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ORIGINAL ARTICLE

Effect of Nettle oil (*Urtica dioica*) Consumption on levels of Blood Glucose and Lipids in Alloxan-induced Diabetic rats

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ABSTRACT
Diabetes is the most common endocrine disease which blood sugar and fat increases followed. Research has shown that some plant extracts have anti-diabetic and so people with diabetes to lower blood sugar, be used therefore, in this study it was decided to establish an experimental diabetic rats, the same situation occurs with type 1diabetes in rats. The aim of present study was to assay ethnopharmacological effects of Nettle (Urtica dioica) consumption on levels of blood glucose and lipids in alloxan-induced diabetic rats. 40 male Wistar rats, weighing 200±20 g and 9 to 10 weeks old, were obtained from the animal breeding center of Islamic Azad University. The rats were randomly divided into 4 equal groups of 10 animals including: 1- normal control, 2- normal rats treated with extract, 3- diabetic control, and 4- diabetics treated with extract. For induction of diabetes, after 15 h fasting, the rats were intraperitoneally injected with alloxan monohydrate at a dose of 60 mg/kg body weight (bw), freshly dissolved in distilled water (5%). Animals with fasting blood glucose of 120 to 250 mg/dl were considered diabetic. Results showed a significant difference among animals of groups 3 and 4 with control group during 3rd week. Results showed that blood glucose level on weeks 3 and 6 in groups 3 and 4 was higher than control group significantly. Increased cholesterol level in group 3 was observed on weeks 3 and 6 compared with prior the study. A significant increase in serum triglycerides was observed on weeks 3 and 6 in group 3 compared with prior the study. Measurement of HDL has revealed that this parameter in rats of group 3 decreased significantly in compared with prior the study. Results showed that LDL levels were increased in rats of group 3 in compared with control group.
Keywords: *Nettle (Urtica dioica), blood glucose, cholesterol, LDL, HDL, triglycerides, alloxan, rats.*

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INTRODUCTION

Diabetes mellitus is a metabolic disorder as old as mankind and its incidence (4 to 5%) is considered to be high all over the world [1]. This endocrine disorder results from abnormal metabolism of carbohydrates, fats and proteins and causes the increase in blood glucose values. Hepatic and renal failure is the main cause of death in diabetic patients [2].

Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas (that is beta cells) when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "Alloxan Diabetes") in these animals, with characteristics similar to type 1 diabetes in humans. Alloxan is selectively toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT2 glucose transporter. Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction. One study suggests that alloxan does not cause diabetes in humans [3]. Others found a significant difference in alloxan plasma levels in children with and without diabetes Type 1[4].

Stinging nettle or common nettle, *Urtica dioica*, is a herbaceous perennial flowering plant, native to Europe, Asia, northern Africa, and North America, and is the best-known member of the nettle genus *Urtica*. The plant has many hollow stinging hairs called trichomes on its leaves and stems, which act like hypodermic needles, injecting histamine and other chemicals that produce a stinging sensation when contacted by humans and other animals [5]. The plant has a long history of use as a medicine and as a food source. Stinging nettle is a dioecious herbaceous perennial, 1 to 2 m (3 to 7 ft) tall in the summer and dying down to the ground in winter. It has widely spreading rhizomes and stolons, which are bright

yellow as are the roots. The soft green leaves are 3 to 15 cm (1 to 6 in) long and are borne oppositely on an erect wiry green stem. The leaves have a strongly serrated margin, a cordate base and an acuminate tip with a terminal leaf tooth longer than adjacent laterals. It bears small greenish or brownish numerous flowers in dense axillary inflorescences. The leaves and stems are very hairy with non-stinging hairs and also bear many stinging hairs (trichomes), whose tips come off when touched, transforming the hair into a needle that will inject several chemicals: acetylcholine, histamine, 5-HT (serotonin), moroidin [6], leukotrienes [6], and possibly formic acid [6,7]. This mixture of chemical compounds cause a painful sting or paresthesia from which the species derives its common name, as well as the colloquial names burn nettle, burn weed, burn hazel. Nettle leaf is a herb that has a long tradition of use as an adjuvant remedy in the treatment of arthritis in Germany. Nettle leaf extract contains active compounds that reduce TNF-α and other inflammatory cytokines [8,9]. It has been demonstrated that nettle leaf lowers TNF-α levels by potently inhibiting the genetic transcription factor that activates TNF-α and IL-1B in the synovial tissue that lines the joint [10].

