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Hypoglycemic and hypolipidemic activity of moringa grown in hydroponics and soil in Ararat Valley

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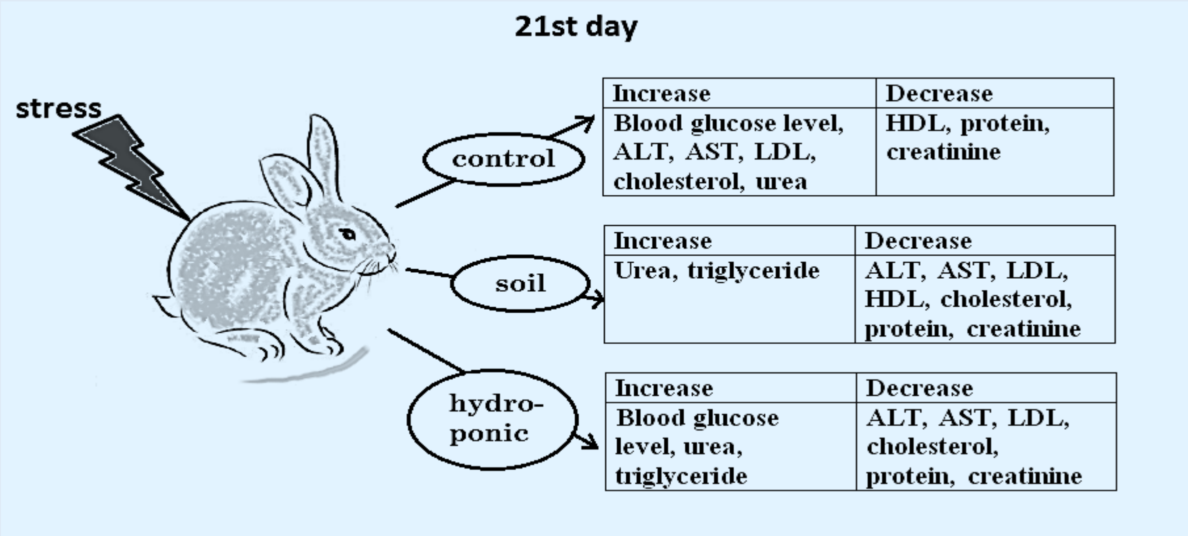
Background: *Moringa* has a hypoglycemic and hypolipidemic influence, and the water extract of its leaves can decrease the amount of glucose during Diabetes Mellitus. *Moringa* leaves in extract or powder form influenced type I and type II diabetes by enhancing insulin secretion and improving glycogen synthesis and glucose uptake in the liver and muscles. This occurred in the case of type I diabetes, as well as by inhibiting glucose uptake and slowing gastric emptying in the case of type II diabetes.

Objective: In our study, we try to evaluate the hypoglycemic, hypolipidemic, and antistress activity of *Moringa* grown for the first time in Armenia in hydroponic and soil conditions to find out the beneficial cultivation method of *Moringa* from the point of view of these activities.

Materials and Methods: The study was done with the usage of a rabbit model, using water extract of *Moringa* leaves made with a 150 mg count of leaves on 1 kg weight of the animal. Hyperglycemia in the rabbits was induced by 21-day immobilization stress (3 hours a day). Rabbits were divided into 4 groups: the first group was treated with the extract from the hydroponic *Moringa* in a dose of 2 ml/day, the second group received the extract from the soil *Moringa* in a dose of 2 ml/day, the third group was the control—which did not receive any extract, but was exposed to stress, like first and second groups— and the fourth group was the norm, which did not receive any extract and was stress free. Each group included 3 animals as means of replication. The glycogen level was measured in the livers, hearts, and muscles of rabbits. The level of glucose, total cholesterol, HDL, and LDL were measured in the serum of animals.

Results: Our results showed that after a 21-day period of stress, the increased level of glucose stays at a high level in control groups, while the use of a hydroponic *Moringa* extract developed stress tolerance in animals and in soil *Moringa* extract stabilizes glucose levels in the blood. The cholesterol exceeded the norm 1.8 times in the control group and was inferior in hydroponic and soil groups by 1.5 and 1.4 times, respectively. These changes reflect the amount of HDL and LDL. Under stress conditions, the quantity of glycogen decreased from the norm in the liver, muscles, and heart muscle in the control group (2.5; 2.4; and 2.4 times, respectively) and increased in hydroponic (1.6; 1.5; and 2.1 times, respectively) and soil (1.6; 1.5; and 2.4 times, respectively) groups. This evidence shows that under stress conditions the glucose level of blood is improved on the 21st day of stress in groups that received the extract of moringa because of glucose exchange to glycogen with high efficiency in different organs.

