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HYPOGLYCEMIC ACTIVITY OF ORIGANUM VULGARE L. AQUEOUS EXTRACT IN HYPERGLYCEMIA INDUCED BY IMMOBILIZATION STRESS IN RABBITS

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Currently diabetes mellitus (DM) is a worldwide problem linked to hyperglycemia and has affected people both in developing and developed countries. DM is divided into Type 1 (T1DM) and Type 2 (T2DM). Moreover, the T2DM is the most prevalence form that encompasses a reduce responsiveness to insulinsensitive cells or defects in insulin secretion. Exposure to chronic immobilization stress contributes to the development of hyperglycemia and hyperlipidemia that can lead to cardiovascular complications. Herbs with hypoglycemic properties, especially *Origanum vulgare* L., would be useful in hyperglycemia treatment. Oral administration of aqueous extract showed significant effect on hyperglycemia and improving glucose tolerance. In addition, extract of *O. vulgare* reduced total cholesterol, low-density lipoprotein cholesterol levels, and vice versa increased high-density lipoprotein cholesterol levels, and so decreased liver enzymes levels (alanine aminotransferase and aspartate aminotransferase) compared with untreated group and might be recommended as herbal remedy in the treatment of stressinduced hyperglycemia.

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Keywords: hyperglycemia, hyperlipidemia, immobilization stress, medicinal plant.

Introduction. Diabetes mellitus (DM) is one of chronic metabolic disorder altered carbohydrate, lipid and protein metabolism lead to hyperglycemia, hyperlipidemia and polyuria due to the inability of the insulin-sensitive cells to respond to the secreted insulin, resulting to a lack secretion of insulin from pancreatic β -cells. Moreover, hyperglycemia can provoke a number of pathogenesis such as neuropathy, cardiomyopathy, peripheral arterial disease and coronary artery disease [1]. Hence, controlling hyperglycemia decreases the risk of developing cardiovascular complications and improves diabetes management. In case of hyperglycemia, the cells cannot metabolize glucose correctly due to insufficient production of insulin and is a diminished ability of the tissue to utilize glucose [1, 2]. Glucose is the main source of energy for most organs and tissues. Nowadays, different therapeutic approaches for treatment of Type 2 DM (T2DM) are utilized, which focuses on

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maintaining normal blood glucose levels. An oral antidiabetic agents and insulin currently used in clinical practice as glucose-lowering agents. Insulin is a polypeptide hormone that secreted from β -cells of the pancreas and regulates the utilization of glucose by the tissues. Apart from insulin, the oral antidiabetic drugs, the sulphonylureas such as glibenclamide, and the antidiabetic agents, which enhances the sensitivity of insulin such as metformin, have been developed. Synthetic drugs, like metformin, can arise side effects such as lactic acidosis that can occur in diabetic patients [1, 3]. Furthermore, this glucose-lowering pharmaceutical drugs and insulin are expensive and have adverse effect such as severe hypoglycemia, liver cell injury and diarrhea [2]. Due to side effects of synthetic drugs, there is a need to discover novel drugs for the regulation of blood sugar and the treatment of hyperglycemia. Presently, for diabetic patients there are several available treatment strategies, either through synthetic drugs or natural sources like medicinal plant. As known, the vast majority of diabetic patients in developing countries use medicinal natural products that have an antidiabetic properties [1-6]. Most people at high risk of developing type T2DM usually take plant-based food that could be enriched with phytochemicals such as phenolic compounds. Thus, medicinal plants continue to provide a potential sources from natural products that could help in the normalization of blood glucose levels. Medicinal plants can protect β-cell function and enhance insulin resistance. Consequently they could be effective for prevention and treatment of diabetes mellitus.

Armenian flora is rich in various medicinal plants, which are widely used for treatment of diabetes [4, 5]. Plants may contain biomolecules with antyhyperglycemic activities. It is known more than 800 plants used in folk medicine for treatment of hyperglycemia have high clinical potential. However, only 30% of these plants are studied [2]. Overall, secondary metabolites derived from plants such as flavonoids, alkaloids, terpenoids and glycosides are responsible for increase of insulin output and decrease of glucose absorption.

