Protective role of *Urtica dioica* L. (Urticaceae) extract on hepatocytes morphometric changes in STZ diabetic Wistar rats

Urtica dioica L. (Urticaceae) ekstraktının STZ diyabetik Wistar sıçanlarda hepatositlerdeki morfometrik değişiklikler üzerine koruyucu etkisi

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Background/aims: *The present investigation was carried out to evaluate the protective effect of the hydroalcoholic extract of Urtica dioica leaves on the quantitative morphometric changes in the liver of streptozotocin-induced diabetic rats.* ***Methods:*** *Thirty male Wistar rats were divided into control (G1), diabetic (G2), diabetic + Urtica dioica (G3) groups. The control group received only sham injections of intraperitoneal saline; the diabetic group received intraperitoneal saline for 5 days followed by streptozotocin (80 mg/kg) on the 6th day; and the diabetic + Urtica dioica group received 100 mg/kg Urtica dioica intraperitoneal (7) injections for 5 days and streptozotocin injection on the 6th day. After five weeks, the animals were sacrificed and whole livers removed. Liver specimens were used for quantitative morphometric analysis after hematoxylin and eosin staining. All data were statistically analyzed by one-way ANOVA and expressed as the mean with standard error of means.* ***Results:*** *In the G3 (diabetic + Urtica diocia) group, the mean surface area of hepatocytes in the periportal zone (Z1) was greater than in G2 (diabetic) and G1 (control) groups, but this difference was not significant. No alteration was observed in the surface area of hepatocytes in the perivenous zone (Z3) in the diabetic+Urtica dioica (G3) group compared to the diabetic (G2) group. The mean nuclear area of hepatocytes of the rats in the diabetic+Urtica dioica (G3) group was higher in Z1 and lower in Z3 than that of rats in the diabetic (G2) group. The mean diameter of hepatocyte nuclei in the diabetic + Urtica dioica (G3) group was lower than that of diabetic (G2) and control (G1) groups in both Z1 and Z3.* ***Conclusions:*** *This study revealed that the administration of extract of Urtica dioica leaves before induction of diabetic with streptozotocin has a protective effect on the morphometric alterations of hepatocytes in the periportal and perivenous zones of the liver lobule in rats.*

Key words: Streptozotocin diabetes, Urtica dioica extract, rat liver, morphometry, periportal/perivenous zone

Amaç: *Bu çalışmada, streptozin ile diyabet geliştirilen sıçanlarda, Urtica dioica yapraklarının hidroalkol ekstraktının, karaciğerde gözlenen kantitatif morfometrik değişiklikler üzerine olan koruyucu etkisinin araştırılması amaçlanmıştır.* ***Yöntem:*** *Otuz adet erkek Wistar cinsi sıçan kontrol (G1), diyabetik (G2) ve diyabetik+Urtica dioica (G3) olmak üzere 3 gruba ayrıldılar. Kontrol grubuna sadece intraperitoneal salin enjeksiyonu yapılırken, diyabetik gruptaki sıçanlara 5 gün salin enjeksiyonun ertesinde 6. gün intraperitoneal olarak 80 mg/kg streptozosin uygulandı. diyabetik+Urtica dioica grubunda ise 5 gün boyunca 100 mg/kg Urtica dioica enjeksiyonunu takiben 6. günde 80 mg/kg streptozosin uygulandı. Beş hafta takibin ertesinde tüm sıçanlar sakrifiye edilerek karaciğerleri bütün olarak çıkartıldı. Karaciğer örnekleri hematoksilen ve eosin ile boyandıktan sonar kantitatif morfometrik analizler için kullanıldılar. Tüm veriler ortalama ± standard sapma kullanılarak tanımlandı ve tek yönlü ANOVA ve değerlendirildi.* ***Sonuç:*** *G3'de (Diyabetik+Urtica dioica) periportal bölgedeki (Z1) hepatositlerin ortalama yüzey alanlar G2 (Diyabetik) ve G1'dekilerden daha büyüktü ancak fark istatistiksel olarak anlamlı bulunmadı. Ayrıca, perivenöz (Z3) bölgedeki hepatositlerin yüzey alanları karşılaştırıldığında G3 (Diyabetik+Urtica dioica) ile G2 (Diyabetik) arasında anlamlı farklılık tespit edilmedi. Ortalama çekirdek yüzey alanları G3 (Diyabetik+Urtica dioica) grubunda G2 (Diyabetik) ile karşılaştırıldığında, Z1'de daha yüksek ve Z3'de daha düşük bulundu. Ortalama hepatosit çekirdek çapı Z1 ve Z3 bölgelerinde, G3'de (Diyabetik+Urtica dioica), G2 (Diyabetik) ve G1 (Kontrol) gruplarına göre daha düşük bulundu.* ***Yorum:*** *Bu çalışmanın sonuçarına göre; sıçanlarda, Urtica dioica yapraklarının ekstraktının uygulanmasının, streptozosin ile diyabetik geliştirilen sıçanlarda karaciğer lobulündeki periportal ve perivenöz bölgelerdeki hepatositlerde gözlenen morfometrik değişiklikler üzerinde koruyucu etkisi mevcuttur.*