Keywords: antistress activity, diabetes, HDL, glucose, *Moringa oleifera*, total cholesterol



INTRODUCTION

Moringa oleifera (*Moringa*) is native to India and is used in many areas of human life due to the macro and microelements it contains [1-3]. It is known that *Moringa* has a hypoglycemic influence [4] and the water extract of its leaves may be used for Diabetes mellitus to reduce the blood glucose level [5]. It was shown that the extract of the *Moringa* leaves inhibits intestinal α-glucosidase, pancreatic cholesterol esterase, and pancreatic lipase activities, thus helping in the treatment of diabetes mellitus [6]. The decrease in theactivity of pancreatic cholesterol esterase supports lowering the level of cholesterol in the organism.

With Diabetes mellitus, lipid concentration is increased in the blood, enhancing the risk of premature atherosclerosis. Crude leaf extract of *Moringa* along with a high-fat diet reduces the level of cholesterol and triacylglycerol in serum. It was shown that the use of *Moringa* extract decreased the lipid profile of the liver, heart, and aorta [7]. The use of powder of *Moringa* leaves decreased low-density lipoproteins (LDL) levels, atherogenic risk, and glycemia in HIV-negative children [8].

Moringa leaf extract had a hepatoprotective action against acetaminophen-induced hepatotoxicity in mice and rats in a dose-depended manner. It did so by decreasing the levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and increasing antioxidant enzymes in the liver and glutathione level (GSH) [9]. The increases in the AST/ALT level is conditioned by the increase in the death level of cardiovascular patients [10] and the increase of the cancer probability [11], which may be formed because of stress. In mice fed with a high-fat diet, the leaf extract of *Moringa* protects against liver damage by decreasing levels of AST, alkaline phosphatase (ALP), ALT, lipid peroxidation (LPO) and increasing GSH [9]. Oral administration of *Moringa* leaf extract caused a maximum reduction of 26.7% in fasting blood glucose level (FBG), and a maximum reduction of 30% in glucose tolerance after 3 hours of glucose consumption in normal and streptozotocin (STZ)-induced sub, mild, and severely diabetic albino rats. After 21 days of intake, FBG and postprandial blood glucose levels have a maximum reduction of 69.2% and 51.2%, respectively [11]. *The use of Moringa leaves extract or powder* may positively influence type I and type II diabetes by enhancing insulin secretion and both improving glycogen synthesis and glucose uptake in the liver and muscles. This pertains to type I, and functions in the case of type II by inhibiting glucose uptake and slowing gastric emptying.[9].

Nowadays, stress is one of the causes of the high number of diabetic patients, especially with type II diabetes. It is mentioned that stress-induced hyperglycemia may take place because of diseases leading to insulin resistance and decreased insulin secretion. This leads to an increase in blood glucose level, and the use of external insulin because of the lack of insulin in the organism [12]. It is shown that chronic stress may increase the level of LDL, and cholesterol, causing hyperlipidemia with subsequent development of atherosclerosis [13], which is one of the complications of diabetes [14]. High cholesterol level in the blood plasma adversely influences the progress of cardiovascular diseases and liver disorders (non-alcoholic fatty liver disease, non-alcoholic steatoses hepatitis) that is conditioned by the activation of oxidative stress and endoplasmic reticulum stress. Studies done on animal models and humans have shown that high cholesterol level is included in the development of many metabolic diseases [15].

In our study, we tried to estimate the hypoglycemic and hypolipidemic influences of the water extract of *Moringa* leaves on the rabbit model using leaves of *Moringa* grown in hydroponic and soil conditions. Like this, we try to estimate the effectivity of these two *Moringa* groups and understand which growing method is more beneficial for the treatment of stress-activated hyperglycemia.

MATERIAL AND METHODS

Study design and animal model: The hypoglycemic activity of the extract was studied on male rabbits with a body mass of 1800-2100g. The initial body mass of the animals was recorded 1 day before the start of the experiment. The animals were kept under standard environmental conditions (22±2°C temperature, light/dark 12-hour cycle). During the experiment, rabbits had free access to eat and drink. All studies were done according to the modern ethical norms confirmed by the "International Recommendation on Carrying out of Biomedical research with Use of Animals" and the research plan was validated by the Armenian National Center of Bioethics.