Origanum vulgare L. is an important aromatic plant belonging to *Lamiaceae* family and have various pharmacological properties The herb and its oil are a valuable source of secondary metabolites and have been used in Armenian folk medicine for treatment of many diseases [7]. Besides, the herb contains the bioactive lipids such as linoleic and oleic acids [8]. This plant recognized for its wide health-promoting and antibacterial effects [7, 8]. In relation with a wide range of biologically active compounds, the herb indicates various pharmacological activities, especially antimicrobial, choleretic and antioxidant. *O. vulgare* from Armenian flora contains a large number of natural bioactive compounds, such as terpenes and flavonoids, and have marked antioxidant, antiradical and antibacterial activities [7, 8]. Nonetheless, the antihyperglycemic and antihyperlipidemic properties of *O. vulgare* are not study clearly.

Long-term immobilization stress leads to various malfunctions, including increasing the blood glucose level, blood pressure and cardiovascular reactivity [4, 5]. Immobilization stress induces memory impairment and depressive-like behavior. Chronic immobilization stress by immovability to alter the antioxidant balance, leading to the development of various pathological states such as mitochondrial dysfunction, disruption of energy pathway and neuronal damage [9, 10].

The present study was undertaken to evaluate antihyperglycemic activities of *O. vulgare* leaf extract and some biochemical parameters in hyperglycemia induced by immobilization stress in rabbits.

Materials and Methods.

Plant Material, Growth Condition and Preparation of Plant Extract. O. vulgare was grown under hydroponic conditions [11]. The dry material was supplied by the Institute of Hydroponic Problems, NAS RA. Sprouts of this plant were transplanted in conditions of classical hydroponics (seating density was 1 $plant/m^2$). Particles of volcanic slag with diameter of 3–15 mm served as substrate for plant, nutrition solution was used as described [11].

5 g of O. vulgare leaf powder was added to 100 mL distilled water and the mixture was extracted at 70°C for 20 min. Aqueous extract was filtered through Watman filter and used for determination of hypoglycemic activity.

Animals. Nine male rabbits (*Californian rabbit*), weighing 2000–2200 g, were acclimatized to standard laboratory conditions for 3–5 days prior to experimentation. The animals were housed under standard environmental conditions (temperature $22\pm2^{\circ}$ C in a light/dark cycle of 12 h) and had free access to food and water during the experimental period. The experiment was authorized by the "International Recommendation on Carrying out of Biomedical Researches with use of Animals," and the study plan has been approved by the National Center of Bioethics (Armenia).

Induction of Hyperglycemia in Experimental Rabbits and Blood Sampling. Rabbits were immobilized for 3 h per day for 21 days using the fixing board [4, 5]. Nine animals were divided into three groups. Group 1: normoglycemic; group 2: hyperglycemic control (undergo immobilization stress); group 3: hyperglycemic experimental which, in common with immobilization stress, received 2 mL of O. vulgare aqueous extract (150 mg/kg body weight) [4, 5]. Biochemical analyses were measured at the beginning of the experiment and then on the 1st, 7th, 14th and 21st days of oral treatment.

Biochemical Analyses. The biochemical analysis was performed to measure the serum level of glucose, total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), triglycerides (TG), total protein (TP), urea and creatinine. All parameters were assayed using enzymatic kit. Serum glucose level (*mmol/L*) was determined by the glucose oxidase method (Dialab Glucose, GOD-PAP, Austria), as described [12]. Total cholesterol and triglycerides were estimated by the method, as developed before [13]. HDL and LDL were measured by the described method [14]. The atherogenic index (AI) was determined by the formula, AI = (TC - HDL)/HDL [14]. Blood samples were taken from the aural vein and collected in serum separation tubes (Clot Activator & Gel, Turkey), then was centrifugated at 3000 g for 10 min.

Liver enzyme markers [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] were determined by kinetic UV assay. Analytical tests were conducted using automatic biochemical analyzers VITROS-5.1/FS (Germany) and MINDRAY B1-120 (China).

Data Processing. All values were expressed as \pm standard error of the mean. Data processing was done using "Statistica 6.0" software for Windows. The

differences between the results of different series were considered valid if Student's criteria (p) were < 0.05.

