Pakistan Journal of Biological Sciences 10 (8): 1200-1204, 2007
ISSN 1028-8880

The Protective Activity of *Urtica dioica* Leaves on Blood Glucose Concentration and β-cells in Streptozotocin-Diabetic Rats

[1]Mohammad Jafar Golalipour and [2]Vahid Khori
[1]Department of Embryology and Histology,
[2]Department of Pharmacology, Gorgan University of Medical Sciences, Gorgan, Iran

Abstract: This study was done to determine the protective activity of the hydroalcholic extract of *Urtica dioica* leaves on Hyperglycemia and β-cells in hyperglycemic rats. Thirty Wistar rats were allocated in groups of normal, Diabetic and treatment. Hyperglycemia in Rats induced by 80 mg kg^{-1} streptozotocin. In treatment group, animals received hydroalcholic extract of *Urtica dioica* 100 mg kg^{-1} day^{-1} for five days, intraperitoneally and then hyperglycemia induced by streptozotocin. The blood glucose concentration was measured by using a Glucometer in 1st, 3rd and 5th weeks. In the end of 5th weeks the animals in each group were sacrificed by anesthesia and whole pancreas in three groups extracted and fixed in bouin's fluid and stained by chromealum hematoxiline-phloxine and beta cells were counted in three groups by Olympus microscope. Mean±SE of blood glucose concentrations in the end of fifth weeks were 99.4±5.0, 454.7±34.5 and 303.6±100.6 in control, diabetic and treatment groups, respectively ($p<0.05$). The percentages of β-cells in control, diabetic and treatment groups were 73.6, 1.9 and 22.9%, respectively. The percentage of β-cells in treatment group comparing with diabetic group was significant ($p<0.05$). This study showed that the protective administration of hydroalcholic extract of *Urtica dioica* has hypoglycemic effect and protective activity of β-cells of langerhans in hyperglycemic rats.

Key words: Hyperglycemia, *Urtica dioica*, β-cells, streptozotocin

INTRODUCTION

Diabetes is a complex and multifarious group of disorders characterized by hyperglycemia reached epidemic proportions in the present century (Chen *et al*., 2005).

More than 1% of the entire world populations are victims of diabetes and their numbers are gradually increasing (Kar *et al*., 2003).

In Iran high occurrence of the disease is noted, especially in urban populations and is therefore a major health problem. Plants used in folk medicine to treat diabetes mellitus represent a viable alternative for the control of this disease (Maroo *et al*., 2002).

Urtica dioica (*U. dioica*), an annual and perennial herb of family *Urticacea* is commonly known as medical herb for a long time in the world.

This herb is known for its anti-inflammatory activity (Obertreis *et al*., 1996; Riehemann *et al*., 1999). Furthermore, there have been also other reports indicating the benefits of using the extract of the leaves or other parts of the plant for the use in different conditions, i.e., diabetes (Kavalali *et al*., 2003; Roman Ramos *et al*., 1992; Petlevski *et al*., 2001; Farzami *et al*., 2003) as well as other disorders like prostatic hyperplasia (Hirono *et al*., 1994; Lichius and Muth, 1997; Kayser *et al*., 1995) rheumatoid arthritis, hypertension and allergic rhinitis (Miltman, 1990) and stimulation of proliferation of human lymphocytes (Wagner *et al*., 1989) and decreasing the lipid peroxidation and liver enzymes (Kanter *et al*., 2003).

Althought, there are some reports about the hypoglycemic activity of *U. dioica* in folk medicine (Petlevski *et al*., 2001; Farzami *et al*., 2003) but, in other hand, several investigations have detected hyperglycemic activity of this herb (Neef *et al*., 1995; Swanston-Flatt *et al*., 1989). Besides in our previous study (Golalipour *et al*., 2006) chronic administration of the extract of *U. dioica* leaves showed no hypoglycemic effect of it in STZ diabetic rats. With regarding the lack of report about the protective effect of administration of this herb (before making diabetes) this research carried out to evaluate protective effect of the hydroalcholoic extract of *U. dioica* leaves on blood glucose concentration and potential protective roles in β-cells of pancreas in diabetic rats.

