoxic activity (12). It has been reported that STZ caused cataracts, necrosis of kidney tubules, mesangial proliferation and hyalins of vessels in rats (14).

This paper describes a study of the effect of *Rumex patientia* L. (*Polygonaceae*) and *Urtica dioica* L. (*Urticaceae*) on the blood glucose and amino acid levels, some serum and urine parameters and the liver and kidney histology of diabetic rats induced by STZ.

Materials and Methods

Drugs and Chemicals. Commercial plant materials, *Rumex patientia* grains and *Urtica dioica* leaves were purchased from a herbalist in Çanakkale. The *R. patientia* grains (2 g) were boiled for 10 minutes in distilled water (100 ml) and filtered. *U. dioica* leaves (0.5 g) were cut into pieces, and these were put into a funnel lined with filter paper. Boiled distilled water (100 ml) was added to the funnel and the extract was collected. Streptozotocin (STZ) and other chemicals from Sigma Chemicals: alkaline phosphatase (ALP), serum glutamic oxalocetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) were purchased from Knickerbocker S.A.E. Lab. Cromotest Kits.

Animals. Male albino rats, *Rattus rattus* norvegicus, aged 3 months and weighing 150-300 g were used. They were kept in an air-conditioned room and fed a standard commercial diet and tap water ad libitum.

Treatment. The rats were grouped according to the following schema:

1. Group: Control, 0.3 ml/rat physiologic saline (0.9%) intravenous (i.v) and water orally
2. Group: 0.3 ml/rat citrate buffer i.v. and water orally
3. Group: 2% decoction of the grain of *R. patientia* orally
4. Group: 0.5% infusion of the leaf of *U. dioica* orally
5. Group: 65 mg/kg streptozocin (STZ) i.v. and water orally
6. Group: 65 mg/kg STZ i.v. and 2% *R. patientia* orally
7. Group: 65 mg/kg STZ i.v. and 0.5% *U. dioica* orally

Methods. Glucose levels from the blood of the tail veins of the rats were measured with a glucometer (Ames) before the experiment. Saline and citrate buffer were injected intraperitoneally. STZ dissolved in citrate buffer was injected intravenously. Oral treatment started 48 hours following STZ injection and lasted for 8 days. At 24 and 48 h and on the 5th and 10th days after the administration of STZ the blood glucose was measured in all animals.

On the 9th day of the experiment, urine was collected during a 24-hour period from all animals. The urine creatinine, urea nitrogen and uric acid were determined spectrophotometrically (spectronic 20 D). At the end of the treatment (10th day), all the rats were anesthetized with ether, and blood from the heart was collected into heparinized tubes and allowed to clot at room temperature. Plasma, separated by centrifugation, was deproteinised for the analysis of amino acids (15). Amino

acids were determined using HPLC, and ALP, SGOT, SGPT, and creatinine was determined spectrophotometrically. For the determination of amino acids, supernatants were stored at -80°C until assayed. The HPLC system consisted Shimadzu LC 6A HPLC, SIL 6A automatic injector unit, Shimadzu RF 535 Fluorescence detector and CR4A integrator. Fluorimetric measurements were taken at an excitation wavelength of 335 nm and an emission wavelength of 445 nm. The analysis was performed with a flow rate of 1.5 ml/min. An adsorbosphere 5 m OPA-HR-RP (4.6 mmx15 cm) reversed phase column was used in conjunction with a C8 guard column (5µ, 4.6 mmx2 cm). Five hundred µl of OPA/2-ME (o-phthaldialdehyde/2-mercaptoethanol) reagent was added to the vial containing 100 µl of amino acid standard or sample. The vial was capped, shaken and, after derivatization for 2.5 min, 10 µl aliquots of the sample was introduced into the HPLC column for analysis.

After the animals had been sacrificed, the kidneys and livers were removed and fixed in buffered formaldehyde solution for histological analysis. Paraffin sections (6 µm) were cut, and stained with haematoxylin-eosin (16).

Statistical analysis. The results are expressed as the mean±SE. The statistical analysis was performed by one-way analysis of variance using the Instad 2.0 programme (Graph Pad Software, San Diego, USA), running on a Macintosh Computer.

