proceedings

MDPI

Proceedings

Herbs Extracts in the Treatment and Prevention of Experimental Metabolic Disorders: Synergistic Hypoglycemic Activity of Ethanol Extracts of *Hypericum alpestre* and *Rumex obtusifolius* †

Anush Aghajanyan, Mikayel Ginovyan and Armen Trchounian *

Department of Biochemistry, Microbiology and Biotechnology, Faculty of Biology, Yerevan State University, 1 A. Manoogian Str., Yerevan 0025, Armenia; A.Aghajanian@ysu.am (A.A.); mikayel.ginovyan@ysu.am (M.G.)

* Correspondence: Trchounian@ysu.am

† Presented at Natural Products and the Hallmarks of Chronic Diseases—COST Action 16112, Luxembourg, 25–27 March 2019.

Published: 15 April 2019

Abstract: Hyperglycemia is a metabolic condition characterized by high blood glucose level due to the disturbances of carbohydrate, lipids and protein metabolism. These disorders lead to impaired of insulin secretion and β-cell dysfunction. It is known that continuous and long-term use of synthetic anti-diabetic drugs can have side effects including hepatic impairment. Thus, the identifications of potent hypoglycemic agents from natural sources of both medicinal and edible plants with minimum side effects, is desirable. Synergetic hypoglycemic activity in experimental animals (rabbits) was shown with ethanol extracts of a mixture of the plant species *Hypericum alpestre* and *Rumex obtusifolius*. The chemical composition of these extracts was identified by gas chromatography-mass spectrometry analysis. The plant extracts mixture and natural products extracted from medicinal plant sources have potential for treatment and prevention of diabetes mellitus and metabolic disorders.

Keywords: immobilization stress; hyperglycemia; plant extract; Armenian folk medicine

1. Introduction

The diversity of plants in Armenia is due to the singularity of the natural environment. Previous studies have reported that *Rumex obtusifolius* separately possesses significant antihyperglycemic and antihyperlipidemic activities. However, we are interested in synergistic hypoglycemic activity of ethanol extract of a mixture of *Hypericum alpestre* and *Rumex obtusifolius* (HR). Our findings showed that *H. alpestre* and *R. obtusifolius* have high concentration of phenolic compounds, flavonoids and tannins, which might be responsible for hypoglycemic effects [1]. In Armenian folk medicine these species are used as antimicrobial agents [2]. Therefore, the purpose of this study was to investigate the biochemical properties and antihyperglycemic activities of the ethanol extract of the herbal mixture in hyperglycemia induced by immobilization stress in rabbits after 21 days of oral treatment.

2. Materials and Methods

2.1. Plant Material and Preparation of the Extract

Plants have been collected in Armenia according to recommendations. Briefly, *H. alpestre* subsp. *polygonifolium* was harvested in the flowering period. *R. obtusifolius* L. seeds were harvested after

maturation. Plant materials were identified and deposited in the herbarium of Yerevan State University. A voucher specimen for *H. alpestre* was ERCB13206, for *R. obtusifolius*—ERCB13208. The *R. obtusifolius* seeds and *H. alpestre* aerial part were extracted with 40% ethanol for 20 min at 60 °C. After that the extract was filtered and was orally administrated after cooling.

2.2. Study Design and Induction of Hyperglycemia in Experimental Rabbits

Hypoglycemic activity of HR ethanol extract was carried out in rabbits (*Oryctolagus cuniculus domesticus*) with the same sex (1800–2000 g). The experiments were performed in accordance with the ethical norms authorized by "International Recommendation on Carrying out of Biochemical Researchers with use of Animals" and study plan has been approved by the National Center of Bioethics (Armenia).

The animals were kept under standard environmental conditions (temperature 22 ± 2 °C, light/dark cycle of 12 h) and were randomly divided into three groups; I—normoglycemic; II—hyperglycemic control (putting immobilization); III—hyperglycemic experimental (received HR extract). Hyperglycemia was induced by immobilization stress in rabbits for 21 days (5 h daily). Immobilization stress leads to disorder of the endocrine system and lipid metabolism. Moreover, prolonged immobilization stress increases the risk of cardiovascular diseases and atherosclerosis [3].

2.3. Biochemical Analysis

The biochemical analysis was performed to measure the serum level of glucose, total cholesterol (TC), high-density lipoprotein (HDL), law-density lipoprotein (LDL), triglycerides (TG). All parameters were assayed using enzymatic kit. Serum was collected and liver enzymes markers (ALT, AST) were determined by kinetic UV assay using kit. Liver and muscle glycogen contents were also carried out by histopathological examination of tissue samples.