Corresponding Author: Mohammad Jafar Golalipour, Department of Histology and Embryology, Gorgan University of Medical Sciences, P.O. Box 49175-553, Gorgan, Iran
Tel/Fax: +98(171)4425165, 4425660

MATERIALS AND METHODS

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 plant extract: Powder of *U. dioica* leaves was perculated 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 (each including 10 rats).

Experimental design: Hyperglycemia was induced with a single intrperiteonally (IP) injection of Streptozotocin (STZ) (80 mg kg^{-1}) to overnight fast rats. Streptozotocin (STZ) purchased from Sigma was dissolved in saline immediately use and intrperiteonally injected (80 mg kg^{-1}). 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. Saline daily for 5 days.
Group II: Diabetic group. Saline daily for 5 days before STZ injection.
Group III: Treatment Group: animals received 100 mg kg^{-1} daily hydroalcholic extract of *U. dioica*, for 5 days, intraperitoneally and then, hyperglycemia induced by intrperiteonally injection of 80 mg STZ.

Glucose tolerance test: Intraperitoneal Glucose Tolerance Test (GTT) was performed on 16 h fasted rats using 2 g glucose/kg 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.

Histopathologic examinations: The animals of three groups are sacrificed by ether anesthesia. Whole pancreas was dissected. The tissue samples were fixed in bouin's fluid and paraffin embedded for microscopic examination in accordance with routine laboratory procedures. Histopathologic examination and grading were carried out on Chromealum hematoxiline-phloxine (Gomori, 1941) stained. The number of islets and the number of β-cells of each islet were counted by OLYMPUS microscope and we used OLYSIA software. The islet diameters were measured using calibrated micrometer by taking of fixed number of islet from the two experimental groups and one normal group.

Statistical analysis: All the grouped data were statistically evaluated using Student's t-test, expressed as the Mean±SE from ten rats in each groups and λ^2 test.

RESULTS

The mean±SE of blood glucose concentrations before of STZ injection and before administration of *U. dioica* (in treatment group) were 85.5±1.7, 102.9±3.7 and 95.6±3.7 mg dL^{-1} in control, diabetic and treatment groups, respectively. One week after injection of STZ, the mean±SE of blood glucose concentration were 214.1±23.8 and 277±45.7 mg dL^{-1} in diabetic and treatment groups, respectively (Table 1).

The effect of STZ in diabetic group showed a gradually increasing of blood glucose level which it was (214.1±23.8) in 1 week and it reached to (454.7±34.5) mg dL^{-1} in fifth week after injection of STZ.

The mean±SE of Blood Glucose concentration level in treatment group decreased from 309.1±37.4 to 303±100 mg dL^{-1} in the fifth week.

In control group the mean±SE of Blood Glucose concentration had not any changes. Statistically analysis showed that there was a significant difference between treatment and control groups ($p<0.05$). Also this difference between diabetic and treatment group was significant ($p<0.05$).

Table 1: Blood glucose concentration in control, diabetic and treatment groups

		Blood glucose (mg dL^{-1})			
1	2	Initial	1 week	3 week	5 week
I	Control	85.5±1.7	87.5±1.9	90.2±2.7	99.4±5.0
II	Diabetic	102.9±3.7	214.1±23.8	243.3±34.8	454.7±34.5*
III	Urtica-Diabetic	95.6±3.7	277.1±45.7	309.1±37.4	303.6±100.6*

*The significant difference between group II and group III relative to control in regard to glucose concentration, 1 = Groups, 2 = Treatments

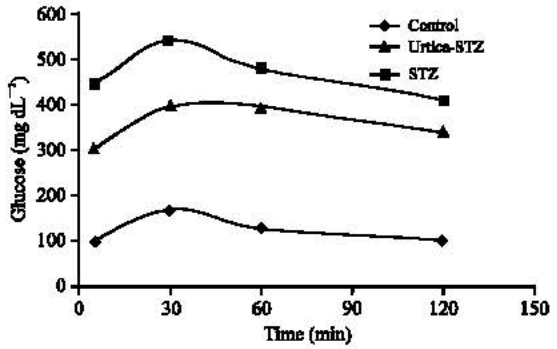

Fig. 1: The result of Glucose Tolerance Test (GTT) that the *Urtica dioica* can cause shift down of GTT carve related to STZ group.[Control: Group (I), STZ: Group (II) and Urtica-STZ: Group (III)]