Nettle is used in shampoo to control dandruff and is said to make hair more glossy, which is why some farmers include a handful of nettles with cattle feed [11].

Nettle root extracts have been extensively studied in human clinical trials as a treatment for symptoms of benign prostatic hyperplasia (BPH). These extracts have been shown to help relieve symptoms compared to placebo both by themselves [12] and when combined with other herbal medicines [13].

Because it contains 3,4-divanillyltetrahydrofuran, certain extracts of the nettle are used by bodybuilders in an effort to increase free testosterone by occupying sex-hormone binding globulin [14].

As Old English *Stiðe*, nettle is one of the nine plants invoked in the pagan Anglo-Saxon *Nine Herbs Charm*, recorded in the 10th century. Nettle is believed to be a galactagogue, a substance that promotes lactation [15].

Urtication, or flogging with nettles, is the process of deliberately applying stinging nettles to the skin in order to provoke inflammation. An agent thus used is known as a rubefacient (something that causes redness). This is done as a folk remedy for rheumatism, providing temporary relief from pain. The counter-irritant action to which this is often attributed can be preserved by the preparation of an alcoholic tincture which can be applied as part of a topical preparation, but not as an infusion, which drastically reduces the irritant action. The aim of present study was to assay ethnopharmacological effects of Nettle (Urtica dioica) consumption on levels of blood glucose and lipids in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Experimental plan

This experimental study was carried out in Islamic Azad University Research Center. All procedures were conducted under supervision of Animal Rights Monitoring Committee of Islamic Azad University Research Center.

TREE preparation and maintenance

Nettle was collected from Azerbaijan Province in North of Iran, during April 2012. The plant was identified by Pharmacognosy Department of Islamic Azad University. Fresh roots were cut and their content extracted three times with ethanol. The extracted solutions were filtered and dried using a rotary evaporator under reduced pressure.

The ethanolic extract yields after vacuum evaporation was 10.6 g per 100 g of fresh root material. Dried extract was kept in the refrigerator at 4°C.

Chemicals

All chemicals used in this study were of analytical grade and obtained from Nanjing Jiancheng Bioengineering Institute, Nanjing, China and Ziest Chemi Co., Iran.

Animals

40 male Wistar rats, weighing 200±20 g and 9 to 10 weeks old, were obtained from the animal breeding center of Islamic Azad University. The rats were randomly divided into 4 equal groups of 10 animals including: 1- normal control, 2- normal rats treated with extract, 3- diabetic control, and 4- diabetics treated with extract. Management and husbandry conditions were identical in all groups with 12/12 h light/dark cycle at 21±2°C. Food and water were provided *ad libitum*. At the beginning of study, prior to induction of diabetes, blood glucose was measured in all experimental rats after 12 h of fasting.

For induction of diabetes, after 15 h fasting, the rats were intraperitoneally injected with alloxan monohydrate at a dose of 60 mg/kg body weight (bw), freshly dissolved in distilled water (5%). Animals with fasting blood glucose of 120 to 250 mg/dl were considered diabetic (Gupta et al., 2005). Blood glucose was estimated by commercially available glucose kit (Ziest Chemi Co., Iran) based on glucose oxidase method. Thereafter, TREE (200 mg/kg in 10 ml/kg normal saline) was gavaged for eight

consecutive weeks to groups 2 and 4 [16]. Simultaneously, groups 1 and 3 were gavaged with similar volume of normal saline solution.

At the end of the experiment, blood samples were collected from the retro-orbital plexus and the sera prepared through centrifuging at 2500 × g for 15 min at 30°C. After 12 h fasting, blood glucose and serum lipid profiles were measured using commercially available kits.

Statistical analysis

The statistical package for social sciences (SPSS Inc., Chicago, IL, USA), version 13.0, was used for statistical analysis. All data are presented as mean ± SEM. Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. $P<0.05$ was considered statistically significant.

RESULTS

Results showed a significant difference among animals of groups 3 and 4 with control group during 3rd week ($P<0.05$). This weight loss also was significant on week 6 ($P<0.01$). On the other hands, the difference between groups 3 and 4 was not significant however, the weight average in group 4 was higher than group 3. Also, this evidence was observed among groups 1 and 2.