Results and Discussion. This study was designed to investigate the protective effects of *Origanum vulgare* L. against chronic immobilization stress. Exposure to chronic immobilization stress contributes to the development of hyperglycemia, and it has been found to increase fasting glucose level. Immobilization stress leads to malfunctions of the endocrine, cardiovascular and immune system, which are characterized by hyperglycemia and hyperlipidemia [4, 5, 10] and disorder of some biochemical parameters such as total protein, creatinine and urea. Carbohydrate and lipid metabolism disorders lead to the increase of LDL-cholesterol and the decrease of HDL-cholesterol levels. For regulation of metabolic disorders, phytotherapy is preferred. This study was designed to investigate the protective effects of *O. vulgare* aqueous extract and its pharmacological activity as antihyperglycemic and antihyperlipidemic agent on immobilization stress in rabbits.

Effect of O. vulgare on Blood Glucose Levels. Immobilization stress leads to disorder of the endocrine and immune system. In addition, immobilization stress characterized by hyperglycemia and hyperlipidemia. Disorders of carbohydrate and lipid metabolism lead to the increase of LDL-cholesterol levels and the decrease of HDL-cholesterol levels. For regulation of metabolic disorder, medicinal plants are preferred.

Hypoglycemic effect of *O. vulgare* aqueous extract was evaluated on hyperglycemic rabbit model in duration of 21 days. During the first immobilization stress, the fasting blood glucose levels in the hyperglycemic control and hyperglycemic+extract groups were increased at 43.8% and 19.3%, respectively (Fig. 1). This increasing of blood glucose level proved that stressful pressure could provoke hyperglycemia. The rabbits that are placed in immobilization stress (control group) on the 21st day showed significant increasing the fasting glucose level (51.2%, p<0.05), while single dose of oral treatment with extract (150 *mg/kg*) showed insignificant increase (13.2%, p<0.05), compared to the 1st day value.



Fig. 1. Effect of *O. vulgare* aqueous extract on fasting blood glucose levels in normoglycemic and hyperglycemic animals. Data are represented as mean ± SEM for 3 animals per group.
* Significantly different levels compared to the normoglycemic group (p<0.05);
** Significantly different levels compared to the hyperglycemic control group (p<0.05).

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On the 10^{th} day of treatment, an oral glucose tolerance test (OGTT) was carried out. The animals were fasted overnight before commencing to experiments. 20% glucose solution (2 g/kg body weigh) was administrated with signal oral dose to all groups of rabbits. The blood glucose level was measured by portable glucometer (Contour TS, Bayer, Switzerland). Blood samples were collected from aural vein at 0, 30, 60, 90 and 120 *min* after glucose loading.

Our findings observed that *O. vulgare* leaf extract showed a significant effect on hyperglycemia of treated group, compared to the hyperglycemic group (Fig. 2). Blood glucose in all groups were increased at a 30 *min* time point after glucose load, and then gradually decreased in the following hours. Particularly, in the normoglycemic group glucose level was increased at 42.8%, hyperglycemic control group at 45.7% and hyperglycemic+extract group – at 27.2%.





* Significantly different levels compared to the normoglycemic group (p<0.01).

** Significantly different levels compared to the hyperglycemic control group (p<0.01).

At 120 *min*, blood glucose levels were significantly reduced in the treated group of rabbits (25.9%) compared to the values at 30 *min* (Fig. 2). Thus, glucose tolerance was significantly improved in the *O. vulgare* treated animal group, compared to the hyperglycemic control group (p<0.01).

Effect of Ethanol Extract of O. vulgare on Serum Lipid Profiles and Some Biochemical Parameters. The results in Tab. 1 represented the serum lipid profile includes TC, TG, HDL, LDL and VLDL levels by immobilization stress of rabbits. In hyperglycemic control group there was a significant increase in lipid profile, especially LDL level (62.0%, p<0.05) as compared to the normoglycemic group. Treatment with O. vulgare extract demonstrated significant reduction in TC, TG, LDL and VLDL levels compared to the hyperglycemic control group. Moreover, after the treatment with extract, HDL level increased in treated animals (44.2%, p<0.05) as compared to the normoglycemic animals. As concerns to atherogenic index (AI), the hyperglycemic control group of animals demonstrated increase (\approx 9-fold), whilst the animals treated with extract showed increase by 2.5-fold, compared to the normoglycemic group.