Table 2: The number of islets, total cells, the number and percents of β cells and islets area (μ) in pancreatic tissues in control, diabetic and treatment groups

1	2	Islets (n)	Total cells (n)	β-cells (n)	β-cells %	Islets area (μ)
I	Control	14.7±2.2	277.3±17.7	204±15	73.6	588.8±28.7
II	Diabetic	5.8±1.1*▲	140.9±18.6*▲	2.7±0.7*▲	1.9	352.7±36*▲
III	Urtica-Diabetic	13.0±2.6*▲	128.3±18.6*▲	29.4±11*▲	22.9	399.7±37.4*▲

*In regard to β-cells, differences were shown between group II and III. ▲(Mean±SE, $p<0.001$), 1 = Groups, 2 = Treatments

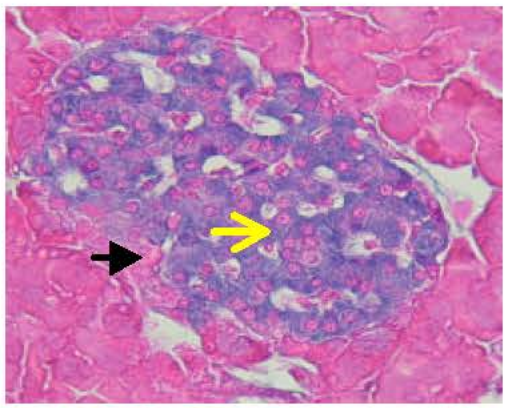

Fig. 2: Pancreatic section from normal rat (group I). The beta cells are shown blue and alpha cells are red. Staining Gomori's method, original magnification X400 [beta cells (➔) alpha cells (➔)]

The same finding was appeared glucose tolerance test such as; we had shift-down (recovery) of GTT curve after 4 weeks injection of STZ (Fig. 1).

The histopathologic results depicted in Table 2 and Fig. 2-4.

In this study the number of islets and number of β-cells in pancreas tissue were 14.7±2.2 and 204±15 in control group, respectively. These were 5.8±1.1 and 2.7±0.7 in diabetic groups and 13±2.6 and 29.4±11 in treatment group, respectively.

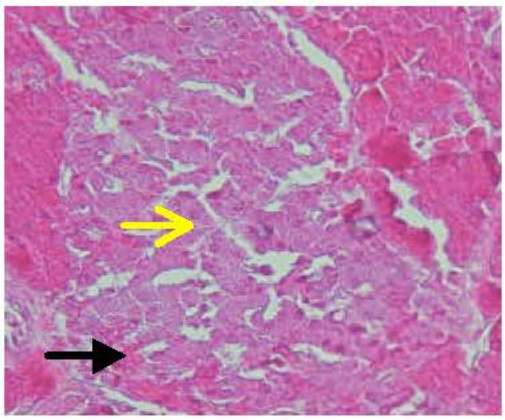

Fig. 3: Rat pancreatic tissue prepared from the Diabetic group (group II). The islet is atrophied and beta cells are decreased. Staining Gomori's method, Original magnification X400 [(➔) beta cell, (➔) alpha cell]

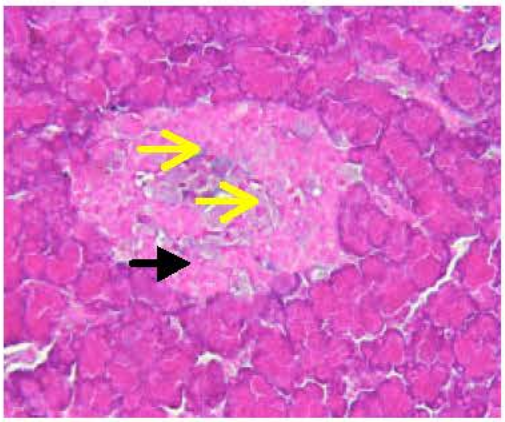

Fig. 4: Rat pancreatic tissue prepared from the protective group (group III).The beta cells are shown blue and alpha cells are red. Staining Gomori's method, original magnification X 400 [beta cells (➔) alpha cells (➔)]