Anahtar kelimeler: Streptozosin diyabeti, Urtica dioica ekstraktı, sıçan karaciğeri, morfometri, periportal/perivenöz bölge

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Manuscript received: 02.12.2008 **Accepted:** 04.02.2010

doi: 10.4318/tjg.2010.0098

INTRODUCTION

Diabetes is a common endocrine disorder associated with various structural and functional liver abnormalities that affect glycogen and lipid metabolism (1-3).

The liver utilizes glucose as a fuel and also has the ability to synthesize it from non-carbohydrate precursors (glyconeogenesis) and store it in the form of glycogen (4).

Recent decades have shown a resurgent interest in traditional plant treatments for diabetes. Plants often contain substantial amounts of antioxidants including alpha-tocopherol (vitamin E), carotenoids, ascorbic acid (vitamin C), flavonoids, and tannins (5), and it has been suggested that antioxidant activity may be an important property of plant medicines used in diabetes. *Urtica dioica* L. (Urticaceae), a folk-medicine known as nettle, has been identified as a contributor to the observed medicinal effect of this plant. *Urtica dioica* (*U. dioica*) has been used as a remedy for diabetes mellitus (DM) (6-9), rheumatoid arthritis, hypertension and allergic rhinitis (10-12), and cardiovascular disease (13,14).

The extracts of *U. dioica* leaves and seeds are suggested to be useful for patients suffering from neutrophil function deficiency (15). It was reported that *U. dioica* prevented damage in the rat liver (16). Some researchers studied hypoglycemic, inhibitory and antitoxic effects of *U. dioica* on the liver, biochemically and physiologically (8,17-20); however, quantitative morphometric studies of its effects are sparse.

The present investigation was carried out to evaluate the protective effect of the hydroalcoholic extract of *U. dioica* leaves on the quantitative morphometric changes in the liver of streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

Plant Material

Urtica dioica L. (Urticaceae) leaves were collected from the cultivated plant, from the suburb of Gorgan, northern Iran (Golestan, Iran) and taxonomically identified in the Department of Pharmacognosy, Mazandaran University of Medical Sciences. A voucher specimen (5-77-1) was deposited in the herbarium of Mazandaran University.

Preparation of the Hydroalcoholic Extract of *U. dioica*

Powder of *U. dioica* leaves was percolated by hydroalcoholic (60°) solvent for 48 hours (h). The extract was filtered and concentrated under vacuum at 40°C to make a jellied material by vacuum spray dryer. In addition to thin layer chromatography and purity tests (foreign matter, total ash, acid insoluble ash and water insoluble ash) for qualification analysis, monosaccharide-linked spectrophotometric assay was carried out to determine the concentration of polysaccharides in *U. dioica* leaves for standardization of the extract. The results of phytochemical analysis showed the presence of a high percentage of tannins and steroids and low percentage of flavonoids, carotenoids and saponins in the leaves of *U. dioica*.

Animals

Thirty male 8-10 postnatal Wistar rats (125-175 gram) were used for this study. Ethical approval and animal care were in accordance with the principles of the regulations in use at Gorgan University of Medical Sciences, Gorgan, Iran. The rats were housed in groups of three in standard animal cages and kept under standard laboratory conditions in Gorgan University of Medical Sciences. Animals had free access to rat pelleted chow and tap water.

