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REGULAR ARTICLE

EFFECT OF ISCADOR ON SELECTED PARAMETERS OF THE METABOLIC BLOCK IN THE ANIMAL TYPE DIABETES INDUCED BY ALLOKSAN

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ABSTRACT

Diabetes is a disease which induces changes in the metabolism of the whole organism. Alloksan is a compound which damages β cells of the islets of Langerhans within the pancreas inducing experimental diabetes in animals. The cytotoxic action of alloksan can affect other types of cells leading to their injuries. The known treatment of diabetes has not resulted in its total cure so far.

Numerous experiments are carried out in order to find substances of preventive effect as well as substances which can relieve the negative impact of this disease. In this study the effect of iscador the substances of wide-range antioxidative and immunostimulating action on selected metabolic parameters during the course of diabetes in mice is presented.

The experiments were carried out on male mice, average body weight 25 – 26g, bred in the constant light conditions LD 12:12 and fed with standard diet with unlimited access to water. The concentration of glucose, cholesterol and triglycerides was estimated in the blood serum with STAMAR kits. The statistical analysis of the results was carried out with Statistica program version 8.

The results indicate that the application of iscador reduced glucose concentration, cholesterol and triglyceride concentrations in blood serum of mice with induced experimental diabetes.

Keywords: Diabetes, Iscador, Glucose, Cholesterol, Triglycerides

INTRODUCTION

Numerous factors can influence the risk of the occurrence of diabetes type 1. The most important seem to be virus infections, mother age above 40 years at the time of childbirth, early inclusion of cow milk in the diet, exposure to toxic substances (among others N-nitrozo derivatives) and other stress factors **(Bingley et al., 2000; Soltesz, 2003)**. The low serum 25-hydroxyvitamin D (25(OH)D) concentrations are associated with increased risk of developing type 2 diabetes mellitus (DM) **(Grimnes et al., 2010)**. The aim of the conservative treatment of diabetes type 1 is the metabolic control leading to the maintenance of the optimal values of blood glucose level and indicators of the lipid metabolism which represent parameters defining the so-called metabolic block. However, progressively wider knowledge concerning the pathogenesis of diabetes type 1 increases the spectrum of studies concerning the prevention and relieve of symptoms of the disease. The studies include: 1 – the application of immunosupressive drugs cyclosporine **(Bougneres et al., 1990)**; nicotinamide **(Gale et al., 2004)**; azathioprine **(Silvestrin et al., 1988)**; interleukin 2 **(Kuzel, 2000)**; 2 – pancreas transplants **(Gruessner and Sutherland, 2005)**; 3 – pancreas islets transplants **(Korsgren et al., 2005)**; 4 – designs of an artificial pancreas **(Deiss et al., 2006)**. It has been also found that reactive oxygen species (ROS) act as intermediaries in the development of diabetes induced by the administration of alloksan to laboratory animals. The selective action of this compound on the β cells of Langerhans islets of the pancreas is a result of three factors. At the beginning, β cells absorb alloksan. Then, it enters, together with intracellular reductors (mainly ascorbic acid, reduced glutathione, cysteine and thiol groups of proteins) and thioredoxin (participating in the biosynthesis of insulin), the redox cycle. This leads to the production, in the first phase, of superoxide radical anion (O2•), and then to the occurrence of other ROS. As a result, the produced reactive oxygen species affect β cells which are poorly protected by enzymes and antioxidants **(Szaleczky et al., 1999)**. Hyperglycemia and ketosis interfere with the metabolism and lead to the decrease in the antioxidative resistance of the organism.

Therefore, it seems that the preventive administration of the 75 plant compounds of antioxidative properties can efficiently protect an organism against pathological changes known as the metabolic block. Literature data point to the protective action of tocopherols and tocotrienols (vitamin E), ascorbic acid (vitamin C) and polyphenols (anthocyanins and flavonoids) **(Foti et al., 1996; Kakhonen et al., 1999; Kalt et al., 1997; Leja et al., 2003)**. Iscador is a water extract obtained from the common mistletoe parasitizing on the oak (Iscador Qu), apple tree (Iscador M), pine (Iscador P) and elm (Iscador U). Many biologically active substances isolated from iscador, such as viscumins (lectins), viscotoxins and flavonoids seem to have the greatest therapeutic significance. Studies carried out on different lines of cancer cells proved the cytotoxic effect of iscador on transformed cells and the simultaneous lack of or significantly lower toxicity to normal cells **(Park et al., 1999)**. It has been found that viscumin ML-I, apart from the cytotoxic activity directed mainly at cancer cells, exhibits a wide-ranging immunostimulating action which leads to an increase in the release of inflammatory cytokines such as TNF-α, IFN-γ, IL-2, IL-12, IL-6 **(Gorter et al., 2003)**. Additionally, iscador shows antioxidative properties **(Greń et al., 2009)**.