The percentage of β-cell in control, diabetic and treatment groups were 73.6, 1.9 and 22.9%, respectively. The percentage of β-cells in treatment group with comparing to diabetic group was significant ($p<0.05$) (Table 2).

DISCUSSION

This study showed that the administration of *U. dioica* leaves before induction of hyperglycemia in rats can prevent of severe increasing blood glucose concentration and disruption of β-cells in comparing with no treatment hyperglycemic rats.

The screenings of medical herbs in two studies have not shown the hypoglycemia activity of aqueous extract of *U. dioica* (Neef *et al*., 1995; Roman Ramos *et al*., 1992).

Also hypoglycemic activity of *U. dioica* in normal rats has been shown by other researches (Bnouham *et al*., 2003; Petleveski *et al*., 2001). Bnouham concluded that anti-hyperglycemic activity of this herb is due to the decrease of transport from small intestine (Bnouham *et al*., 2003).

Acute and chronic hypoglycemic activity of *Utrica* was demonstrated by Farzami in Iran (Farzami *et al*., 2003) and Kavalali in Turkey (Kavalali *et al*., 2003). Farzami showed that a fraction from *U. dioica* was a potent stimulator of insulin release of β-cells. Kavalali tested the effect of lectin fraction of extract of *U. pilulifera* seed for their hypoglycemic activity and found hypoglycemic activity and β-cell regenerative potency with seed of this herb.

In our previous study the leaves extract of *U. dioica* had no hypoglycemic activity and ability to regenerate pancreatic β-cells after four weeks of treatment in streptozotosin- diabetic rats (Golalipour *et al*., 2006).

In our previous study, the animals probably the β cells damage is so extensive that no increase in insulin secretion is possible. However, in the present study we show a partial amelioration of β-cell in diabetic rats. If STZ-induced diabetes is reversible, then it could be suggested that the active content of the leaves extract might play a protective role in preventing free-radicals actions, that may destroy β-cell, those would then cause more secreatory ability of β-cells this, decrease plasma glucose concentration.

U. dioica probably prevented the disruption of β-cells of islets in the pancreas. In recent study, our results indicated that decreased blood glucose concentration by *U. dioica* pretreatment might be due to protective roles or partial regeneration in the β-cells. However, other extra pancreatic mechanisms such as enhanced glucose transport into the cells, inhibition of the endogenous glucose production can not role out by the results of present study.

STZ produce hyperglycemia in a concentration dependent model by selective β-cell cytotoxic effect (Saini *et al*., 1999). The mechanism of action of STZ on rodent β-cell is related to uptake of STZ into β-cells and DNA strand breaks which then causes a lethal depletion of NAD, in the β-cells (Swanston-Flatt *et al*., 1989; Saini *et al*., 1996).

It has been shown that inhibition of free radical scavenger enzymes and enhancing production of the superoxide radical are the mechanism of STZ on pancreatic β-cells (Swanston-Flatt *et al*., 1989).

The role of free radicals in disruption of insulin action and impairing glucose tolerance test was shown by Ford (Ford *et al*., 1996).

Our findings suggest that *U. dioica* may have antioxidant or free radical scavenger properties. Thus, *U. dioica* may have a role in preventing of diabetes or slowing the progress of diabetic disease.

Antioxidant and free radical scavenging properties of *U. dioica* leaves has been established by several studies (Kanter *et al*., 2005; Cetinus *et al*., 2005; Mavi *et al*., 2004). Preliminary phytochemical of the ethanolic extract of *U. dioica* was achieved according to the Trease and Evans method (Trease and Evan, 1983). Previous studies showed that antihyperglacemic activity of the *U. dioica* related to active component of *U. dioica* leaves such as flavonid, peptids and coxmarins (Ellnain *et al*., 1986; Adamski and Biegonska 1984; Rossiikaya *et al*., 1985). It has been suggested that the active natural compounds might act by antioxidant and free radical scavengering effect to cause the hypoglycemic activity.

