Pakistan Journal of Biological Sciences 9 (8): 1482-1485, 2006
ISSN 1028-8880

Chronic Effect of the Hydroalcholic Extract of *Urtica dioica* Leaves on Regeneration of β-cells of Hyperglycemic Rats

[1]Mohammad Jafar Golalipour, [2]Vahid Khori, [3]Soraya Ghafari and [3]Anneh Mohammad Gharravi
[1]Department of Embryology and Histology, [2]Department of Pharmacology,
[3]Department of Embryology and Histology, Gorgan University of Medical Sciences, Gorgan, Iran

Abstract: *Urtica dioica* has been introduced with hypoglycemic activity in Iranian traditional medicine. There are some reports with different results about the hypoglycemic activity of *Urtica dioica*. This study was done to determine the chronic effect of the hydroalcholic extract of *Urtica dioica* leaves on Hyperglycemia and regeneration of β-cells in hyperglycemic rats. 30 Wistar rats were allocated in groups of normal, Diabetic and treatment. Hyperglycemia in Rats induced by 80 mg kg^{-1} streptozotocin. One week after injection of STZ Animals in treatment group received hydroalcholic extract of *Urtica dioica* 100 mg kg^{-1} day^{-1} for 4 weeks, intraperitoneally. The blood glucose 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 ether anesthesia and whole pancreas in three groups extracted and fixed in bouin and stained by chrome alum hematoxiline-phloxine stain and beta cells percentage were counted in three groups by Olympus microscope. Mean±SE of blood glucose concentrations in end of fifth weeks were 99.4±5.0, 454.7±34.5 and 447.4±42.5 in control, diabetic and treatment groups, respectively ($p<0.05$).The percentage of β-cells (beta cells) in control, diabetic and treatment groups was 73.6, 1.9 and 1.3, respectively. The percentage of β-cells in diabetic and treatment groups comparing with control group was significant ($p<0.05$). This study showed that chronic administration of hydroalcholic extract of *Urtica dioica* has not hypoglycemic effect and regeneration of β-cells of langerhans in hyperglycemic rats.

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

INTRODUCTION

Plants used in folk medicine to treat diabetes mellitus represent a viable alternative for the control of this disease (Maroo *et al*., 2003).

Urtica L. Stinging nettle (*Urticacea*) is annual and perennial herb, distinguished with stinging hairs. Leaves are opposite. Flowers are green with yellow stamens, the male and female flowers on separate plants. Fruits are achene. These are the characters of *Urtica* genus which belong to the family *Urticacea*. The main varieties identified under the *Urtica dioica* L, *U. urens* L, *U. pilulifera* L., *U. cannabina* L., *U. Memberanacea* Poiret, *U. kiovensis* Rogoff (Kavalali *et al*., 2003).

Among these *Urtica dioica* (*U. dioica*) has already been known for a long time as medicinal plants in the world.

The blood sugar lowering effect of *U. dioica* as a medicinal herb has been introduced in old script such as those written by Avicenna (Farzami *et al*., 2003).

There have been also other reports indicating the benefits of using the herb for the use in different conditions, i.e. diabetes (Roman Ramos *et al*., 1992), prostatic hyperplasia (Hirono *et al*., 1994; Kayser *et al*., 1995). Inflammation (Obertrries *et al*., 1996), rheumatoid arthritis, hypertension and allergic rhinitis (Miltman, 1990).

For instance *U. dioica* is amongst several species listed for their use against hypoglycemic and diabetes in folk medicine (Rahman and Zaman, 1989; Fazami *et al*., 2003) in a large pharmacological screen of European species with knows potential anti-diabetic effects. Also, two earlier studies have detected hyperglycemic activity of *U. dioica*.

With the existence of controversy of reports about the glycemic activity of *U. dioica*, this study was done to evaluate chronic hypoglycemic effect of the hydroalcholic extract of *U. dioica* leaves and potential roles in regeneration of β-cells of pancreas.

Corresponding Author: Mohammad Jafar Golalipour, Department of Histology and Embryology, Gorgan University of Medical Sciences, Gorgan, Iran P.O. Box 49175-553
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 October 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 of ten animals each.

Experimental design: Diabetes was induced with a single 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 4 weeks

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

Group III: Treatment group. diabetic rats administered 100 mg kg^{-1} daily hydroalcholic extract of *U. dioica*, for 4 weeks.