Results showed that blood glucose level on weeks 3 and 6 in groups 3 and 4 was higher than control group significantly ($P<0.001$) and group 2 showed no significant difference from control group. Also, treatment with nettle in diabetic rats (group 4) made no significant blood glucose lowering effects compared with control group (table 1).

Increased cholesterol level in group 3 was observed on weeks 3 and 6 compared with prior the study ($P<0.05$, $P<0.01$). Also, total cholesterol level in group 4 on weeks 3 and 6 was significantly lower than group 3 ($P<0.05$). Also, treatment with extract yields to decrease in total cholesterol level in group 2 in compared with prior the study (figure 1).

A significant increase in serum triglycerides was observed on weeks 3 and 6 in group 3 compared with prior the study ($P<0.05$). On the other hands, the difference between groups 3 and 4 on weeks 3 and specially 6 was significant ($P<0.05$, $P<0.01$). Also, this change was not observed in group 2 (figure 2).

Measurement of HDL has revealed that this parameter in rats of group 3 decreased significantly in compared with prior the study ($P<0.01$) and treatment with extract resulted in significant increase in HDL in compared with group 3 ($P<0.05$, figure 3).

Results showed that LDL levels were increased in rats of group 3 in compared with control group ($P<0.01$) and treatment with extract decreased its levels ($P<0.01$, figure 4).

Table 1: effect of extract on blood glucose level (mg/dl) in animals

Time of the study / Groups	0	3	6
1 (control)	117.4±6.7	109.2±9.1	121.6±9.4
2 (control+extract)	101.5±8.2	128.5±7.3	92.5±8.1
3 (diabetics)	108.8±12.4	387.7±14.1	408.8±12.4
4 (diabetics+extract)	90.1±7.5	340.5±13.7	345.6±13.3

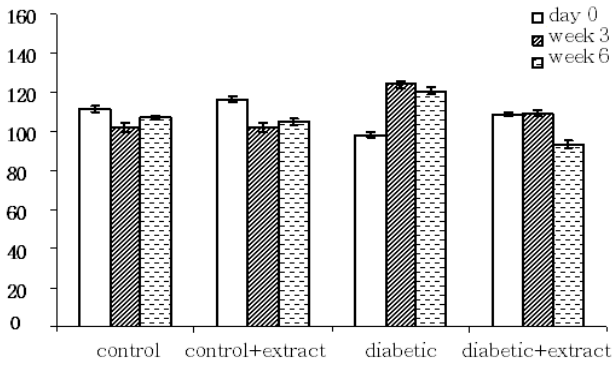

Figure 1: comparison of serum values of triglycerides in groups.

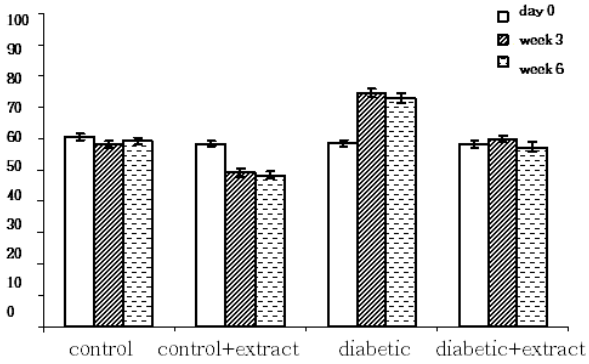

Figure 2: comparison of serum values of cholesterol in groups.

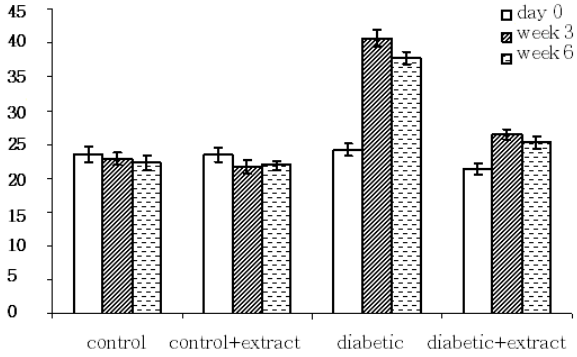

Figure 3: comparison of serum values of LDL in groups.