On the basis of the above facts, target of this study was to monitor the effect of iscador Qu, rich in viscumins and flavonoids, on selected parameters indicating changes in an organism during diabetes type 1.

MATERIAL AND METHODS

Experimental animals

The experiments were carried out on male mice (n=32), average body weight 25 – 26g, bred in the constant light conditions LD 12:12 and fed with standard diet with unlimited access to water. All the experiments were performed with the acceptance (No. 36/2010) of the Local Ethical Committee, Cracow, Poland.

Experimental design

The animals were divided into four groups (8 animals each) one control and three experimental groups. The control mice were administered physiological salt. The first experimental group was administered iscador Qu at 5 mg/kg body weight (b. w.) for 4 days, the second group was administered alloksan at 75 mg/kg b. w. for 4 days, while the third

group was administered both iscador Qu at 5 mg/kg b. w. and alloksan at 75 mg/kg b. w. also for 4 days. All injections were administered intraperitoneally (i. p.) at the volume of 100 µL. Thirty minutes after the fourth injection animals were anaesthetized and decapitated. The blood samples were collected from the carotid artery.

Biochemical analysis

The concentrations of glucose, cholesterol and triglycerides were estimated in the blood serum with STAMAR kits. Glucose estimation was based on the enzymatic reaction of glucose with oxygen catalyzed by glucose oxidase. The reaction leads to the formation of D-gluconate and hydrogen peroxide. Hydrogen peroxide in the presence of peroxidase reacts with phenol and 4-aminoantipyrine producing chinone dye and water. The intensity of the colour spectrophotometrically at the wavelength 500 nm was measured. The estimation of cholesterol was based on the reaction of cholesteryl ester with water. The reaction was catalyzed by cholesterol esterase. As a result of this reaction cholesterol and fatty acids are produced. At the second stage, cholesterol is oxygenised by cholesterol oxydase producing 4-cholestenon-3 and hydrogen peroxide. Hydrogen peroxide reacts with the participation of peroxidase with phenol and 4-aminoantipyrine. As a result, chinone dye and water are produced. The intensity of the color was estimated spectrophotometrically at the wavelength 500 nm.

The enzymatic estimation of triglycerides involves three stages. First, lipase hydrolyses triglycerides to glycerol and fatty acids. Then, glycerol, at the presence of glycerol kinase and ATP, is phophorylised to 3-P-glycerol. 3-P-glycerol oxidase (GPO) catalyses the formation of hydrogen peroxide which reacts with p-chlorophenol and 4-aminoantipyrine (4-AA) producing a coloured complex. The intensity of the colour is directly proportional to the concentration of triglycerides. All the spectrophotometric measurements were performed using Marcel s 330 spectrophotometer (Marcel Poland).

Statistical analysis

The statistical analysis of the results was carried out with Statistica program version 8. The distribution was tested using Shapiro-Wilk test. Differences between consecutive groups were analysed using one-way ANOVA followed by post hoc analysis with Tukey test.

Statistical significance was defined at $p<0.05$. The data are expressed as mean±SD (standard deviation).

RESULTS

During the experiments, a statistically significant increase in the concentration of glucose, cholesterol and triglycerides in the animal model of diabetes type 1 (induced by the administration of alloksan) was found ($p<0.001$). However, the administration of iscador caused a decrease in the concentration of the studied substances in all experimental groups. It was also observed that simultaneous administration of iscador limits the negative effects of alloksan on the concentration of glucose, cholesterol and triglycerides. In animals from the control group, the concentration of glucose was 6.04±0.38 mmol/L. The highest and statistically significant increase ($p<0.001$) of 67% was observed after the administration of alloksan (10.10±0.23 mmol/L). However, after the injection of iscador Qu a decrease of 42% in the concentration of glucose in comparison with the 149 control values was found ($p<0.001$). Moreover, it was observed that combined administration of iscador Qu and alloksan caused an increase of 41% (8.56±0.57 mmol/L) in the concentration of glucose in comparison with the control animals ($p<0.001$). On the other hand, the average concentration of glucose in animals from this experimental group was lower in comparison with the values for the experimental group treated only with alloksan ($p<0.001$) (Figure 1,Table 1).