The hyperglycemia in rabbits was induced by the 21-day-long immobilization stress (3 hours a day). They were fixed on board [16]. The rabbits were divided into four groups with 3 animals in each group. The mentioned number of animals was chosen since it provides reliable reproducible results. The first group received 2 ml of water extract from the hydroponically cultivated plant once a day. The second group received 2 ml water extract of plant grown in soil once a day. The control group was the third one, which included healthy animals that did not receive any plant extract. The fourth group was set as the norm , which did not undergo stress and did not receive any treatment. The amount of the given extract was calculated at the rate of 150 mg per 1 kg of body weight. The extract was given by mouth with a syringe. All experiments were done based on the current ethical norms stated by "International Recommendation on Carrying out of Biomedical Research with Use of Animals," and have been approved by the National Center of Bioethics (Armenia).

Biochemical analyses: The blood glucose level, lipid profile, and body mass of rabbits were measured at the beginning of the study and the 1st, 7th, 14th, and 21st days of the oral injection experiment. Blood samples were taken from the ear vein and were collected in serum separation tubes (Huma Tube K3E, Germany). A blood clot was removed by 10 min centrifugation at 3000 g under 4 °C. The received supernatant was the serum.

At the end of the study animals were decapitated: the glycogen level in the liver and muscle of animals was analyzed.

The levels of glucose, total cholesterol, high-density lipoproteins (HDL), LDL, and triglycerides were measured in the serum. All indicators were measured using enzyme kits. Glucose level (mmol/L) in serum was defined using a glucose test kit based on the glucose oxidase method (Dialab Glucose, GOD-PAP, Austria) [17]. Total cholesterol and triglycerides were estimated by the method provided earlier [18]. HDL and LDL were measured using the described method [19]. Analytical tests were carried out using a UV-Vis spectrophotometer (Genesys 10S, USA). The glycogen content was determined by the described method [20]. The creatinine level was estimated by the modified Jaffe's method, and the urea level was measured using Berthelot reaction [19]. Protein analyses were done based on biuret reaction [21].

Statistical analyses: Statistical analyses were done with the help of GraphPadPrism 8 program and Excel. $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Glucose: In our study before the stress, the glucose level was 5.8mmol/L in the rabbits of the control group, 5.7mmol/L in the hydroponic group, 4.9mmol/L in the soil group, and 4.9 mmol/L in the norm group. On the first day of the stress the highest level of glucose was observed in the soil group, followed by the control. On the 21st day, the lowest level of glucose was in the soil group from the stress-influenced ones, and the highest in the control. In the soil and hydroponic groups, the level of glucose on the 21st day increased by 1.14 times compared with its level before the experiment started, while it increased in the control group by 1.5 times. In the control group, the stress consequences existed after 21 days (Fig. 1). Feeding animals with hydroponic *Moringa* generates tolerance in animals and the use of the soil *Moringa* extract stabilizes the glucose level in the blood after stress.

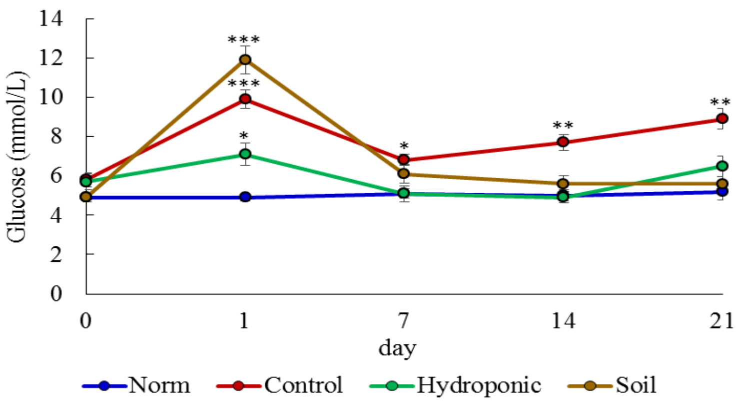


Fig. 1. Glucose level (mmol/L) on the 1st, 7th, 14th, and 21st days after stress in norm, control, hydroponic, and soil groups (stress was induced on the 1st day). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to norm group.

The glucose tolerance test shows that in groups with soil and hydroponic *Moringa* extracts, the glucose amount regulation was also observed within 120 minutes, while in the control group any regulation was not observed and was significantly higher from the norm group (Fig. 2). This shows that the tincture of *Moringa* leaves has the ability to regulate glucose amount.

