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ANTIHYPERGLYCEMIC PROPERTIES OF THE HERBAL MIXTURE COMPOSED OF EXTRACTS FROM *SALVIA OFFIC*INALIS L., *CALENDULA OFFICINALIS* LINN., *GLYCYRRHIZAE RADIX* L. AND *ECHINACEA PURPUREA* L. ON HYPERGLYCEMIA INDUCED BY IMMOBILIZATION STRESS IN RABBITS

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Hyperglycemia is the major risk factor for atherosclerosis and cardiovascular diseases, which is associated with several cardio-metabolic risk factors such as diabetes, hypercholesterolemia and high blood pressure. The antihyperglycemic properties of the herbal mixture (HM) from *Salvia officinalis* L., *Calendula officinalis* Linn., *Glycyrrhizae radix* L. and *Echinacea purpurea* L. in rabbits with hyperglycemia induced by immobilization stress have been evaluated, hypoglycemic activity, improved lipid profile and body weight, to restore liver and muscle glycogen levels by HM aqueous extract were revealed.

***Keywords*:** herbal mixture, immobilization stress, hypoglycemic effects, lipids.

Introduction. Hyperglycemia is a metabolic disorder of the endocrine system characterized by abnormal glucose metabolism, which is demonstrated by high blood sugar (hyperglycemia), blood lipid disorders (hypercholesterolemia), high blood pressure and cardiovascular disease due to the disturbances of carbohydrate, lipid and protein metabolism resulting from insulin resistant and β-cell dysfunction [1, 2].

Dyslipidemia is a very frequent metabolic disorder, which is characterized by an increase of the rates of triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL) and reduction of high-density lipoprotein (HDL) [3–5]. It is known that hypercholesterolemia contributes to the development of atherosclerosis and subsequent of hypertension, ischemic heart disease and renal failure [5].

Immobilization stress can increase the blood glucose level and the risk of cardiovascular disease due to impaired endothelial function. Chronic immobilization stress leads to atherosclerosis development, which has an endothelial dysfunction at early stages [6]. The oxidative damage and inflammatory mediators induced by chronic psychological stress play a key role in this process. Furthermore, durable immobilization can contribute to the formation of unstable atherosclerotic lesions as a result of immune system cells accumulation and molecules adhesion, leading to thrombosis and cardiac complications [6, 7].

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There are many drugs currently used in the treatment of diabetes. Some reports indicate that treatments with synthetic anti-diabetic drugs have toxicity and cause adverse effects, such as hypoglycemia, gastrointestinal and liver problems [8]. Tissues of many plant species contain secondary metabolites such as flavonoids, glycosides, saponins, steroids, tannins, alkaloids and terpenes. Extracts of different plant organs, including roots, leaves and flowers may contain pure natural bioactive compounds. In fact, one part of the plant may exert a beneficial medicinal property, while other parts of the same plant may be ineffective or even toxic.

Herbal mixture (HM) is composed of *Salvia officinalis* L., *Calendula officinalis* Linn., *Glycyrrhizae radix* L. and *Echinacea purpurea* L. The latter was used as an immunomodulatory agent. Several studies have reported that each herb or various combinations of these herbs have helpful properties, including antioxidant [9–11], hypoglycemic, hypolipidemic [9, 11], anti-inflammatory, antibacterial [9, 10], anticancer [9, 12] effects in multiple diseases.

In recent years, many research studies have been conducted to document the traditional uses of *S. officinalis* L. and to find new biological effects for this plant. These studies have revealed a wide range of pharmacological activities including anticancer, anti-inflammatory, antioxidant, antimicrobial, anti-mutagenic, hypoglycemic, and hypolipidemic effects [9]. The major phytochemicals in flowers, leaves, and stems of *S. officinalis* L. are well identified. A wide range of constituents include alkaloids, carbohydrate, fatty acids, glycosidic derivatives (e.g., cardiac glycosides, flavonoids, glycosides, saponins), phenolic compounds (e.g., coumarins, flavonoids, tannins), poly acetylenes, steroids, terpenes/terpenoids (e.g., monoterpenoids, diterpenoids) [9].