Results

As shown in table 1, no statistical differences were observed in the glucose levels of the control group animals (group 1) at the beginning and in the course of

Table 1. Glucose levels of controls and experimental groups

GROUPS	n	Before injection	GLUCOSE (mg/dl)				
			24 hours	48 hours	5th day	10th day	
Control Groups	1st Group Physiologic saline	7	112.6±2.43	102.0±3.18	103.4±6.57	101.4±5.09	82.6±7.79
	2nd Group Citrate buffer	7	117.3±7.32	103.0±3.75	104.6±7.28	107.0±4.62	87.5±5.73
	3rd Group 2% <i>R. patientia</i>	7	122.9±6.86	104.1±6.19	107.3±5.93	103.0±6.94	95.1±7.69
	4th Group 0.5% <i>U. dioica</i>	7	105.3±4.85	103.1±8.07	96.2±11.07	95.8±5.57	117.6±8.74
	5th Group Water	6	107.7±7.19	237.9±9.83	337.8±35.13	317.5±14.84	258.5±23.31
Diabetic Groups (65 mg/kg STZ)	6th Group 2% <i>R. patientia</i>	6	98.3±8.23	257.8±10.56	249.8±9.83	244.8±17.03	182.0±25.26
	7th Group 0.5% <i>U. dioica</i>	7	119.0±4.91	271.4±11.97	237.9±10.61	264.4±15.56	261.3±10.92

** p<0.01; *** p<0.001

the experiment (24 h, 48 h, 5th and 10th day). STZ significantly increased the blood glucose levels in the 5th, 6th and 7th groups (p<0.01 and p<0.001) compared to the control groups. Although *R. patientia* decreased the high glucose levels induced by STZ, these decreased levels were also high in comparison with the control groups. *U. dioica* had no effect on the high glucose levels induced by STZ.

As shown in table 2, no statistical differences were determined in the blood and urine parameters of the 1st, 2nd, 3rd and 4th groups. In blood; Creatinine in the 5th group (p<0.01), ceratinine clearance in the 5th group (p<0.05), ALP in the 5th, 6th and 7th groups (p<0.001), SGOT in the 5th group (p<0.01), SGPT in the 5th and 7th groups (p<0.001) were significantly increased compared to the control groups. Urine volume in the 5th (p<0.001)

Table 2. The levels of some blood and urine parameters in the experimental groups.

GROUPS	n	BLOOD					URINE			
		ALP (U/L)	SGOT (U/L)	SGPT (U/L)	Creatinine (mg/dl)	Creatinine Clearance (ml/min)	Creatinine (mg/dl)	Volume (ml)	Urea Nitrogen (mg/dl)	Uric Acid (mg/dl)
1st Group (Physiologic saline)	7	43.86±4.83	54.86±2.81	9.43±1.09	2.99±0.07	0.15±0.02	83.87±4.85	7.07±1.19	2145.1±175.3	8.00±1.35
2nd Group (Citrate buffer)	7	37.80±3.76	62.28±3.57	13.00±0.85	2.96±0.10	0.19±0.02	81.49±7.22	9.29±1.30	1968.6±108.2	7.43±1.25
3rd Group (2% <i>R. patientia</i>)	7	23.80±3.76	62.28±3.57	10.00±0.53	3.63±0.25	0.13±0.03	85.40±10.40	3.79±0.66	2470.9±235.1	15.75±2.47
4th Group (0.5% <i>U. dioica</i>)	7	46.00±1.67	67.4±8.37	12.20±1.46	3.11±0.06	0.10±0.03	35.74±5.63	8.20±1.83	1876.6±203.5	3.80±1.02
5th Group (water)	6	143.6±6.7	81.16±13.6	28.30±6.03	4.10±0.30	0.35±0.02	20.63±2.40	17.75±2.40	1891.7±63.59	4.00±0.45
6th Group (2% <i>R. patientia</i>)	6	95.90±19.7	50.16±7.18	11.50±0.89	2.99±0.27	0.15±0.03	63.13±7.70	3.71±1.06	2303.0±249.2	10.86±1.75
7th Group (0.5% <i>U. dioica</i>)	6	82.90±11.9	74.16±2.55	25.50±3.07	3.86±0.32	0.28±0.15	48.56±9.65	11.58±1.86	2121.3±286.8	7.17±2.63

*: p<0.05 **: p<0.01 ***: p<0.001

Table 3. Effect of *R. patientia* and *U. dioica* on plasma amino acids levels in streptozotocin (STZ) induced diabetes in rats (nmol/ml).