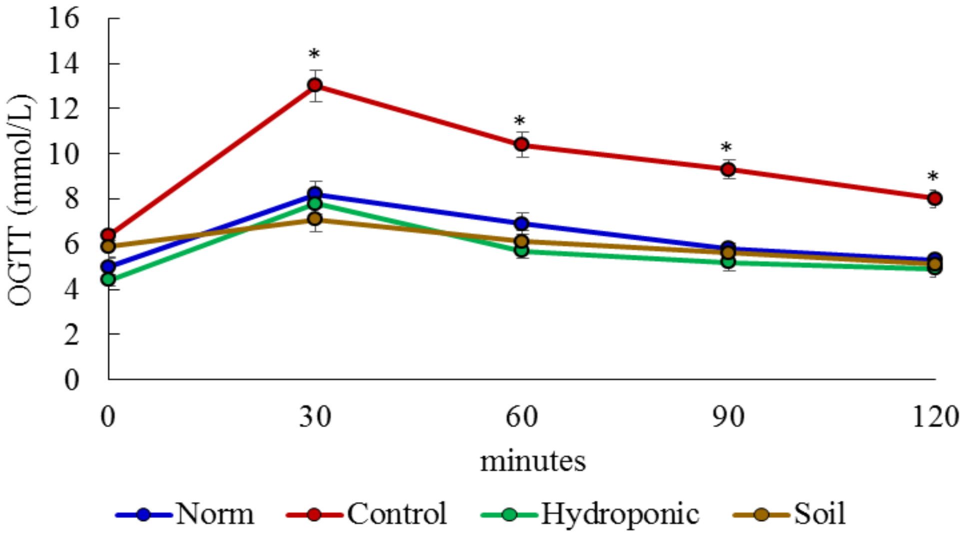


Fig. 2. Glucose tolerance test (OGTT, mmol/L), done after stress during 120 min with 30 min intervals in control, hydroponic, and soil groups compared with the norm. * p<0.01 compared to norm group.

Influence on ALS and AST: ALT and AST are the assessment markers of liver function. During diabetes, there can be disorders of the liver functioning. Many studies showed that the AST and ALT values may be used for the diagnosis of diabetes [22].

In our study, in the control group, the AST and ALT values increased by 1.8-1.9 times on the 21st day of the stress, compared with the start day of the experiment, while in hydroponic and soil groups these indicators were reduced 1.4 times. Despite these existing differences, the values of the AST and ALT stayed between the ranges of the norm values reported for the rabbits in the literature (AST 35-130 IU/L and ALT 45-80 IU/L) [23], except for the 21st day's ALT value of the control.

According to the reports, there is a positive correlation between the amount of AST, ALT, and inulin and glucose [24]. In our study, in the animals taking *Moringa* the amount of ALT and AST were reduced (Fig. 3), consequently, the glucose amount was also reduced.

The AST/ALT ratio of De Ritis is interconnected with oxidative stress and systemic inflammation [25]. Normally, in humans, it should be in the range of 0.91-1.75 (1.33±0.42) and values less than 1 provide evidence for liver lesion [26]. The ratio of the AST and ALT average values given in Mellilo's article is 1.32, which is near the AST/ALT ratio value for human beings [24].

The AST/ALT ratio of less than 1 and the increase in the ALT level are connected with insulin resistance [27]. It has been shown that the development of type 2 diabetes is probable when AST/ALT values are less than 0.882 [28]. According to other data, this threshold is higher and the AST/ALT≤0.93 ratio suffices for the development of type 2 diabetes [29].

