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## Effect of *Urtica dioica* on Morphometric Indices of Kidney in Streptozotocin Diabetic Rats-A Stereological Study

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**Abstract:** The aim of the present study was to investigate the effect of *Urtica dioica* on Morphometric indices of kidney in diabetic rats. Thirty male adult albino wistar rats of 125-175 g divided into control, diabetic and *Urtica dioica* treatment groups. In treatment Group, diabetic rats received 100 mg kg<sup>-1</sup> daily hydroalcoholic extract of *U. dioica* intraperitoneally for 4 weeks. After the animals had been sacrificed, the kidneys were removed and fixed by formaldehyde, cut horizontally into 1 mm slices and processed, Stained with H and E. Stereological study performed using light microscope and the image projected on a table of olysa software. Cavalieri principle was used to estimate the volume of cortex, medulla and whole kidney. All the grouped data statistically evaluated using Student's t-test, expressed as the Mean±SE. Ration of kidney weight /body weight in diabetes (0.51) and diabetes-extract group (0.67) were higher then control group (0.42). Ratio of kidney volume /body weight in diabetes (350) and diabetes-extract group (348) were higher then control group (323). Volume Ratio of cortex /medulla in diabetes-extract group (1.65) was higher then control (1.34) and diabetes group (1.33). Glomerular area and diameter and proximal tubule diameter in diabetes-Extract group was higher than control and diabetes groups. This study revealed that *Urtica dioica* has no effect on renal morphometric indices in induced diabetic rats.

**Key words:** *Urtica dioica*, diabetic rats, streptozotocin, stereology

### INTRODUCTION

Various organs in the body such as kidney is affected with metabolic disturbance diabetes. The most important factor in the development of diabetic complications in both type 1 and type 2 diabetes is poorly controlled blood glucose (American Association of Diabetes Educators, 2002).

Many of the same factors are involved in the development of diabetic problems and the development of other common diabetic complications such as kidney diseases (American Association of Diabetes Educators, 2002).

Studies indicated that one-third of diabetic patients take alternative medications, of which *Urtica dioica* is the most commonly used (Ryan *et al.*, 2001).

*Urtica dioica* (Urticaceae) is used to treat rheumatic pain and for colds and cough, also is known as antiasthmatic, diuretic, haemostatic, hypertensive, hypoglycemic and antidandruff (Sezik *et al.*, 1997; Tahri *et al.*, 2000; Testai *et al.*, 2002), But there are deprived documents about effect of *Urtica dioica* on

Morphometric indices of kidney in streptozotocin-induced diabetic rats. Thus the aim of the present study was to investigate the treatment effect of *Urtica dioica* on Morphometric indices of kidney in streptozotocin-induced diabetic rats.

### MATERIALS AND METHODS

This study performed in faculty of medicine, Gorgan University of Medical Sciences, Iran during 2004-2005.

**Plant material:** *U. dioica* leaves were collected from cultivated plant, from suburb of Gorgan, northern Iran (Golestan, Iran) in OCT 2004 and taxonomically identified by Department of Pharmacognosy, Mazandaran University of Medical Sciences. A voucher specimen (5-77-1) was deposited in the herbarium of Mazandaran University.

**Preparation of hydroalcoholic extract of *Urtica dioica*:** Powder of *U. dioica* leaves was percolated by hydroalcoholic (60°) solvent for 48 h. The extract was

filtered and concentrated under vacuum at 40°C to made a jellies material. 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 another reagent assay (spectrophotometry) have been carried out to determine the concentration of polysaccharides in *U. dioica* leaves for standardization of the extract. The results of phytochemical analysis showed the existence of high percentage of Tannins, Steroids and low levels of Flavonids, Carotenoids and Saponins in leaves of *U. dioica*.

**Animals:** Male adult albino rats (wistar strain) of 125-175 g were fed on pellet diet and tap water for full acclimatization. The animals were kept in air-conditional animal room (22±2°C) under a 12 h light/dark cycle. The rats were divided into three groups of ten animals each.

**Experimental design:** Diabetes was induced with a single IP injection of Streptozotocin (STZ) (80 mg kg<sup>-1</sup>) to overnight fast rats. Streptozotocin (STZ) purchased from Sigma was dissolved in saline immediately use and intraperitoneally injected (80 mg kg<sup>-1</sup>).