C. officinalis Linn. of the family Astraceae has been widely used in homeopathic medicine for treating many diseases. It has been reported to possess many pharmacological activities, including antioxidant, anti-inflammatory, antibacterial and cytotoxic effects as well as tumor reducing potential [10, 12]. Extracts of *C. officinalis* flowers of different polarities exhibited antioxidative effects on liposomal lipid peroxidation induced by Fe^{2+} and ascorbic acid [12].

G. radix L. includes many constituents: triterpene glycosides (glycyrrhizin), chalcones and flavonones [13]. Glycyrrhizin is a glycoside of glycyrrhetinic acid and is abundantly present in the roots. This saponin is the major sweet constituent and the main bioactive compound in *G. radix*, which has hepatoprotective properties in humans and animals organisms [13].

E. purpurea L. is a perennial medicinal herb with important immunostimulatory and anti-inflammatory properties. Biological activities of the plant such as antioxidant, antibacterial, antiviral and larvicidal activities have been reported in experimental studies. A number of studies revealed that alkamides are involved in the immunomodulatory properties of Echinacea extracts *in vitro* and *in vivo* [11].

The aim of this study was to investigate antihyperglycemic properties of the herbal mixture (HM) in rabbits with hyperglycemia.

Materials and Methods.

*Plant Materials and Preparation of Herbal Extracts***.** The dried samples of the plant species were supplied by the Institute of Hydroponic Problems, National Academy of Sciences, Yerevan (Armenia). The plants were grown using hydroponics method [14]. Sprouts of these plants were transplanted in conditions of a

classical hydroponics (seating density was 1 plant per cm^2). As substrate for plants, particles of volcanic slag with diameter of 3–15 *mm* served; nutrition solution was used, as described [14]. To prepare the aqueous extract, the dry leaves and roots were extracted at 70°*C* for 20 *min*.

Animals. Hypoglycemic activity of HM extract was carried out on rabbits with the same sex (weighing 2000–2200 *g*). The animals were kept under standard environmental conditions (temperature 22±2°*C* in a light/dark cycle of 12 *h*). The rabbits had free access to food and water during the experimental period. All experiments were performed in accordance with the current ethical norms stated by "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. Hyperglycemia was induced by immobilizing stress in the rabbits during 15 days (3 *h* daily) [6, 7]. They were roughly fixed on the board. Group 1 served as normoglycemic, group 2 was the hyperglycemic control (putting immobilization), group 3 in common with immobilization was administrated in single orally doses in 2 *mL* aqueous extract of HM (100 *mg*/*kg* body weight). Blood glucose levels, lipid profile and body weight of rabbits were measured at the beginning of the experiment and then on the 1st, 5th, 10th and 15th days of oral treatment.

At the end of the experiment the animals were sacrificed and analysis of liver and muscle glycogen content was carried out.

Blood samples were taken from the aural vein and collected in serum separation tubes (Huma Tube K3E, Human, Germany). Blood clot was removed by centrifugation at 3000 *g* for 10 *min* in a centrifuge at 4°*C*. The resulting supernatant was designated as serum.

Biochemical Analysis. Biochemical analysis was performed determining glucose level of serum, TC, TG, HDL-cholesterol, LDL-cholesterol. All parameters were assayed using enzymatic kit. Serum glucose level (*mmol*/*L*) was determined using glucose test kit based on the glucose oxidase method (Dialab Glucose, GOD-PAP, Austria), as described [15]. TC and TG were estimated by the method, developed before [15]. HDL and LDL were measured using the method, as described [16]. The atherogenic index (AI) was determined by the formula, as suggested [16]. Briefly, AI = (TC–HDL) / HDL. Analytical tests were conducted using an UV-VIS spectrophotometer (Genesys 10S, USA).

Histopathological Examination. The livers and muscles of experimental animals were harvested and followed by the histopathological examination; glycogen contents were determined by the method, as described [17].

Data Processing. All values were expressed as ± standard error of the mean. Data processing was done using "Statistica 6.0" software of Windows. The differences between the results of different series were considered valid, if Student criteria (p) was < 0.05. A difference of $p < 0.05$ or less in the mean values was considered as statistically significant.

Results and Discussion.