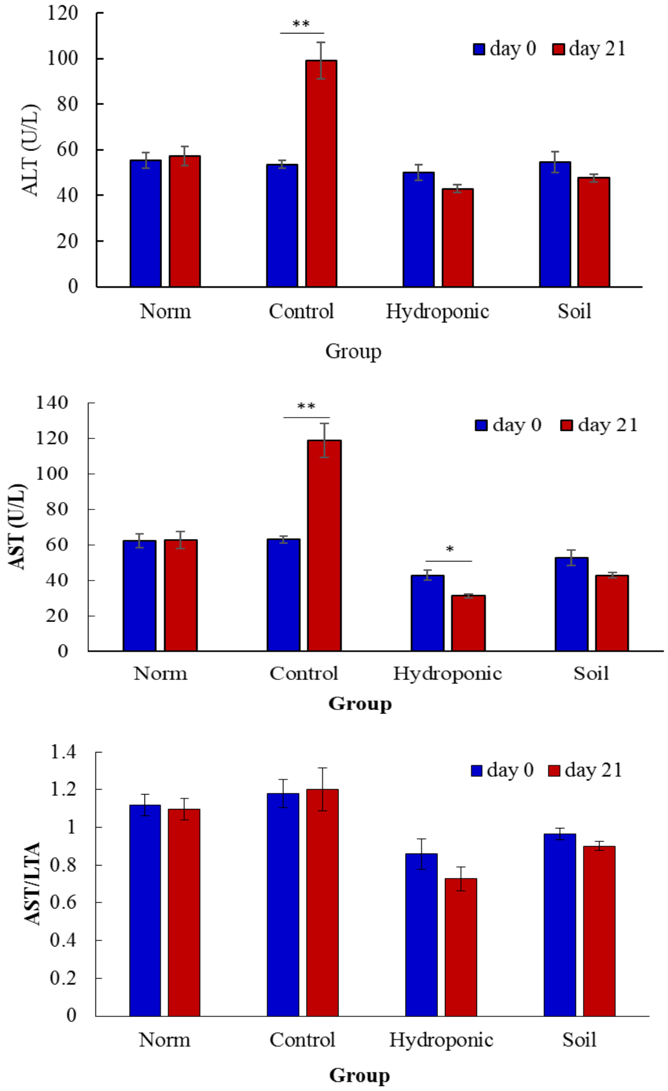


Fig. 3. The levels of ALT and AST and their ratio before the experiment and on the 21st day of the experiment in control, hydroponic, and soil groups and norm. * p<0.05, ** p<0.01.

In our study, in the control group on the 21st day, the levels of glucose, AST, and ALT increased, respectively, 1.5, 1.9, and 1.9 times (Fig. 3). It provides evidence for the development of diabetes [30]. In the hydroponic and soil groups on the 21st day of stress, the AST/ALT value decreased.

Table 1. The amounts of the cholesterol and triglycerides, as well as total proteins, urea, creatinine, HDL, and LDL, measured in control, hydroponic and soil groups of rabbits and in the norm.

Indices	mmol/L			
	Norm	Control	Hydroponic	Soil
Cholesterol	1.93 ± 0.08	3.53 ± 0.16	1.3 ± 0.05	1,4 ± 0,05
Triglycerides	0.89 ± 0.06	0.85 ± 0.06	1.32 ± 0.42	1.27 ± 0.36
HDL	1.33 ± 0.51	1.0 ± 0.15	1.32 ± 0.34	1.17 ± 0.43
LDL	1.04 ± 0.19	1.49 ± 0.08	0.95 ± 0.12	0.90 ± 0.22
Creatinine	197.0 ±1.81	149.0 ±1.21	113.0 ±1.29	114.0 ± 1.42
Urea	5.9 ± 0.46	8.9± 0.34	7.6 ± 0.62	6.5 ± 0.42
	g/L			
Total proteins	111.0 ±1.09	50.0 ± 0.65	97.0 ± 0.33	95.0 ± 0.51

Cholesterol: The increase of cholesterol in the pancreatic β-cells leads to the decrease in insulin exocytosis from these cells with further development of type I diabetes mellitus and stimulation of apoptosis of these cells. Because of this, diabetic patients are often suggested to take cholesterol-lowering drugs to protect β-cells [31, 32].

In our study, the cholesterol amount increased by 1.83 times in the control group compared with the norm and decreased by 1.5 and 1.4 times, respectively, in groups that received hydroponic and soil *Moringa* extracts (Table 1). This proves that under stress, the cholesterol amount increases in the organism, and the use of the *Moringa* extract forms the stress-resistance in the organism, protecting it from different diseases conditioned by stress, such as cardiovascular diseases [33-35].

HDL and LDL and Moringa extract: The change in the amount of cholesterol is reflected in the amount of HDL and LDL. LDL is defined with a high content of cholesterol, while HDL is not. In our study, compared with the norm group data, the amount of LDL increased in the control group, while the amount of HDL is enhanced in the groups that received *Moringa* extract, coinciding with our cholesterol results. In the control group an increase in cholesterol level is expressed by an increase in LDL. It is mentioned in the literature that oxidation of LDL is one of the main causes of inflammation and atherogenesis, and plasmic HDL has antiatherogenic, antioxidant, and anti-inflammatory influences. The activity of some enzymes inhibiting LDL oxidation is due to the presence of HDL [36]. LDL increase and HDL decrease cause the development of cardiovascular diseases, while LDL decrease and HDL increase prevent it [37]. One of the main causes of death in diabetic patients is cardiovascular disease [38]. Our results of HDL and LDL (Table 1) evidence that the use of *Moringa* extract will reduce the probability of cardiovascular disease development in diabetic patients and will reduce the risk of death. Note that according to Kruit et al. HDL protects the β-cells from the disorders induced by cholesterol, from apoptosis generated by stress, and from the type 2 diabetes pathogenesis of Langerhans islands, and the therapeutic normalization of HDL quantity and quality may be a new approach for the type 2 diabetes prevention or treatment [39].