Blood samples for glucose measurements were taken from the tail vein. Diabetes was confirmed by measuring the glucose concentration by using Glucometer method.

In the experiments, ten rats were used in each group.

**Group I:** Normal control group, received Saline daily for 4 weeks.

**Group II:** Diabetic group received Saline daily for 4 weeks after STZ injection.

**Group III:** Treatment Group, diabetic rats that administered 100 mg kg<sup>-1</sup> daily hydroalcoholic extract of *U. dioica* (Kavalali *et al.*, 2003), for 4 weeks.

**Glucose tolerance test:** Interaperitoneal Glucose Tolerance Test (GTT) was performed on 16 h fasted rats using 2 g glucose kg<sup>-1</sup> body weight. In all groups, blood was collected from the animals by tail snipping at 0, 30, 90 and 120 min after glucose load. Also glucose test were performed after IP injection STZ in 1, 3 and 5 weeks

**Fixation and preparation for light microscopy:** The kidneys were fixed by formaldehyde. The kidneys were cut horizontally into 1 mm slices. Approximately 10 slices obtained from each kidney. Slices were arranged in a number of sequences. Then they were processed and embedded in paraffin. Each level, three sections were cut to a thickness of 5 µm by a microtome and were stained

with H and E. Stereological study was performed using light microprojectors and the image was projected on a table of olysa software attached into microscope. Cavalieri principle was used to estimate the volume of cortex, medulla and whole kidney. The first section from each triple was chosen and then each kidney was wholly analyzed at a final magnification of X40 and volume was estimated from the following equation (Gundersen *et al.*, 1988; Schmitz *et al.*, 1990; Hinchliffe *et al.*, 1991; Heidari *et al.*, 2002).

$$V = \frac{\sum_{i=1}^m P.a/p.\bar{t}}{M^2}$$

For measurement of proximal tubules, distal tubules, Glomerulus three slices (slices 2, 5, 8) selected. This slices positioned in 1/3 first, middle and end of each kidney And Outer diameter of proximal tubules, distal tubules, area and diameter of Glomerulus examined.

**Statistical analysis:** All the grouped data were statistically evaluated using SPSS v.11.5 Students t-test, expressed as the Mean±SE from ten rats in each groups.

## RESULTS

One week after injection of STZ, the mean±SE of blood glucose concentration were in diabetic and treatment groups higher than control group. In the end of study the mean body weight for all animals of treatment groups were Lower than controls (230.0±9 g) (p>0.05) (Table 1).

Ratio of kidney/body weight in diabetes (0.51) and diabetes-extract group (0.67) were higher then control group (0.42).

Volume ratio of cortex/medulla in diabetes-extract group (1.65) was higher then control (1.34) and diabetes group (1.33) (Table 2).

Proximal and distal tubule diameter in diabetes-extract groups was higher than control and diabetes groups (Table 3).

Table 1: Body and kidney weight and ratio of kidney/body weight in control, diabetic and treatment (100 mg/kg/daily intraperitoneally) groups

Groups	First body weight (g)	End body weight (g)	Kidney weight (g)	Kidney/body weight (%)
Control	200.2±13.4	230.0±9.0	1.00±0.12	0.42
Diabetes	130.9±10.2	144.7±6.8	0.74±0.09	0.51
Diabetes extract treatment	121.3±6.50	114.1±6.7	0.77±0.11	0.67

Table 2: Volume of kidney and volume ratio of cortex/medulla in control, diabetic and treatment (100 mg/kg/daily intraperitoneally) groups

Groups	Kidney volume (cm <sup>3</sup> )	Kidney volume/ kidney weight	Cortex volume (cm <sup>3</sup> )	Medulla volume (cm <sup>3</sup> )	Cortex/ medulla ratio
Control	323.58±66.50	323	166.08±20.29	123.9200±39.320	1.34
Diabetes	259.04±54.96	350	139.61±34.76	106.2600±46.060	1.33
Diabetes extract treatment	267.09±28.26	348	147.11±1.200	89.9499±13.013	1.65

Table 3: Glomerular area and diameter, proximal and distal tubules diameter in control, diabetic and treatment (100 mg/kg/daily intraperitoneally) groups