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 bouin's fluid fixed and paraffin embedded for microscopic examination in accordance with routine laboratory procedures. Histopathologic examination and grading were carried out on chrome alum hematoxiline-phloxine stain (Gomori, 1941) stained sections. 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 three 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 were 85.5±1.7, 102.9±3.7 and 92±4 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 388.2±17.4. mg dL^{-1} in diabetic and treatment groups, respectively.

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 increased from 388.2±17.4 to 447.4±42.5 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 ($p<0.05$). But this difference between diabetic and treatment group was not significant. Therefore, the treatment with 100 mg kg^{-1} day^{-1} for 4 weeks of hydroalcholic extract of Urtica dioica did not show any hypoglycemic activity.

The same finding was appeared in glucose tolerance test such as; we had no change in trend of GTT curve after 4 weeks injection of U.D (Fig. 1).

The histopathologic results depicted in 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

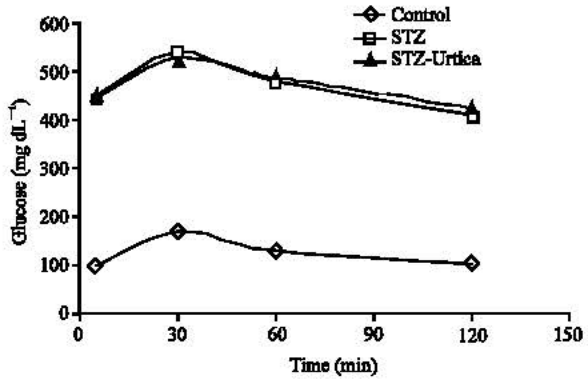

Fig. 1: Glucose tolerance test (GTT) in 5th week in control (I), STZ (II) and STZ-Urtica (III) groups that showed no effect of *U. dioica* on crude of GTT

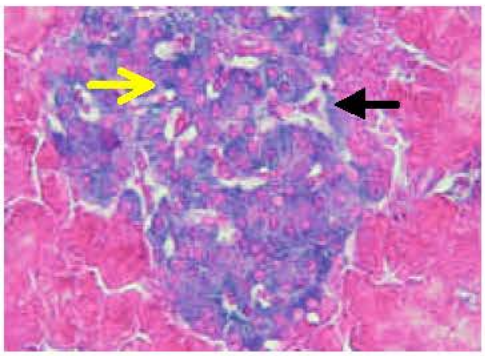

Fig. 2: Pancreatic section from normal rat (group I). The beta cells are shown blue and Alpha cells are red. Staining Gomori's method, original magnification X400 [Alph cells (⟶) beta cells (⟶)]

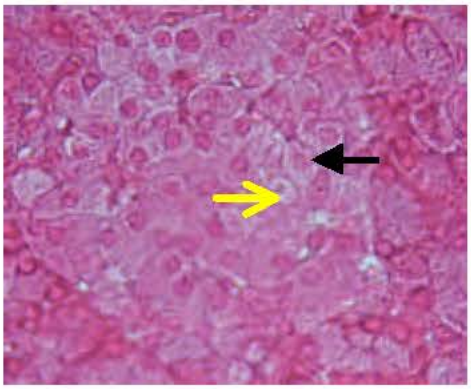

Fig. 3: Rat pancreatic tissue prepared from the Diabetic group (group II) The islet is atrophied and beta cells are decreased. Staining Gomori's method, Original magnification X400 [(⟶) beta cell, (⟶) alpha cell]

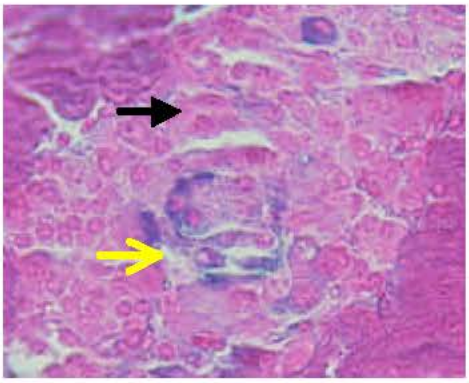

Fig. 4: Rat pancreatic tissue prepared from the treatment group (group III). The beta cells are shown blue and alpha cells are red. Staining Gomori's method, original magnification X400 alpha cells (⟶) beta cells (⟶)

control group, respectively. These were (5.8±1.1) and 2.7±0.7 in diabetic groups and (9±1.8) and 1.8±0.5 in treatment group, respectively.