Table 1

Parameters, mmol/L	Normoglycemic	Hyperglycemic control	Hyperglycemic+extract
Total Cholesterol (TC)	1.4 ± 0.08	$4.5 \pm 0.16*$	$2.0 \pm 0.06*$
Triglycerides (TG)	0.85 ± 0.12	$1.3 \pm 0.4*$	$1.03 \pm 0.16*$
HDL	0.78 ± 0.04	$1.0 \pm 0.15*$	$1.4 \pm 0.3*$
LDL	1.0 ± 0.15	$2.63 \pm 0.09*$	$1.0 \pm 0.13^{*}$
VLDL	0.17 ± 0.03	$0.46 \pm 0.07*$	$0.21 \pm 0.06*$
AI	0.4 ± 0.05	$3.5\pm0.15*$	$1.0 \pm 0.13*$

The effect of O. vulgare extract on lipid profile in rabbits

* Significantly different from normoglycemic group (p<0.05). Data are represented as mean ± SEM for 3 animals per group (also for Tab. 2).

Urea is a product of protein metabolism and commonly known as blood urea nitrogen. Therefore, concentration of urea is dependent on protein intake and capacity of body to catabolized of protein [15]. Creatinine is a waste product and it is produced from the breakdown of creatine and phosphocreatine and generated through muscle and protein diet [15]. The high concentration of creatinine and urea in serum is an evidence for the impairment of kidney functions. The continual immobilization stress provokes the significant increase in the levels of serum creatinine (21.3%) and urea (48.8%) in hyperglycemic control group as compared to the normoglycemic control group (Tab. 2).

Table 2

Parameters	Normoglycemic	Hyperglycemic control	Hyperglycemic+extract
Total protein, g/L	112.4 ±1.21	$67.5 \pm 0.65*$	$88.4 \pm 0.94*$
Albumin, g/L	77.4 ± 0.12	$42.2 \pm 0.4*$	$60.8 \pm 0.16^*$
Urea, mmol/L	$2.2 \pm 0.15*$	$4.3 \pm 0.04*$	$3.1 \pm 0.3*$
Creatinine, mmol/L	77.1 ± 0.19	$98.0 \pm 0.43*$	$84.0 \pm 0.13*$

The effect of O. vulgare extract on biochemical parameters in rabbits

The data reveals that treatment with extract of O. vulgare (150 mg/kg) shows a positive effect on lipid profiles and some biochemical parameters such as total protein, creatinine and urea. Induction of hyperglycemia characterized by increased catabolism of protein as a result of insulin deficiency, which increases muscle wasting and loss of tissue proteins [4, 15, 16]. Our study has shown, as a result of immobilization, total protein and albumin content in serum were significantly decreased in hyperglycemic control group of rabbits at 39.9% and 45.4%, respectively. Nevertheless, our results indicated that administration of O. vulgare extract elevated the total protein and albumin levels when compared with hyperglycemic control group (Tab. 2).

Effects of O. vulgare on Liver Enzymes. The metabolic disorders such as obesity, hyperlipidemia and diabetes mellitus have been associated with mild-to-moderate alanine aminotransferase (ALT) and aspartate aminotransferase (AST) elevation [5, 17–19]. Furthermore, abnormally high serum levels of liver function enzyme ALT and AST, as measure of hepatic damage, has been linked to the T2DM [17]. Both ALT and AST are concentrated in liver, and elevations in their levels are indicating hepatic damage. Compared to the AST, ALT is more specifically originated from liver. Therefore, increased ALT levels in serum have been considered more specific for liver damage than AST [18]. Many authors attribute the elevation in ALT and AST after muscle injury [5, 18, 19]. Our studies were intended to influence hyperglycemia on the ALT and AST levels as markers for hepatic damage.

The Fig. 3 shows the relationship between high level of serum ALT and AST and hyperglycemia. The findings showed that the activity of these enzymes in serum of hyperglycemic control group of animals was higher compared to the hyperglycemic + extract group, which indicates that the immobilization stress had severe liver cell damage. The treatment with *O. vulgare* extract ALT and AST activities decreased by 2.2-fold and 2.1-fold, respectively (Fig. 3, a and b). However, after the treatment of animals, these levels were corrected which proved the positive effect of *O. vulgare* extract on enzymes level and could demonstrate an attenuation effect on hepatic damage.