	Arpartic acid	Glutamic acid	Serine	Histidine	Glycine	Threonine
1st Group (control) Physiologic saline	9.21±0.24	83.11±3.16	142.41±7.15	37.83±2.16	191.27±4.66	165.28±6.71
2nd Group Citrate buffer	10.08±0.53	83.14±3.81	131.40±6.83	42.02±2.84	194.02±8.66	150.21±6.07
3rd Group 2% <i>R. patientia</i>	7.59±0.43	83.58±5.15	128.18±9.32	39.07±2.03	167.27±6.74	174.17±8.51
4th Group 0.5% <i>U. dioica</i>	8.42±0.26	80.68±5.11	140.22±7.75	49.40±3.97	159.52±4.68	172.67±5.95
5th Group STZ+Water	*	*	*	*	*	*
6th Group STZ+2% <i>R. patientia</i>	5.43±0.55	40.13±2.65	112.63±5.84	59.51±4.98	146.20±12.4	185.54±7.71
7th Group STZ+0.5% <i>U. dioica</i>	7.19±0.37	45.51±4.19	140.64±3.76	57.25±2.17	182.37±8.34	201.31±8.34
	6.04±0.30	60.07±7.12	130.24±4.09	59.15±5.82	151.40±12.1	153.06±9.68

* p<0.01, ** p<0.001

Table 3 continue

	Tyrosine	Methionine	Valine	Phenyl- alanine	Isoleucine	Leucine	Lysine
1st Group (control) Physiologic saline	59.66±3.65	36.85±2.24	139.78±5.12	48.94±2.07	81.01±3.16	127.19±4.76	334.56±12.98
2nd Group Citrate buffer	57.31±4.19	36.50±2.23	135.03±4.03	47.84±2.65	79.91±2.16	132.53±11.32	296.45±21.23
3rd Group 2% <i>R. patientia</i>	63.23±4.27	35.02±2.09	145.45±8.19	46.51±2.86	83.65±6.58	166.27±42.31	285.00±28.78
4th Group 0.5% <i>U. dioica</i>	77.17±3.64	* 54.57±4.49	145.26±10.87	58.34±3.97	80.64±5.11	126.57±6.83	229.22±24.27
5th Group STZ+Water	48.59±3.58	38.76±2.15	* 245.84±16.98	* 71.07±3.61	* 111.25±6.45	* 192.22±12.10	268.83±19.81
6th Group STZ+2% <i>R. patientia</i>	50.94±2.27	* 51.52±4.15	* 201.53±14.14	62.57±4.75	83.37±4.36	140.65±14.37	282.03±15.16
7th Group STZ+0.5% <i>U. dioica</i>	77.76±8.00	* 60.85±4.93	** 348.13±12.33	* 77.89±7.27	** 182.90±12.52	** 242.38±17.62	** 188.64±18.52

* p<0.01, ** p<0.001

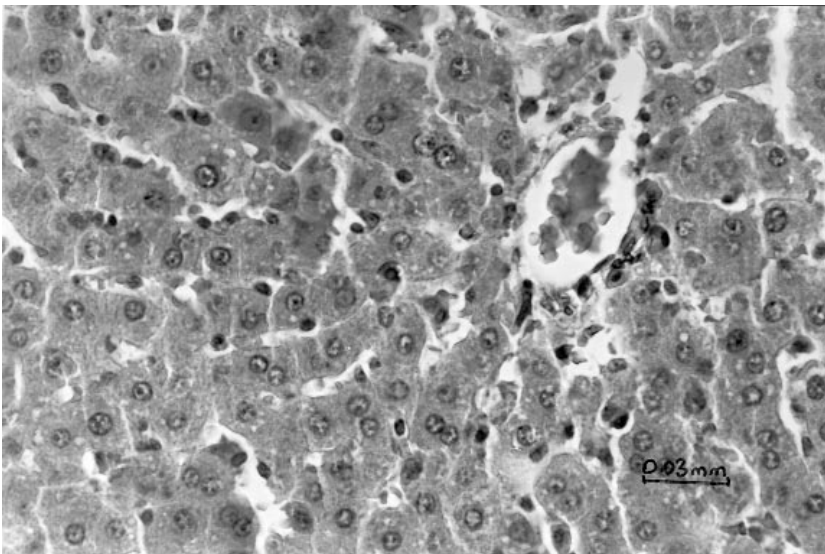


Figure 1. Section of liver from an animal treated STZ+*U. dioica*. Low level hyperaemia, cell infiltrations, hydropic degeneration. H.E.

and 7th groups (p<0.01) had significantly increased, while urine ceratinine was lower in the 5th group (p<0.001).

Table 3 shows the plasma amino acid levels determined by HPLC. STZ caused a decrease in the levels of aspartic acid, glutamic acid, serine and glycine; and increased the levels of histidine, valine, phenylalanine, isoleucine and leucine. *R. patientia* induced a slight, nonsignificant decrease in these parameters. *U. dioica*

caused an additional increase in the levels of valine, phenylalanine, isoleucine, leucine and lysine.