Experimental Design

Thirty male Wistar rats were divided into control (G1), diabetic (DM) (G2), diabetic+UD (G3) groups. The control group received only sham injections of intraperitoneal (IP) saline; the diabetic group received IP saline for 5 days followed by STZ (80 mg/kg) on the 6th day; and the DM+UD group received 100 mg/kg UD IP (7) injections for 5 days and STZ injection on the 6th day.

Glucose concentration in the blood of the tail vein of the rats was measured with an Accu-chek active blood glucose monitor test strip (8). Blood glucose range above 200 mg/dl was considered as hyperglycemia (21).

Glucose Tolerance Test

Intraperitoneal glucose tolerance test (GTT) was performed on rats fasted 16 h using 2 g glucose/kg-body weight. In all groups, blood was collected from the animals by tail snipping at 0, 30, 60 and 120 min after glucose load. Glucose test was performed before injection of *U. dioica* extract and STZ (on 1st and 6th days of experiment) and after IP injection of STZ on the 10th, 20th and 35th days of the experiment.

Five weeks from the start of the experiment, all animals in the three groups were deeply anesthe-

tized with ether. Livers were removed and fixed in natural buffered formaldehyde fixative. Pieces of hepatic tissue were obtained from the left lateral and right posterior lobes and sliced at 5 mm thickness and embedded in paraffin wax after overnight automatic processing. Hematoxylin and eosin-stained sections (22) at 5-μm thickness with 30-μm distance were used for morphological and morphometric analyses (Figure 1).

Morphometric Measurements

The surface areas of the hepatocytes in zone 1 (Z1, close to the portal tract) and in zone 3 (Z3, close to the central vein) of the hepatic acinus were measured using the Olympus BX-51T-32E01 research microscope connected to DP 12 Camera with 3.34 million pixel resolution and Olysia Bio software (Olympus Optical Co. LTD, Tokyo, Japan).

For each hepatocyte, total cell surface, nuclear area and nucleolar area were measured. The outline of each hepatocyte was measured initially followed by moving the computer mouse along the outline of the hepatocytes after tacking an image with 40X objective. A separate measurement for nuclei and nucleoli was performed by the same methodology with 100X oil objective. At least 50 hepatocytes from each zone (total 100) were measured in each liver. For each chosen hepatocyte, total area and perimeter of hepatocyte, nucleus and area of the nucleolus and long and short axes of the nucleus were measured by Olysia Bio software, and then other parameters were obtained from these data with SPSS software.

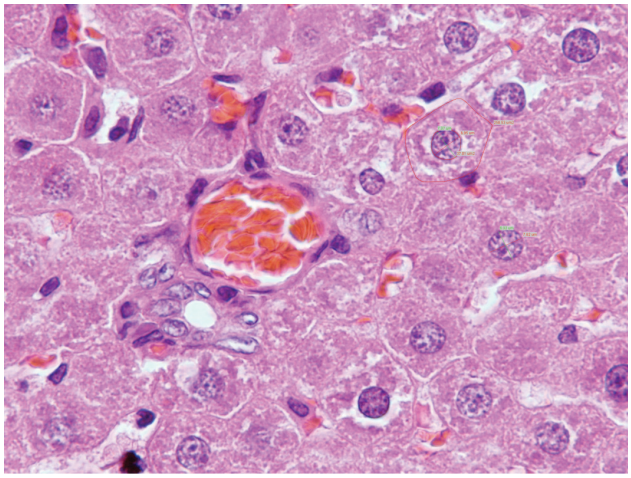

Figure 1. Morphometric characteristics of hepatocytes in periportal zone (Z1) in the control group (hematoxylin & eosin, 1000X).

Morphometric Variables

The following variables were used in this study (23, 24):

Area: total area of hepatocytes, nuclei and nucleoli in mononuclear cells.

Perimeter: perimeter of whole hepatocytes.

Diameter: diameter of nucleus (mean axis lengths).

Long axis: long axis of the nuclei.

Short axis: short axis of the nuclei.

Nuclear/cytoplasmic ratio: the ratio of mean nuclei to the mean area of their cytoplasmic area.

Nuclear area ratio: the ratio of the mean area of nuclei to the mean area of their nucleoli.