Effect of HM on Fasting Blood Glucose Levels. Fasting blood glucose levels in the hyperglycemic control (47.2%) and hyperglycemic + HM extract (26.0%) groups during the first day of immobilization (3 *h*) were significantly increased, compared with the normoglycemic group, $p < 0.05$ (Fig. 1).

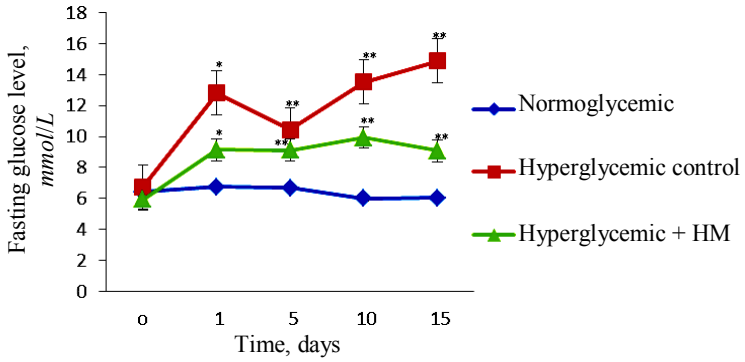

Fig. 1. The effects of HM aqueous extract on fasting blood glucose levels in normoglycemic and hyperglycemic rabbits. Data are represented as mean ± SEM for 3 animals per group:
* significantly different levels compared with the normoglycemic group ($p < 0.05$);
** significantly different levels compared with the hyperglycemic control group ($p < 0.05$).

The results showed significant increases in blood glucose levels after immobilization stress of the rabbits. Therefore, it may be noted that disposable strong stressful pressure provokes hyperglycemia.

Fasting blood glucose level in the hyperglycemic control group significantly increased on the 15th day of immobilization (54.6%) compared with the baseline value. In the group of animals which got the aqueous extract of HM reduction in fasting glucose level was demonstrated at the 15th day reaching the 1st day level (Fig. 1). The blood glucose lowering effect of HM extract could be attributed to the presence of flavonoids and phenolic compounds that have been associated with hypoglycemic activity [9, 10].

It is known that hyperglycemia is commonly associated with disturbance of lipid metabolism, leading to the increased TC and low-density lipoprotein as well as decreased high density lipoprotein levels [18]. Hyperlipidemia is one of the major factors linked with hyperglycemia due to insulin deficiency during diabetes and correlated with carbohydrate metabolism. Insulin resistance and lack of insulin secretion due to pancreatic β-cell failure are among the leading causes of type 2 diabetes.

In the current study, a reduction was observed in the TC, TG, very LDL-(VLDL) and LDL-cholesterol after treating with HM extract (see Table).

The effect of HM aqueous extract on lipid parameters in rabbits

Lipid parameters, *mg/dL*	Experimental groups		
	Normoglycemic	Hyperglycemic control	Hyperglycemic + extract
TC	68.89 ± 0.27	130.88 ± 0.72*	66.38 ± 0.69*
TG	57.89 ± 0.64	85.96 ± 0.98*	40.35 ± 0.21*
VLDL-cholesterol	11.57 ± 0.15	17.19 ± 0.63*	8.07 ± 0.11*
HDL-cholesterol	26.24 ± 0.17	18.14 ± 0.61*	28.56 ± 0.47*
LDL-cholesterol	31.48 ± 0.49	95.55 ± 0.56*	50.24 ± 0.51*
Atherogenic Index	1.62 ± 0.06	6.21 ± 0.44*	1.32 ± 0.03*

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

Hypolipidemic activity of HM extract may be mediated by reducing intestinal cholesterol absorption and increasing reverse cholesterol transport [3, 4]. Therefore, it was also found that HM extract improved the serum lipid profile, which eventually alleviated diabetes complications.

Effect of Aqueous Extract of HM on Liver and Muscle Glycogen Levels. The hyperglycemic control group showed 3.4-fold reduction in liver glycogen levels, compared with the normoglycemic group ($p<0.01$) (Fig. 2). The hyperglycemic + HM group induced significant increases in the liver glycogen levels compared with the hyperglycemic control and normoglycemic groups (3.8 and 1.1 fold respectively, $p < 0.01$) (Fig. 2, a). The hyperglycemic control group showed strong reduction (36 fold, $p < 0.01$) in muscle glycogen levels, compared with the normoglycemic group (Fig. 2, b).