The analysis of the average concentration of cholesterol showed that the administration of alloksan caused a statistically significant increase in its concentration (6.45±0.39 mmol/L) in comparison with the values for the control group (4.97±0.26 mmol/L) ($p<0.001$). The injection of iscador decreased the concentration of cholesterol by 21.3% (3.91±0.26 mmol/L) in comparison with the control ($p<0.001$). It was found that the combined administration of iscador and alloksan caused an increase in the concentration of cholesterol (5.74±0.26 mmol/L) in comparison with the control values ($p<0.01$). On the other hand, the increase was lower comparing to animals treated with alloksan exclusively ($p<0.01$) (Figure 1, Table 1). Similar tendencies were noted in the triglyceride concentrations. The highest increase ($p<0.05$) in the concentration of triglycerides was observed after the administration of alloksan (2.43±0.04 mmol/L), and lower and insignificant increase after the combined administration of iscador and alloksan (2.31±0.04 mmol/L) in comparison with the control (2.21±0.05 mmol/L). However, after the administration of iscador a significant

(p<=0.001) decrease of 32.6% (1.49±0.09 mmol/L) was observed in comparison with the concentration of triglycerides in the control animals (Figure 1,Table 1).

Tab 1 Concentrations of glucose, cholesterol and triglycerides in blood serum (mmol/L±SD) after administration of iscador and alloksan

Control	Iscador	alloksan	iscador+alloksan
	Glucose		
6.04±0.38	3.50±0.21***	10.10±0.23***	8.56±0.57***
	Cholesterol		
4.97±0.26	3.91±0.26***	6.45±0.39***	5.74±0.26**
	Triglycerides		
2.21±0.05	1.49±0.09*	2.43±0.04***	2.31±0.04

* differences statistically significant compared to the control group at $p<0.05$

**differences statistically significant compared to the control group at $p<0.01$

***differences statistically significant compared to the control group at $p<0.001$

DISCUSSION

In physiological conditions, a dynamic equilibrium between the production of reactive oxygen species (ROS) and the antioxidative abilities of an organism exists. This protects against injuries to DNA, cell structure and its functions, tissues and organs. The increased production of free oxygen radicals and changes in antioxidative system results in imbalance in oxidoreductive equilibrium which can lead to the oxidative stress. Oxidative stress plays an important role in the pathogenesis of many diseases, among others in diabetes. Our earlier studies concerning the oxidoreductive properties of iscador pointed to its strong antioxidative properties. It was shown that this substance neutralised reactive oxygen species in cells **(Greń, 2009)**.