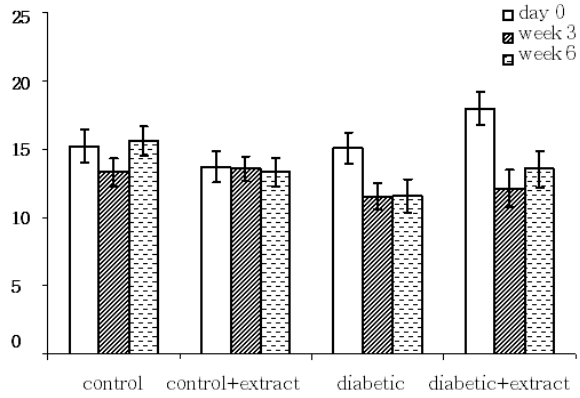

Figure 4: comparison of serum values of HDL in groups.

DISCUSSION AND CONCLUSION

Type 2 diabetes is a multi-factorial disease, frequently associated with a cluster of pathologies including obesity, hypertriglyceridemia, impaired glucose tolerance, and insulin resistance. Fructose intake may be associated with increased risk of type 2 diabetes through several biological mechanisms [17]. A higher fructose intake may play a role in an increase in body weight due to the positive energy balance. Positive energy balance leads to obesity that is associated with a higher concentration of non esterified fatty acids, which may reduce insulin sensitivity, increase hepatic glucose production, and have a deleterious effect on the beta cell function [18].

Golalipour et al showed that the protective administration of hydroalcholic extract of *Urtica dioica* had hypoglycemic effect as well as protective activity on pancreatic beta cells in hyperglycemic rats [19].

Urtica dioica has been used as antihypertensive, antihyperlipidemic and antidiabetic herbal medicine. In one study by Ahangarpour et al., [20-12]. It has been chown that compared to control group, daily administration of fructose was associated with significant increase in FIRI, blood glucose and insulin, significant decrease in lepin, and no significant change in TG, HDL, LDL, LDL/HDL ratio, VLDL, ALT, and

ALP. The extract significantly decreased serum glucose, insulin, LDL and leptin, and LDL/HDL ratio and FIRI. It also significantly increased serum TG, VLDL, and AST, but did not change serum ALP. They concluded that Urtica dioica extract, by decreasing serum glucose, and FIRI, may be useful to improve type 2 diabetes mellitus. Also, by positive effect on lipid profile and by decreasing effect on leptin, it may improve metabolic syndrome.

Bnouham et al., [21] demonstrated that i.p. administration of the water extract (WE) of Ap and Ud (150 mg/kg) 30 minutes before the glucose overload (2 g/kg) showed a significant reduction glycemia, respectively of 36 % at 60 min ($p<0.05$) and 50 % at 180 min ($p<0.05$) after glucose overload compared with controls. In contrast, the effect of WE of Au and Th (150 mg/kg, i.p.) was not significant. The in vitro study of glucose utilization by isolated rat hemidiaphragm suggests that these extracts in combination with insulin potentiate its activity and enhance the utilization of glucose. They concluded that these plants possess antidiabetic activity.

Domola et al., [22] suggested that the antidiabetic component of UD-1 was due to one or more structurally related cyclical peptides that facilitate glucose uptake by forming unique glucose permeable pores. The structure and function of these glucose-conducting pores are discussed herein.

Golalipour and Khori, [23] showed that the percentages of beta-cells in control, diabetic and treatment groups were 73.6, 1.9 and 22.9%, respectively. The percentage of beta-cells in treatment group comparing with diabetic group was significant ($p < 0.05$). Their study showed the protective administration of hydroalcholic extract of Urtica dioica has hypoglycemic effect and protective activity of beta-cells of langerhans in hyperglycemic rats.

Cholesterol is one of the body fats and is an important building block in the structure of biological membranes, and used in the biosynthesis of steroid hormones, bile acids and vitamin D. Moreover, the high cholesterol concentration in the blood increases the risk of developing atherosclerosis and related cardiovascular diseases [24]. Low-density lipoprotein takes the cholesterol from liver to tissues, whereas high-density lipoprotein facilitates the translocation of cholesterol from the peripheral tissues to liver for catabolism. Therefore, HDL has a useful effect in reducing serum cholesterol and the increase of its level in serum is suggested [25]. The LDL/HDL ratio is an important predictor of coronary heart disease risk. Therefore, this dose of extract had more efficacies to decrease liver damage.

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