Cytoplasmic area: the hepatocyte area minus nuclear area.

Area difference: nuclear area minus nucleolar area.

Statistical Analysis

A general lineal model was used to analyze the glucose data. Comparison of morphometric parameters between the groups was made using the one way analysis of variance (ANOVA). Post-hoc comparisons of parameters were performed using the Bonferroni procedure. All the statistical computations were made using the statistical packages SPSS version 11.5. A value of $p<0.05$ was considered to indicate a significant difference between groups.

RESULTS

Blood Glucose Concentrations

The baseline blood glucose concentrations before injections were 92.5±0.9, 99.2±2.1 and 93.4±1.9 mg/dl in control (G1), DM (G2) and DM+UD (G3) groups, respectively.

The mean±SE of blood glucose concentrations after injections of U. dioica extract was 95.50±1.6 mg/dl in the DM+UD (G3) group. The ranges of blood glucose concentration after injection of STZ in control (G1), DM (G2) and DM+UD (G3) groups are depicted in Table 1. In the control (G1) group, the mean±SE of blood glucose concentration did not show any changes. Statistical analysis showed that the interaction between groups and days was significant ($p<0.01$). Multiple post hoc comparisons with Bonferroni revealed some significant differences between groups (Table 2). GTT findings such as shift-down (recovery) of GTT curve on day 1, day 6, day 10 and day 35 of the experiment are shown in Figure 2.

Table 1. Blood glucose level (mg/dl) of rats in Control (G1), Diabetic (G2) and Protective (DM+UD) groups on different days

Groups (n=10)	Blood Glucose Level (mg/dl)					
	Day 1	Day 6	Day 10	Day 20	Day 35	P-value
Control (G1)	92.5± 0.9	89.3±1.2	93.2±1.2	93.5±3.3	94.4±2.4	
Diabetic (DM)	99.2± 2.1	92.3±4.1	269.7±20.3	296.4±49.5	450.7±33.6	**<0.01**
Protective (DM+UD)	93.4±1.9	95.50±1.6	252.6±13.5	299.10± 65.0	298.56±80.1	

P-value group total day <0.01
Control=(G1), Diabetic (DM)=G2, Protective (DM+UD)=G3
Results are expressed as Mean±SE of the mean.

Table 2. Multiple comparisons of glucose level between Control, Diabetic and Protective (DM+UD) groups

(I) Groups	(J) Group	Significance	95% Confidence Interval (CI) Lower Bound	Upper Bound
Control (G1)	Diabetic	.001	-220.13	-78.03
	Protective	.001	-186.31	-44.21
Diabetic (DM)	Control	.001	78.03	220.13
Protective (DM+UD)	Control	.001	44.21	186.31

Bonferroni based on observed means (p<0.05).
Control=(G1), Diabetic (DM)=G2, Protective (DM+UD)=G3