2.4. GC-MS Analysis

Gas chromatography and mass spectrometry (GC-MS) technique (HP 5890 Series II gas chromatograph with HP 5972 Series MS detector) was used for identification of the bio-active constituents from the plants.

3. Results

Our findings indicated that oral administration of HR ethanol extract (300 mg/kg body weight) significantly decreased fasting glucose levels of treated group of animals (66.2%, $p < 0.05$), improving glucose tolerance (29.5%), increasing liver and muscle glycogen contents and corrected of the lipid profile, liver function enzymes compared to the hyperglycemic control group.

After 21 days of oral treatment with the HR ethanol extract, blood lipids parameters demonstrated significantly decreased total cholesterol (66.6%), LDL-cholesterol (64.6%) and TG (70.8%) levels compared to the hyperglycemic control group. There were no differences of HDL-cholesterol level between the treated and the normoglycemic group of animals. Treatment with HR reduced liver enzymes level; ALT and AST (31.8% and 48.6 %, respectively) in comparison with hyperglycemic control group. As the result of immobilization stress, liver and muscle glycogen levels were reduced which might be caused to decrease of glycogen synthase activity due to low insulin level. However, treated animals had increased liver and muscle glycogen level (1.4, 1.2-fold, respectively, $p < 0.01$) compared to the untreated rabbits. The rabbits in the hyperglycemic control group also demonstrated loss of body weight (21.6%, $p < 0.01$) in comparison with the treated group of animals (8.6%).

The *R. obtusifolius* seeds have significant concentration of phenolics, particularly, flavonoids and tannins [1,2]. These biologically active compounds may be responsible for the hypoglycemic effects of HR. Treatment with ethanol extract of herbal mixture single dose for 21 days can correct a carbohydrate and lipids metabolism.

In the extracts of selected tested plant materials, various biologically active compounds were identified by GC-MS technique, which could play important role in total hypoglycemic effect. GC-MS analysis of the extract of *H. alpestre* revealed the presence of 24 compounds. The major compounds with potential biological activities were several phenolic compounds (Catechol (2.9%), guaiacol (0.56%), vanillic acid (3.70%)), some fatty acids (tetradecanoic acid (7.69%)), palmitic acid (9.46%), linolenic acid (17.44%), octadecanoic acid (2.15%), two terpenoids (phytol (0.52%), trans-farnesol (0.48%)), furfural (1.44%) and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (8.62%) compounds. In the extract of *R. obtusifolius* 32 compounds were identified. Some of the identified compounds are considered to possess biological activities. These were linoleic acid (41.32%), cis-vaccenic acid (24.43%), palmitic acid (12.25%), oleic acid (3.65%) 1,2,4-benzenetriol (0.46%), N-[4-bromo-n-butyl]-2-piperidinone (0.64%), (Z)—9-octadecenal (0.53%), methyl linoleate (0.45%), cis-9-hexadecenoic acid (0.40%) and supraene (0.21%). The combination of these compounds is suggested to be responsible to the hypoglycemic effects determined. Possible mechanisms would be revealed.

4. Conclusions

In conclusion, the present study has shown a positive effect of the synergetic action between *H. alpestre* and *R. obtusifolius* plants that contributes to higher antihyperglycemic activity in comparison to *R. obtusifolius.*

Author Contributions: A.A. and M.G. have carried out experiments, prepared the abstract; A.T. supervised the study and edited the abstract.

Funding: This research was funded by Committee of Science, Ministry of Education and Science of Armenia.

Acknowledgments*:* This work is based upon work from COST Action NutRedOx-CA16112 supported by COST (European Cooperation in Science and Technology).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Aghajanyan, A.; Nikoyan, A.; Trchounian, A. Biochemical activity and hypoglycemic effects of *Rumex obtusifolius* L. seeds used in Armenian traditional medicine. *BioMed Res. Int*. **2018**, *2018*, ID4526352.
2. Ginovyan, M.; Petrosyan, M.; Trchounian, A. Antimicrobial activity of some plant materials used in Armenian traditional medicine. *BMC Compl. Altern. Med*. **2017**, *17*, 50.
3. Lim, S.M.; Park, S.H.; Sharma, N.; Kim, S.S.; Lee, J.R.; Jung, J.S.; Suh, H.W. Blood glucose regulation mechanism in depressive disorder animal model during hyperglycemic states. *Brain Res. Bull.* **2016**, *12*, 116–122.