Triglycerides: Triglycerides are part of very low-density lipoproteins (VLDL). The latter become intermediate-density lipoproteins (IDL), later transforming into LDLs [40]. It is proposed that hypertriglyceridemia is an important trigger of β-cells insulin excretion in young organisms during fast, as well as during good nutrition, while excess production of insulin and chronic hyperinsulinemia have a pathogenic influence in the development of type 2 diabetes [41]. In our study, compared with the norm, the level of triglycerides increased only in groups that received *Moringa* extract (Table 1), which evidences the increase of insulin production in these groups. This by itself proves the fact that in these groups on the 21st day of stress, the amount of glucose is near to the norm. As in these groups, the amount of LDL is less than the norm, which means that the transformation of VLDL to LDL does not take place, thus the diabetes development does not trigger.

Creatinine, urea, and protein: In our study, compared with the norm, the level of creatinine decreased in control (1.3 times), hydroponic (1.7 times), and soil (1.7) groups (Table 1). The literature data is controversial. Harita and others mention that a low level of creatinine in blood serum enhances the probability of type 2 diabetes generation [42], which was confirmed for male population by Song and others [43]. According to Chutani and Pande, a statistically significant increase of the urea and creatinine in blood serum is detected in patients with type 1 and type 2 diabetes, compared with the healthy population [44]. The increase in the urea level in patients with type 2 diabetes was detected also by Azeez and others [45]. In our study, the level of urea was also increased in the control (1.5 times), hydroponic (1.3 times), and soil (1.1 times) groups, compared with the norm. In groups with the use of *Moringa* extract the increase was low.

The total proteins decreased strictly in the control group compared with the norm (2.22 times). In hydroponic and soil groups the decrease was less (1.14 and 1.17 times, compared with the norm). As a great portion of the HDL are proteins, the decrease of the protein amount may be combined with the HDL. The total protein/HDL ratio in the norm, control, hydroponic and soil groups were 83.5, 50, 73.5, and 81.2, respectively.

Glycogen: In our study under stress conditions, compared with the norm, the amount of glycogen in the liver, skeletal muscles, and heart muscle was reduced in the control group (2.5, 2.4, and 2.4 times, respectively), increased in hydroponic (1.6, 1.5, and 2.1 times) and soil (1.6, 1.5, and 2.4 times) groups (Table 2). This testifies that under stress conditions on the 21st day, the blood glucose level is regulated in the groups receiving an extract of *Moringa,* due to its transformation to glycogen in different organs with high efficiency. It was shown in the literature that the strategy to increase the content of liver glucose in mice leads to the long-term inhibition of the diabetes phenotype expression regardless of the circulating insulin amount [46]. It is also mentioned that in the case of type 2 diabetes, the amount of glycogen decreases in muscles [47].

Table 2. Glycogen amount in the liver, skeletal, and heart muscles of control, hydroponic, and soil groups' rabbits and norm.

Tissues	Glycogen concentration (mg/g tissue)			
	Norm	Control	Hydroponic	Soil
Liver	5,4 ± 0,44	2,2 ± 0,32	8,8 ± 0,24	8,9 ± 0,35
Skeletal muscle	3,1 ± 0,16	1,3 ± 0,42	4,8 ± 0,12	4,8 ± 0,14
Heart muscle	2,2 ± 0,15	0,9 ± 0,16	4,6 ± 0,12	5,2 ± 0,12

CONCLUSION: Generally, it may be assumed that the use of *Moringa* extract decreases the undesirable consequences of stress by regulating the glucose level and decreasing the amount of LDL in the blood. Hydroponic and soil grown *Moringa* are equivalent in hypoglycemic and hypolipidemic activities.

Abbreviations: ALT - alanine aminotransferase; AST - aspartate aminotransferase; HDL - high-density lipoproteins; LDL - low-density lipoprotein

Authors Contribution: Each author contributed to the planning of the experiment, its implementation, processing the results and writing the article.

Competing Interests: There are no competing interests.

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