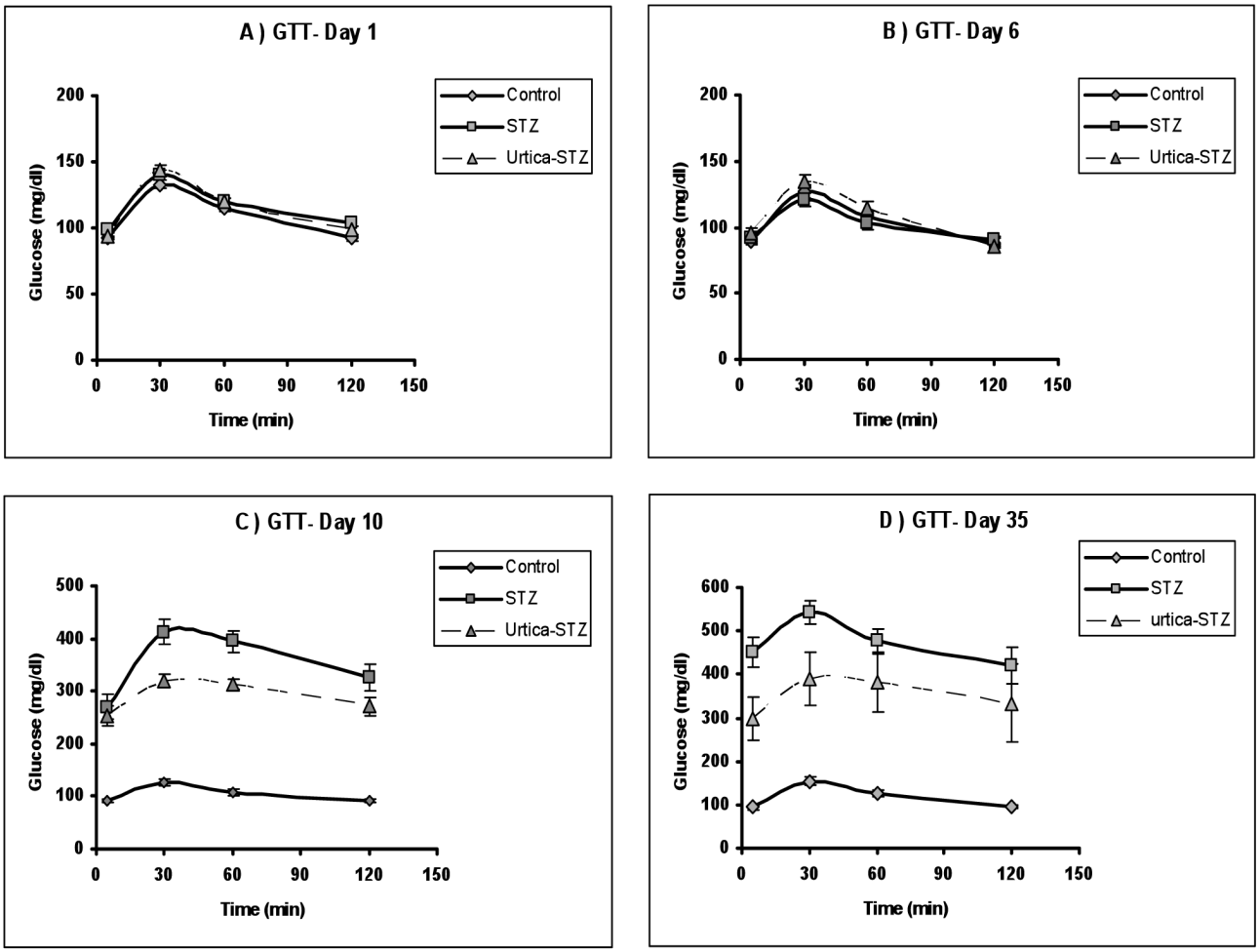

Figure 2. The curves of Glucose Tolerance Test (GTT) in Control, Diabetic (STZ) and Protective (DM+UD) groups.
A) Day 1 (before injection of *U. dioica*), B) Day 6 (after injection of *U. dioica* and before injection of STZ), C) Day 10 (5 days after injection of STZ), and D) Day 35.

Table 3. Quantitative parameters of hepatocyte area, perimeter, cytoplasmic area and nuclear/cytoplasmic ratio in hepatocytes of Zone 1 and Zone 3 in Control, Diabetic and Protective (DM+UD) Groups (n =10)

Groups	Hepatocyte (μm)^2		Hepatocyte perimeter (μm)		Cytoplasmic area (μm)^2		Nuclear/cytoplasmic ratio	
	Z1	Z3	Z1	Z3	Z1	Z3	Z1	Z3
Control (G1)	260.82±4.92	263.90±4.80	67.24±0.67	68.09±0.64	212.05±4.58	216.11±4.50	0.24±0.00*	0.22±0.05
Diabetic (DM)	257.45±4.08	277.77±3.45	65.68±0.51	69.02±0.53	212.24±3.54	228.78±3.19	0.21±0.00*	0.21±0.00
Protective (DM+UD)	261.46±5.07	263.58±6.77	66.67±0.76	68.04±0.89	215.02±4.80	216.53±6.30	0.23±0.01	0.22±0.00

Results are expressed as Mean±SE of the mean (p value <0.05) Control=(G1), Diabetic (DM)=G2, Protective (DM+UD)=G3 * Diabetic and Control group are significant Protective and Diabetic group are significant

Changes in the Total Area of Hepatocytes

Quantitative parameters of hepatocyte area, perimeter, cytoplasmic area, and nuclear/cytoplasmic ratio in Z1 and Z3 of hepatocytes in control (G1), DM (G2) and DM+UD (G3) groups are given in Table 3. The mean of these parameters in Z1 of hepatocytes in the DM+UD (G3) group was higher than in DM (G2) and control (G1) groups, but this difference was not significant. Furthermore, the area of hepatocytes and cytoplasmic area in Z3 in the DM (G2) group were higher than in the other groups, and the nuclear/cytoplasmic ratio in Z3 in the DM+UD (G3) group was higher than in the control (G1) and DM (G2) groups.