Groups	Glomerular area (cm <sup>2</sup> )	Glomerular diameter (µm)	Distal tubule (µm)	Proximal tubule (µm)
Control	1112.78±394.42	139.31±22.43	14.37±3.01	11.36±2.28
Diabetes	1123.85±248.29	140.47±16.70	15.26±3.01	11.27±2.39
Diabetes extract treatment	1277.59±342.57	154.08±20.86	16.47±3.35	11.38±2.47

## DISCUSSION

This study revealed that administration of *Urtica dioica* following diabetes induction with 100 mg kg<sup>-1</sup> b.w. streptozotocin has not any treatment effects on Morphometric parameters of kidney in diabetic rats, such as kidney weight, kidney/body weight ratio, volume ratio of cortex/medulla volume, parameters of glomerular area, glomerular diameter and diameter of proximal and distal tubule.

The kidney has become the focus of investigation in studies of diabetic complications because many of the same factors are involved in the development of diabetic problems and the development of other common diabetic complications, such as microvascular disease and retinopathy (American Association of Diabetes Educators, 2002).

In present study ratio of kidney/body weight in diabetic and treatment group were higher than control group that revealed size of kidney increased and *Urtica dioica* has not treatment effects.

In diabetic patients, the size of the kidney is increased during treatment and in most studies it remains 20-30% larger. (Mogensen and Andersen 1973; Murray *et al.*, 1993; Tuttle *et al.*, 1991). Complete normalization of kidney size is not achieved in patients even after intensive treatment (Rasch, 1986; Wald *et al.*, 1986; Maunsbach *et al.*, 1962). In experimental diabetes, preserving kidney weight by treatment from the beginning of the disease is possible (Rasch *et al.*, 1979a), but if treatment is started later the kidney weight cannot be completely normalized (Gotzsche *et al.*, 1981; Stackhouse *et al.*, 1990).

Volume ratio of cortex/medulla in diabetes-extract group was higher then control and diabetes group. This ration can be explained with increasing of volume in cortex and subsequently volume of kidney. Also glomerulus and proximal -distal tubules are responsible for increasing or decreasing of kidney, cortex and medulla volume.

The present study has shown a smaller relative volume growth of glomeruli as compared with the control

groups. The glomerulus is a major site of functional impairment in diabetic kidney problems and end stage renal disease is associated with an increase in mesangium and in basement membrane material and subsequent closure of the glomerulus (Lundbaek, 1965; Mogensen *et al.*, 1983; Olsen, 1969; Rasch, 1979b).

The development of new capillaries involves formation of new endothelial cells and basement membrane, whereas the podocytes probably do not participate as they rarely divide (Rasch and Norgaard, 1983), consequently the podocytes with their complicated foot processes must reorganize to cover the new capillary surfaces. One mechanism to accomplish this, known both from human and experimental studies, is broadening of the epithelial foot processes (Gunderson, 1980b) but other mechanisms are also possible. The two studies suggest a slow growth of glomeruli mainly by hyperplasia. As stated previously, results of present study showed a smaller relative volume growth of glomeruli as compared with the control groups. The glomerular volume in diabetes decreases after insulin treatment. However in present study the glomerular area and diameter did not decrease in treatment groups that revealed that *Urtica dioica* has not treatment effects such as insulin.

Mechanism of glomerular volume decreasing after insulin treatment is not clear, so that it is not known whether the number of capillaries decreases, but if this were the case, the excess basement membrane might accumulate in the glomerulus and in the mesangium. It can be speculated whether many periods of growth and regression may lead to repetitive accumulation of basement membrane.

Studies clearly showed that the Proximal Tubules (PT) have the potential to enlarge mainly by hyperplasia in diabetes. The reason for the growth of the PT is not fully understood, but several factors could be working in concert. Growth factors, including insulin like growth factor, which possibly stimulates growth in the PT, are increased in diabetes (Flyvbjerg *et al.*, 1990a, b).

The increased glomerular filtration rate imposes an extra workload on the tubules for reabsorption of filtered

water and solutes and the increased amount of Na, K-ATPase in the kidney in the STZ-diabetic kidney confirms an increase in sodium absorption (Rasch, 1986; Wald *et al.*, 1986).

Also glomerulus and proximal-distal tubules are responsible for increasing or decreasing of kidney, cortex and medulla volume.

In present study these parameters increased subsequently ratio volume of cortex/medulla affected.

In conclusion *Urtica dioica* has not cure effect on renal morphometric indices following streptozotocin induced diabetes.

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