* Significantly different levels compared to the normoglycemic group (p<0.05).

** Significantly different levels compared to the hyperglycemic control group (p<0.05).

Conclusion. In the current study we found a positive effect of the aqueous extract of *O. vulgare* L. on blood glucose levels, lipid levels, liver function enzymes (ALT, AST) and some biochemical parameters in hyperglycemia induced by immobilization stress in rabbits during the 21 days of treatment. These investigations

corroborated the *O. vulgare* extract ameliorated glucose homeostasis, corrected lipids and proteins levels. These findings indicate that the plant might be a good source for delay of development of stress-induced hyperglycemia and correct metabolic disorders.

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ORIGANUM VULGARE L. ՉՐԱՅԻՆ ԼՈԻԾԱՄՉՎԱԾՔԻ ՀԻՊՈԳԼԻԿԵՄԻԿ ԱԿՏԻՎՈԻԹՅՈԻՆԸ ԻՄՈԲԻԼԻՉԱՑԻՈՆ ՍԹՐԵՍԻ ՊԱՅԱՄՆՆԵՐՈԻՄ

Ներայումս շաթարախտը համաշխարային խնդիր է, որն ազդում է ինչպես զարգացող, այնպեես էլ զարգացած երկների բնակչության վրա։ Տարբերում են շաթարային դիաբետի երկու տեսակ՝ ՇԴ1Տ և ՇԴ2Տ։ Առավել տարածված է 2-րդ տիպի շաքարախտը, որի ժամանակ դիտվում է ինսուլինի արտազատման խանգարումներ։ Իմոբիլիզացիոն սթրեսը նպաստում է հիպերգլիկեմիայի զարգգացմանը, ինչը կարող է առաջ բերել սրտանոթային բարդություններ: Origanum vulgare L. ջրային յուծամզվածթով կենդանիների բուժումը դրական ազդեցոություն է ունեցել գլլուկոզի մակարդակի վրա։ Ավելին, O. vulgare յուծամզվածքը նվազեզրել էր ընդհանուր խոլեստերինի և ցածը խտության լիպոպրոտեինների մակարդակը, և հակառակը, բարձրացրել բարձր խտության լիպոպրոտեինների մակարդակը։ Լուծամզվածքը ունեզել է դրական ազդեցություն լյարդի ֆերմենտների ֆունկցիայի, ինչպես նաև որոշ կենսաքիմաիական գուզանիշների վրա իմոբիլիզագիոն սթրեսի ենթարկված ճագարների արյան շիճուկում 21 օր բուժման ընթագքում։ Այսպիսով, բույսը կարող է առաջարկվել որպես բուսական միջոց սթրեսի հետևանքով առաջացած հիպերգլիկեմիայի բուժման համար։

А. А. АГАДЖАНЯН, А. О. ТАДЕВОСЯН

ГИПОГЛИКЕМИЧЕСКАЯ АКТИВНОСТЬ ВОДНОГО ЭКСТРАКТА ORIGANUM VULGARE L. В УСЛОВИЯХ ИММОБИЛИЗАЦИОННОГО СТРЕССА

В настоящее время сахарный диабет является глобальной проблемой, затрагивающей население как развивающих, так и развитых стран. Различают два типа сахарного диабета – (СД1Т) и (СД2Т). Наиболее распространенным является диабет 2-го типа, связанный с нарушением секреции инсулина. Иммобилизационный стресс способствует развитию гипергликемии, что может привести к сердечно-сосудистым осложнениям. Лечение животных водным экстрактом Origanum vulgare L. оказало положительное влияние на уровень глюкозы. При этом экстракт O. vulgare снижал уровень общего холестерина и липопротеинов низкой плотности, и наоборот, повышал уровень липопротеинов высокой плотности. Экстракт оказывал положительное влияние на функцию ферментов печени, а также на некоторые биохимические показатели сыворотки крови кроликов в условиях иммобилизационного стресса в течение 21-дневного лечения. Таким образом, растение можно рекомендовать в качестве растительного лекарственного средства для лечения гипергликемии, вызванной стрессом.