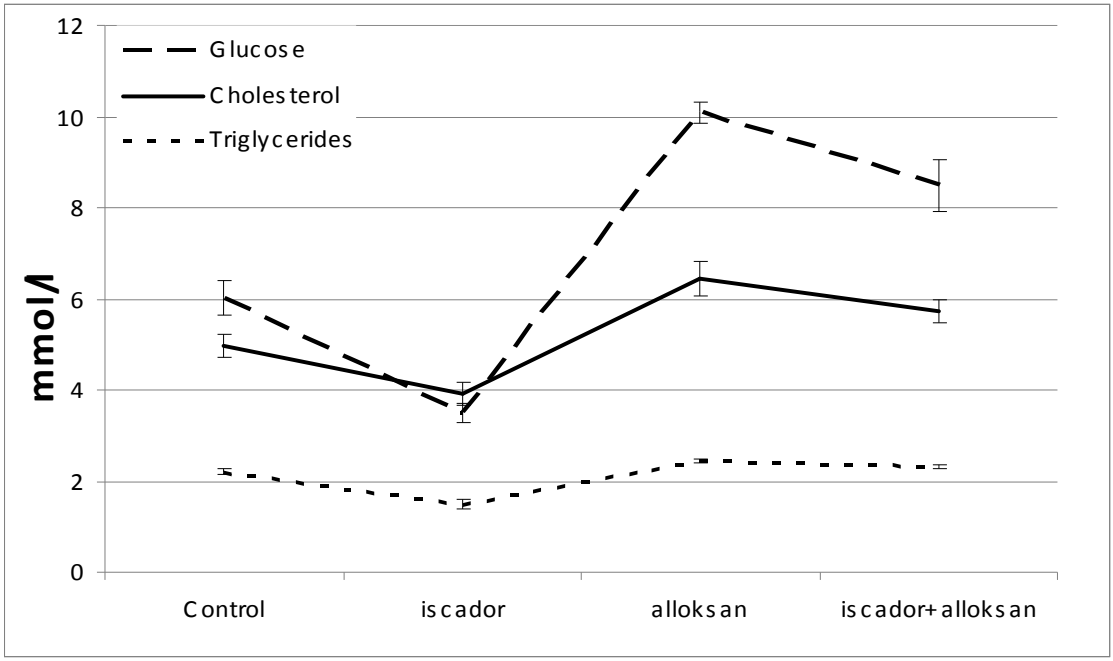

Fig 1 Concentrations of glucose, cholesterol and triglycerides in blood serum (mmol/L±SD) after administration of iscador and alloksan

Iscador stimulates immune system and antioxidative system (total antioxidant status; TAS) on intracellular level **(Greń, 2009)**. The neutralisation of free radicals is essential to prevention of diabetes because ROS play a role in the decrease of tissues sensitivity to insulin. Additionally, iscador improves the functioning of liver, an organ which, apart from the pancreas, plays an important role in the metablism of glucose and insulin in the organism. Liver is the place where insulin is metabolised. Oxidative stress mostly affects lipid membranes. Oxidation of the lipoproteins of the vessel wall leads to the formation of lipid superoxides. Glucose catalyses this reaction. Intensive oxygenation reactions cause the peroxidation of lipids which stimulates phospholipase, the cycle of the metabolism of arachidonic acid, and consequently an increase in the activity of cyclooxygenase and lipooxygenase. Free radicals also damage other membrane enzymatic systems which results in the influx of Ca^{2+} ions into the cell and the activation of proteases and phospholipases which increases the dysfunction of the cell membrane and membrane receptors. Hence, biologically active substances present in iscador and exhibiting hypoglycemic effects in animals with the experimental diabetes can also play a protective role towards biological membranes.

Plants are a source of a number of biologically active chemical 199 compounds which due to the non-specific reaction with the surface of cells of the immunological system can, somehow “accidentally” activate cells (for example, some plant polysaccharides can be

identified by lectin cell receptors). Aglutinin *Viscum* – VAA *(Viscum album aglutinin)* induces the expression of genes for cytokinins IL-1, IL-6, IL-12, TNF-α **(Harmsma et al., 2004)**. Free fatty acids released from the fat tissue enter the blood of the hepatic portal vein. They enter the liver inducing the production of lipoproteins of a very low density (VLDL, *very low-density lipoproteins*) and in this way cause hypertriglyceridemia. Dyslipidemia and uric acid metabolism disorders are characteristic for the insulin resistance syndrome **(Wilcox et al., 2005)**. The obtained results point to the potential participation of iscador in the process of lowering the concentration of triglycerides and, thus, to its potential preventive action protecting the organism against the negative effects of diabetes. **Gray and Flatt (1999)** showed that water extract from the mistletoe stimulated the secretion of insulin by β cells of the pancreas. The results of these investigations showed that the stimulation of insulin secretion was not due to the action of lectins because they lost their activity in extracts treated with heat. Hence, it can be suggested that the components of iscador which are resistant to heat, mainly viscotoxins and flavonoids, influence the glucose metabolism. Modulating effect of iscador on the insulin action is based on phosphorylation/dephosphorylation processes. It is suggested that insulin stimulates MAP2K (MAP kinase kinase) which through the phosphorylation cascade activates the ribosomal protein S6 (the component of 40S subunit) which, in turn, is an inhibitor of autophage processes in the cell **(Blomaart et al., 1995)**. During our preliminary studies it was observed that iscador administration limits the negative effects of experimental diabetes induced by alloksan. This effect was reflected by a decrease in the value of selected indicators of the laboratory diabetes. Hence, studies aiming at the application of iscador as a substance showing the preventive action and/or limiting the symptoms of diabetes should be continued.

CONCLUSIONS

Results of this study clearly indicate that application of iscador during the animal type diabetes results in decrease in the concentration of glucose, triglycerides and cholesterol. Thus it is suggested that iscador may be useful in attenuation of some diabetic symptoms and prevention of health complications occurring in diabetes.

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