CONCLUSIONS

This study showed that the extracts of *Urtica dioica* leaves can prevent from severe increasing of blood glucose concentration and also it protect of β cells, if it is used before induction of hyperglycemia. Therefore, the usage of this plant can prevent from severity of diabetes in people with background of diabetes.

ACKNOWLEDGMENT

We thanks from research department of Gorgan University of medical sciences, Mr. Behnampour and Dr. Soleimani, Miss Ghafari and Mr. Gharavi.

REFERENCES

Adamski, R. and J. Bieganska, 1984. Investigation on substances present in *Urtica dioica* L. leaves. Part 2. Analysis of proteins, amino acids and nitrogen non-protein substances. Herba Pol., 30: 7-26.

Bnouham, M., F.Z. Merhfour, A. Ziyat, H. Mekhfi, M. Aziz and A. Legssyer, 2003. Antihyperglycemic activity of the aqueous extract of *Urtica dioica*. Fitoterpia, 74: 677-681.

Cetinus, E., M. Kilinc, F. Inanc, E.B. Kurutas and N. Buzkan, 2005. The role of *Urtica dioica* (Urticaceae) in the prevention of oxidative stress caused by tourniquet application in rats. Tohoku. J. Exp., 205: 215-221.

Chen, H., S. Brahmbhatt, A. Gupta and A.C. Sharma, 2005. Duration of streptozotocin induced diabetes differentially affects p38-mitogen-activated protein kinase (MAPK) phosphorylation in renal and vascular dysfunction. Cardiov. Diabetol., 4: 3.

Ellnain, W.M., W. Bylka and Z. Kowalewski, 1986. Flavonil compounds in *Urtica dioica*. Herba. Pol., 32: 131-137.

Farzami, B., D. Ahmadvand, S. Vardasbi, F.J. Majin and S.H. Khaghani, 2003. Induction of insulin secretion by a component of *Urtica dioica* leave extract in perfuse islets of langerhans and its *in vivo* effects in normal and streptozotocin diabetic rats. J. Ethnopharmacol., 89: 47-53.

Ford, E.S., J.C. Will, B.A. Bowman and K.M. Narayan, 1999. Diabetes mellitus and serum carotenoids: Findings from the third national and nutrition examination survey. Am. J. Epidemiol., 149: 168-176.

Golalipour, M.J., V. Khori, S. Ghafari and A.M. Gharravi, 2006. Chronic effect of the hydroalcholic extract of *Urtica dioica* leaves on regeneration of β-cells of hyperglycemic rats. Pak. J. Biol. Sci., 9: 1482-1485.

Gomori, G., 1941. Observations with differential stains on human islets of langerhans. Am. J. Pathol., 17: 395-406.

Hirono, T., M. Homma and K. Oka, 1994. Effects of stringing Nettle root extract and their steroidal components on the Na, K ATPase of the bening prostatic hyperplasia. Plantaedica, 60: 30-33.

Kanter, M., I. Meral, S. Dede, M. Cemek, H. Ozbek, L. Uygan and H. Gunduz, 2003. Effects of *N. sativa* L. and *Urtica dioica* L. on lipid peroxidation, antioxidant enzyme systems and some liver enzymes in Cc l4-treated rats. J. Vet. Med. A., 50: 264-268.

Kanter, M., O. Coskun and M. Budancamanak, 2005. Hepatoprotective effects of *Nigella sativa* L. and *Urtica dioica* L. on lipid peroxidation, antioxidant enzyme systems and liver enzymes in carbon tetrachloride-treated rats. World J. Gastroenterol., 11: 6684-6688.

Kar, A., B.K. Choudhary and N.G. Bandyopadhyay, 2003. Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. J. Ethnopharmacol., 84: 105-108.