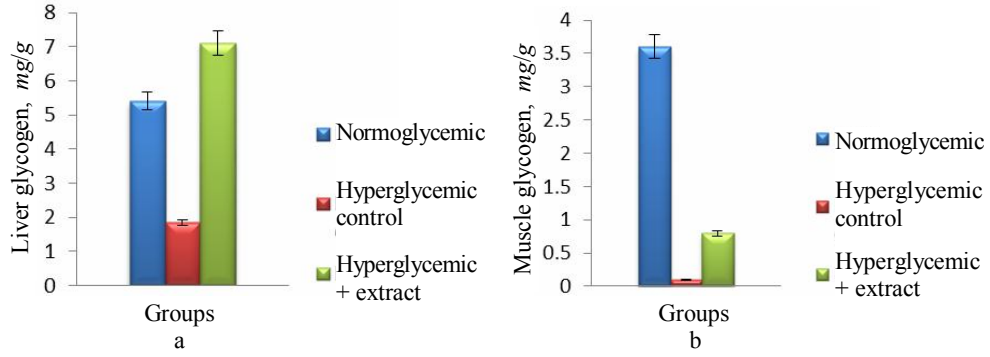

Fig 2. The effects of HM aqueous extract on liver (a) and muscle glycogen (b) levels. Data are represented as mean ± SEM for 3 animals per group.

Our study has shown that, as a result of immobilization, liver and muscle glycogen content was reduced, which could be linked to an inactivation of glycogen synthesis. However, the results showed that administration of HM significantly increased liver glycogen level (Fig. 2, a), compared with untreated hyperglycemic rabbits. It may suggest that administration of HM stimulated insulin secretion from pancreatic β-cells, thereby liver glycogen synthesis increased.

Effect of HM on body weight changes. It is known that HM leads to severe body weight loss. The body weight lowering often is associated with hyperglycemic conditions, as a result of insulin deficiency, which produces degeneration of structural proteins and muscle wasting [19, 20]. During the 15 days of the study period, treatment with HM extract prevented weight loss in hyperglycemic rabbits, whereas untreated hyperglycemic group continuously lost weight (13.6%, $p < 0.01$) (Fig. 3) due to immobilization stress it is compared with the initial day.

However, it was observed that during the experimental period, body weight of HM treated rabbits did not change compared with the initial date (Fig. 3). It is suggested that the HM extract could be protective against the degradation of structural proteins.

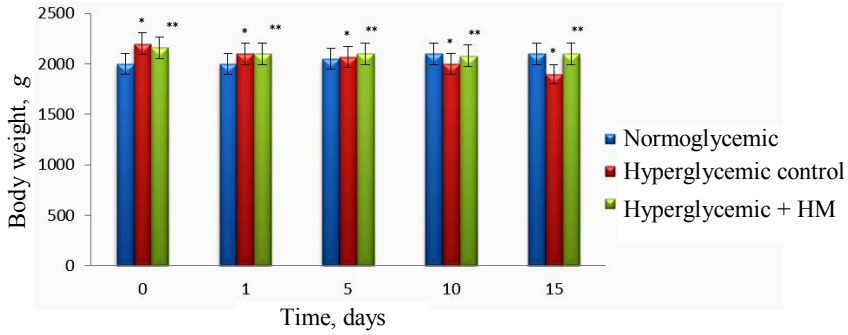

Fig. 3. The effects of HM aqueous extract on the body weights of rabbits. Data are represented as mean ± SEM for 3 animals per group:
* significantly different levels compared with normoglycemic group ($p < 0.01$);
** significantly different levels compared with hyperglycemic control group ($p < 0.01$).

Conclusion. To sum up, the present study has shown the efficiency and safety of the aqueous extract of HM composed of *Salvia officinalis* L., *Calendula officinalis* Linn., *Glycyrrhizae radix* L. and *Echinacea purpurea* L. for treating hyperglycemia. That revealed hypoglycemic activity, improved lipid profile and body weight, restored liver and muscle glycogen levels in hyperglycemia induced by immobilization stress in rabbits. This herbal mixture might be recommended for treating of diabetes mellitus.

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