Changes in the Nuclei

The changes in the nuclei in the three groups of animals are shown in Table 4.

The mean nuclear area in the DM+UD (G3) group was higher in Z1 and lower in Z3 than that of rats in the DM (G2) group. The mean diameter of hepatocyte nuclei in the DM+UD (G3) group was lower than that of DM (G2) and control (G1) groups in both Z1 and Z3.

Changes in the Nucleolar Area

The measurements of nucleolar areas are shown in Table 5. The mean nucleolus area in the DM+UD (G3) group was higher in Z1 and lower in Z3 than that of rats in DM (G2) and control (G1) groups.

DISCUSSION

Microscopic measurements revealed morphometric changes in the liver due to the hydroalcoholic extract of *U. dioica* leaves before inducing diabetes in rats. The results of this study showed that U. dioica extract caused an increase in the area of hepatocytes and nuclear area in Z1 and a reduction in the area of hepatocytes and nuclear area in Z3 in the protective (DM+UD) group in comparison to the diabetic (DM) group. Therefore, administration of *U. dioica* leaves extract before inducing diabetes in rats could prevent reduction of hepatocyte area in the periportal zone and increase in the nucleus area in the perivenous zone in the protective group.

There have been no extensive studies about the therapeutic effects of *U. dioica* on the liver in a diabetic model. Several experimental studies investigated the role of *U. dioica* extract in the liver biochemically and pathologically. Gunes et al. (25)

Table 4. Quantitative parameters of hepatocyte nuclei in Zone 1 and Zone 3 in Control, Diabetic and Protective (DM+UD) groups (n=10)

Groups	Area (μm)^2		Perimeter (μm)		Diameter (μm)		Long Axis (μm)		Short Axis (μm)	
	Z1	Z3	Z1	Z3	Z1	Z3	Z1	Z3	Z1	Z3
Control (G1)	48.76±0.81 *	47.78±0.80	29.50±0.26 *	28.89±0.26 *	7.75±0.04 *	7.56±0.06 *	8.15±0.05 *	7.96±0.07 *	7.35±0.05 *	7.17±0.07
Diabetic (DM)	45.21±0.85 *	48.99±0.74	27.59±0.33 *	28.24±0.26	7.45±0.06 *	7.47±0.06	7.78±0.07 *	7.83±0.07	7.13±0.07	7.12±0.06
Protective (DM+UD)	46.44±0.88	47.05±0.81	28.72±0.29	27.07±0.30 *	7.38±0.07 *	7.29±0.07 *	7.82±0.08* *	7.63±0.07 *	6.95±0.07 *	6.96±0.07

Results are expressed as Mean±SE of the mean (p value <0.05).

Control=(G1), Diabetic (DM)=G2, Protective (DM+UD)=G3 * Protective and Diabetic groups with Control group are significant Protective and Diabetic group are significant

Table 5. Quantitative parameters of nucleolar areas, area difference (nuclear minus nucleolar areas) and nuclear/nucleolar ratio from hepatocytes in Zone 1 and 3 in Control, Diabetic and Protective (DM+UD) groups (n=10)

	Nucleolus area $(\mu m)^2$		Area difference $(\mu m)^2$		Nuclear / Nucleolar ratio	
Groups	**Z1**	**Z3**	**Z1**	**Z3**	**Z1**	**Z3**
Control (G1)	5.25±0.12	5.16±0.13	43.51±0.77*	42.62±0.74	9.85±0.22*	9.87±0.21
Diabetic (DM)	5.21±0.14	5.32±0.13	39.99±0.77*	43.66±0.69	9.25±0.20	9.80±0.22
Protective (DM+UD)	5.56±0.15	4.91±0.13	40.87±0.80	42.14±0.87	9.01±0.22*	10.52±0.28

Results are expressed as Mean±SE of the mean (p value <0.05) Control=(G1), Diabetic (DM)=G2, Protective (DM+UD)=G3* Protective and Diabetic groups with Control group are significant

reported that *U. dioica* extract had no significant effect on histological changes in the liver of STZ-induced diabetic rats. He observed cell infiltration, low-level sinusoidal congestion and hydropic degeneration in the STZ-induced diabetic rats as well as the *U. dioica* extract protective group after inducing diabetes. Gunes concluded that *U. dioica*, when used as a traditional medicine, has no effect on diabetes. Moreover, it causes some side effects in the liver.