Kavalali, G., H. Tuncel, S. Goksel and H.H. Hatemi, 2003. Hypoglycemic activity of *Urtica pilulifera* in streptozotocin-diabetic rats. Ethnopharmacology, 84: 241-245.

Kayser, K., J. Bubenzer, G. Kayser, S. Eichhorn, T.V. Zemlyanukhina, N.V. Bovin, S. Andre, J. Koopmann and H.J. Gabius, 1995. Expression of lectin, interleukin-2 and histopathologic blood group binding sites in prostate cancer and its correlation with integrated optical density and syntactic structure analysis. Anal. Quant. Cytol. Histol., 17: 135-142.

Lichius, J.J. and C. Muth, 1997. The inhibiting effect of Urtica dioica root extracts on experimentally induced prostatic hyperplasia in the mouse. Planta Med., 63: 307-310.

Maroo, J., V.T. Vasu, R. Aalinkeel and S. Gupta, 2002. Glucose lowering effect of aqueous of *Enicostemma littorale* Blume in diabetes: A possible mechanism of action. J. Ethnopharmacol., 81: 317-320.

Mavi, A., Z. Terzi, U. Ozgen, A. Yildirim and M. Coskun, 2004. Antioxidant properties of some medicinal plants: *Prangos ferulacea* (Apiaceae), *Sedum sempervivoides* (Crassulaceae), *Malva neglecta* (Malvaceae), *Cruciata taurica* (Rubiaceae), *Rosa pimpinellifolia* (Rosaceae), *Galium verum* subsp. verum (Rubiaceae), *Urtica dioica* (Urticaceae). Biol. Pharm. Bull., 27: 702-705.

Miltman, P., 1990. Randomized double-blind study of *Urtica dioica* freeze drier the treatment of allergic rhinitis. Planta Medica, 16: 35-39.

Neef, H., P. Declereq and G. Laekeman, 1995. Hypoglycaemic activity of selected European plants. Phytother. Res., 9: 45-48.

Obertreis, B., K. Giller, T. Teucher, B. Behnke and H. Schmitz, 1996. Anti-inflammatory effect of Urtica dioica folia extract in comparison to caffeic malic acid. Arzneimittelforschung, 46: 52-56.

Petlevski, R., M. Hadzija, M. Slijepcevic and D. Juretic, 2001. Effect of antidiabetis herbal preparation on serum glucose and fructosamine in NOD mice. J. Ethnopharmacol., 75: 181-184.

Riehemann, K., B. Behnke and K. Schulze-Osthoff, 1999. Plant extracts from stinging nettle (*Urtica dioica*), an antirheumatic remedy, inhibit the proinflammatory transcription factor NF-kappaB. FEBS. Letter, 442: 89-94.

Roman Ramos, R., F. Alarcon Aguilar, A.L. Lemus and J.L. Flores Saenz, 1992. Hypoglycemic effect of plans used in Mexico as anti-diabetics. Arch. Med. Res., 23: 59-64.

Rossiikaya, G.I., T.D. Dargaeva, L.I. Brutko and S.M. Nikoleav, 1985. Study of the extraction process and determination of the same of biologically active compounds in the total bile expelling agent. Farmasiya Moscow, 34: 38-41.

Saini, K.S., C. Thompson, C.M. Winterford, N.I. Walker and D.P. Cameron, 1996. Streptozotocin at low doses induces apoptosis and at high doses causes necrosis in a murine pancreatic beta cell line, INS-1. Biochem. Mol. Biol. Intl., 39: 1229-1236.

Swanston-Flatt, S.K., C. Day, P.R. Flatt, B.J. Gould and C.Y. Bailey, 1989. Glycemic effects of traditional European plant treatment for diabetes, Studies in normal and streptozotocin diabetic mice. Diabetes Res., 10: 69-75.

Trease, G.E. and W.C. Evans, 1983. Pharmacognosy. Bailliiere Tindall Press, London, pp: 309-706.

Wagner, H., F. Willer and B. Kreher, 1989. Biologically active compounds from the aqueous extract of *Urtica dioica*. Planta Med., 55: 452-454.