No significant histological changes in the liver and kidney were seen in the 1st, 2nd and 3rd groups. Although cell infiltrations and a few sinusoidal congestions were seen in the 4th group, the histologic appearance of the livers was similar to the control group. Furthermore in the kidneys of this group there were some hyperaemic

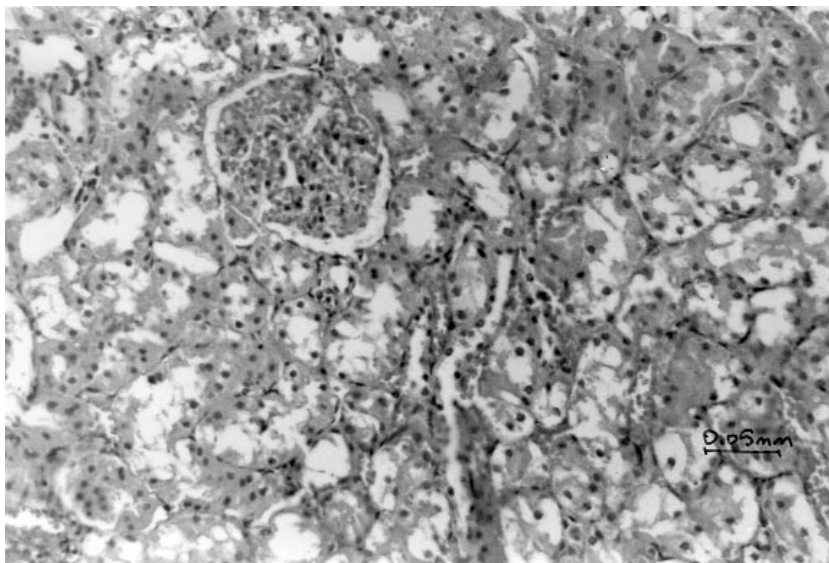


Figure 2. Section of kidney from an animal treated STZ+*U. dioica*. Degeneration of distal and proximal tubules and hyperaemia H.E.

areas. In the livers of the 5th group, cell infiltrations, low level sinusoidal congestions and hydropic degeneration in some hepatocytes were observed. The proximal and distal tubules of kidneys in this group contained some degenerative changes and some hyperaemic areas. In the 6th group, the livers and kidneys were similar to the 5th group. The livers in the 7th group receiving STZ and *U. dioica* exhibited low level hyperaemia and cell infiltration, and hydropic degeneration in some hepatocytes (Fig. 1). The proximal and distal tubules of the kidneys exhibited degenerative alterations and hyperaemia (Fig. 2).

Discussion

Single dose administration of STZ produces diabetogenic effects in the first 24 hours in rats (8, 17). It also showed effects at 30 minutes in studies of perfused rat pancreas (6, 12, 18). In the present study, it was found that glucose levels increased significantly 24 hours after STZ administration. *R. patientia* decreased the high STZ-induced glucose levels. However, these levels were still significantly high compared to the control group (group 1).

Piyachaturawat et al. (1991) reported that STZ exhibits nephrotoxic and hepatotoxic activity. It was suggested that its effects on the kidney and liver and its function in excretion and metabolism respectively. It has been reported that diabetes causes an increase in urine

volume, creatinine, ALP, SGOT and SGPT (7, 9, 13). In the present study, STZ caused increases in these parameters, while these parameters were decreased by *R. patientia* compared to control group levels. *U. dioica* was either less effective. Some authors have reported an increase in valine, isoleucine and leucine concentration, and decrease in the serine, threonine and glycine concentration in the urine and blood of diabetes mellitus patients. Their glycemia levels were not measured (9, 19). STZ also, has similar effects (20). In the present study, an increase in histidine and phenylalanine was observed, and there was a decrease in aspartic acid and glutamic acid levels. This is different from the findings of other studies (9, 19-24). Except for the glutamic acid, histidine and valine, the amino acid levels were similar to the control group levels with *R. patientia*, while *U. dioica* had no effect on these levels changed by STZ. Our histological results confirm the results from urine and blood. *R. patientia* has a much less protective effect on nephrotoxicity and hepatotoxicity induced by STZ. But *U. dioica* had no protective effect, and it even caused an increase in nephrotoxicity.

As a conclusion, *Urtica dioica* used as a traditional medicine has no effect on diabetes. Moreover, it causes some side effects in the kidney and liver. However, *Rumex patientia* has a minor effect on diabetes induced by STZ and has not side effects.

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