The percentage of β-cell in control, diabetic and treatment groups were 73.6, 1.9 and 1.3%, respectively. The percentage of β-cells in diabetic and treatment groups comparing with control group was significant ($p<0.05$).

According to our finding although the IP injection of STZ caused a significant of decrease in the percent of β-cells but the administration of *U. dioica* could not change the number of beta cells.

DISCUSSION

The finding of this study indicated that the chronic administration of the extract of *U. dioica* leaves did not decrease blood glucose concentration and also have not any effect on number of beta cells in pancreatic tissue.

Various studies showed the controversial effects of Urtica species on inducing hyperglycemia (Bnouham *et al*., 2003; Swanston-Flatt *et al*., 1989; Roman Ramos *et al*., 1992).

In recent study extra pancreatic effect of *U. dioica* was observed (Onal *et al*., 2005).

Onal *et al*. (2005) established that antidiabetic effect of *U. dioica* can be related to be inhibition of alpha-glycosidase enzyme in rat intestine (Onal *et al*., 2005)

The results of present study show that the exact mechanism of effect of *U. dioica*. is not dependent on beta cells of pancreas. This finding is accordance to study of Onal *et al*. (2005), showed extra pancreatic effect of *U. dioica*. Earlier study about STZ showed that in high dose we have irreversible damage of beta cell of pancreas (Saini *et al*., 1996). Therefore we can conclude that

U. dioica couldn't regenerate necrotic beta cell of pancreas. The other species of this genus, *U. pilulifera* has been reported to has hyperglycemic effects (Kavalali *et al*., 2003). Kavalali showed that the chronic effect of seed of *U. pilulifera* can induce the hypoglycemia and prevent cellular damage of pancreas on diabetic rats (Kavalali *et al*., 2003).

The major differences between the kavalali study and our study are: 1. in present study we used leaves of *U. dioica* while in kavalali study seed of *U. dioica* was used. 2. Difference between two species may be responsible for different results.

Acute hyperglycemic activity of *U. dioica* was demonstrated in STZ diabetic rats by Farzami *et al.* (2003) and Fathi- Azad *et al.* (2005). Farzami demonstrated that an hydroalcholic extract of *U. dioica* was a potent stimulator of insulin release in short time (Farzami *et al*., 2003) on the basis of present observation, we can not rule out potential short time hyperglycemic activity of *U. dioica*, but this effect dose not observed in long time and dose not solely due to regeneration of beta cells of pancreas.

Taken together we have shown that chronic administrations of the leaves extract of *U. dioica* in Hyperglycemic rats have not hypoglycemic activity. Moreover, we have not any effect on regeneration of beta cells of pancreas

These results put under question on usefulness of *U.dioica* leaves in the treatment of diabetes mellitus in long time. Further pharmacological studies are necessary to confirm potential usefulness of this plant as a prophylactic agent in preventing development of diabetes.

ACKNOWLEDGMENTS

We thanks research department of Gorgan University of Medical Sciences, Mr. Behnampour and Dr. Soleimani for helping.

REFERENCES

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

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 perifused islets of langerhans and its *in vivo* effects in normal and streptozotocin diabetic rats. J. Ethnopharmacol., 89: 47-53.

Fathi-Azad, F., A. Garjani, N. Maleki and S. Ranjdost, 2005. Study of the hypoglycemic activity of the hydroalcoholic extract of *Urtica dioica* in normal and diabetic rat. Pharmac. Sci., 2: 65-69.

Gomori, G., 1941. Observations with difrencial 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.

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. B ubenzer, G. Kayser, S. Eichhorn, T.V. Zemlyanukhina and N.V. Bovin *et al*., 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.

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.

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

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, 6: 52-56.

Onal, S., S. Timur, B. Okutucu and F. Zihnigolu, 2005. Inhibition of alpha-glucosidase by aqueous extract of some potent antidiabetic medicinal herbs. Prep. Biochem. Biotechnol., 35: 29-36.

Rahman, A.U. and K. Zaman, 1989. Medicinal plants with hypoglycemic activity. J. Ethnopharmacol., 26: 1-55.

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

Saini, K.S., C. Thompson, C.M. Windterford, N.I. Wlaker and D.P. Cameron, 1996. Sterptozotocin at low doses induce apoptosis and at high doses cause necrosis in 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 Euroupian plant treatment for diabetes, Studies in normal and streptozotocin diabetic mice. Diabetes Res., 10: 69-75.