Onal et al. (19) conducted a study with *U. dioica* with alpha-glycosidase inhibitor activity to identify a prophylactic effect for diabetes. The inhibitory effects of this plant against the enzyme source (liver, small intestine) were surveyed.

The exact mechanism of the morphometric changes due to *U. dioica* in animal diabetic models is not clear but there are some possible mechanisms of these alterations in the liver parenchymal cells. These morphometric changes may be influenced by components in the extract of *U. dioica*, which contains both organic and inorganic constituents. Some researches have determined the effect of the extract of *U. dioica* on biotransformation enzyme systems, lipid peroxidation, antioxidant enzyme systems, and lactate dehydrogenase in diabetic rats (8, 17, 20) and in carbon tetrachloride-hepatotoxicity rats (18, 26). Furthermore, aqueous infusion of *U. dioica* exhibits antioxidant activity towards iron-promoted oxidation of phospholipids, linoleic acid, and deoxyribose (27).

In addition, the extract of *U. dioica* may affect the diabetes action on hepatic parenchymal cells (STZ-induced diabetes) or affect directly the activity of hepatocytes. In this study, we observed various alterations in Z1 and Z3 of hepatocytes in diabetic rats that may be related to different metabolic capacities and subcellular structures.

Regarding the increase in cytoplasmic area in the protective (DM+UD) group in this study, it may be due to cytoplasmic mechanism action of the extract components resembling the effect of steroids. These changes can be due to smooth endoplasmic reticulum proliferation in hepatocytes, as shown in some studies (23, 28).

Oxidative energy metabolism, gluconeogenesis and biotransformation reactions are catalyzed mainly in the periportal zone, and glycolysis linked to liponeogenesis, glucose utilization, glycogen storage and lipid formations are predominant in the perivenous zone (29-31). Thus, it was suggested that liver cells are functionally heterogeneous, with zones periportal being predominantly gluconeogenic and perivenous glycolytic (29). Also, nuclei are responsible for production of RNA and consequent cytoplasmic protein synthesis (32, 33). Diabetes is associated with loss of heparan sulfate (HS) from the liver, which may impede lipoprotein clearance (34). Diabetes suppresses hepatic N-deacetylase/N-sulfotransferase-1 (NDST) enzymatic activity in the periportal more than perivenous zone in diabetic rats (34). Whereas nuclei are responsible for production of RNA, and consequent protein synthesis such as enzymes (NDST) may cause an increase in nuclei size in the perivenous zone in the diabetic group.

In addition, in diabetes, hyperglycemia is responsible for the development of oxidative stress (8,35). The modified oxidative state and free radical species impair structure of the cell membrane. Lipid peroxidation, oxidative stress and resultant tissue damage are hallmarks of cell death in diabetes (36-38).

Any compound, natural or synthetic, with antioxidant properties that might contribute towards the partial or total alleviation of these damages may have a significant role in the treatment of DM (39). The antioxidant responsiveness mediated by *U. dioica* may be anticipated to have biological significance in eliminating reactive free radicals that may otherwise affect the normal cell functioning. Since the *U. dioica* extract has antioxidant proper-

ties, it can possibly affect the mechanisms of STZ and modulate or limit the effects of diabetes on the liver tissue.

In conclusion, this study revealed that the administration of hydroalcoholic extract of *U. dioica* leaves (100 mg/kg/day for 5 days) before induction of DM with STZ has a protective effect on the morphometric alterations of hepatocytes in periportal and perivenous zones of the liver lobule in Wistar rats.

Acknowledgement

We thank the Research Department of Gorgan University of Medical Sciences for its financial support for this study.

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