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# THE USE OF RESVERATROL AS BIOLOGICAL ACTIVE SUBSTANCE IN STOPPAGE OF PANCREATIC β-CELLS DEGENERATION

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**ABSTRACT**

Nowadays is supported the idea that diabetes has a stage evolution where clinical manifest form is actually the last from those four expressivity stages of this disease. In the final stage, metabolic disorder stands at the most advanced degree, and destruction of β-cells rises above 80%. The fast growth of population morbidity affected by diabetes mellitus enforces identification of optimal solutions in creation and diversification of new pharmaceutical drugs with predetermined functional properties. In this way, achievements by modern biotechnologies lead the attention of today's researchers to use resveratrol as biological active substance from natural source due to high qualities that he owns.

**Key words:** modern biotechnologies, resveratrol, biological effects, degeneration.

## Introduction

The modern biotechnologies, based on cellular and biomolecular processes, develop technologies and products that help improve our lives and the health of our planet. Recent developments in biotechnology include genetically modified plants and animals, nanotechnology and cell therapies. These include applications in various fields: from agricultural practice to the medical sector.

Diabetes mellitus (DM) is a complex metabolic disease characterized by high concentration of blood glucose level and increased insulin resistance (3). It is considered a state of free radicals overproduction due to metabolic stress, as a result of alterations in energy metabolism, elevated non-enzymatic and auto-oxidative glycosylation, inflammatory mediators levels and the status of antioxidant defense (5).

Pancreatic β-cells represent up to 70% of cells in islets of Langerhans, and play a major role in controlling glucose homeostasis by secreting the hormone insulin (8). Nowadays is supported the idea that diabetes has a stage evolution where clinical manifest form is actually the last from those four expressivity stages of this disease. In the final stage, metabolic disorder stands at the most advanced degree, and destruction of β-cells rises above 80%.

Type 1 DM results from damage of pancreatic insulin producing β-cells by autoimmune system through the action of proinflammatory cytokines, while type 2 DM (non-immunogenic type) results from insulin resistance with chronically elevated blood glucose levels that lead to pancreatic β-cell dysfunction and chronic complications, such as: retinopathy, nephropathy and neuropathy (1, 5, 8).

At present, numerous studies are focusing on pharmaceutical drugs production to find more efficient ways of combating different diseases. In this way, a great attention has been attracted to natural compounds due to their many biological effects.

Resveratrol, as a natural compound, performs different therapeutic functions, such as: antioxidant, antitumor, antidiabetic, antiviral and also cardioprotective, neuroprotective, radioprotective activities. It's considered by many researchers as the potential candidate of maintaining human health with non-toxic and well-tolerable properties (9).

Resveratrol (3,4',5 – trihydroxystilbene) is a naturally occurring phytoalexin found in a variety of fruits, such as: grapes, peanuts, cranberries, blackberries, pomegranates etc. For plants, this phenolic compound shows a protective role against microbial and fungal infections, injuries, heavy metals and UV radiations (6).

Being a potential candidate with different pharmacological activities, this natural compound has been extensively studied in diabetes therapy. It is reported that resveratrol eliminates free radicals, minimizing possible radical damage in different types of human tissues (11). This biological active substance protects pancreatic tissue by reducing the expression of inflammatory factors which regulates numerous genes in β-cells (8). Furthermore, it decreases blood glucose levels due to the activation of AMPK – a master regulator of metabolism and

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the lowering expression on hepatic glucose production, as well as on oxidative stress (4).

The present study aims to evaluate the effects of resveratrol on blood glucose level and plasma insulin concentration, as a beginning step in stoppage of pancreatic β-cells degeneration in alloxan induced diabetic rats.

## Materials and method

For this investigation were taken 40 Wistar albino rats, of both sexes with a body weight between 135-175g, which were randomly divided into the following four experimental groups (n=10):

Group I (control group): with physiological solution (0,9% NaCl) administration of 1 ml per animal in the intraperitoneal cavity.

Group II (alloxan group): with alloxan administration in the proportion of 200 mg/ 1 kg body weight, that is diluted in 1 ml of physiological solution per animal in the intraperitoneal cavity. Intraperitoneal injection with alloxan is a common used method in inducing of animal diabetic model.

Group III (resveratrol group): with oral administration of resveratrol extracted from *Vitis vinifera*, in the proportion of 0,015g diluted in 0,3ml distilled water per animal. Oral administration is a route of administration where this biological active substance was taken by investigated rats through the mouth.

Group IV (alloxan+resveratrol group): with intraperitoneally alloxan administration followed by oral administration of resveratrol extracted from *Vitis vinifera*.

Alloxan is a cyclic-urea derivative, which has a highly cytotoxic effect on pancreatic β-cells. It is a diabetogenic agent, being used in a lot of scientific studies as a tool to induce experimental diabetes on animals. In this investigation, alloxan was administered to the group II, for a total of 20 days (diabetic standard group, in which diabetic rats didn't receive any treatment), while to the group IV – for 10 days, followed by 14 days of resveratrol treatment (mixed group). After 3-4 days of alloxan administration appeared typical initial diabetes symptoms: polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger), the investigated animals being hyperglycemic, glycosuric and ketoacidotic.

Resveratrol powder, obtained from grapes skin and seeds, was orally administered to the group III and IV, at the same time for two weeks.

At the end of experimentation, the blood from investigated rats was collected into tubes containing heparin as anticoagulant, and was separated the plasma fraction by centrifugation (3000 rotations per minute). Insulin level was quantitative determined using ELISA method (Enzyme-Linked Immunosorbent Assay). ELISA is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule.

The significant differences between the two groups were analyzed with Student's t – Test, being considered statistically significant if $p<0,05$. Results are expressed as the mean ± standard deviation.

## Results and discussion

It is known that intraperitoneal injection of a single alloxan dose (200 mg/ kg) is shown to produce a modest rise in blood glucose level in rodents due to the action of this diabetogenic agent on the pancreas that consists of: directly disruption of β-cell membrane permeability and irreversible β-cell damage within 12 hours (10). After injection is seen a triphasic blood-glucose response: an initial hyperglycemia (at 1-4 h), followed by a profound hypoglycemia (6-12 h) and finally a persistent hyperglycemia (after 24 h) with a reducing impact on the plasma insulin level (Cooperstein and Watkins, 1981).

In this way, alloxan has the ability to decrease glucose-mediated insulin secretion and to induce selective necrosis of β-cells, which therefore makes this cyclic-urea derivative useful to study the required effects in a diabetic condition (7).

At the end of experimentation, blood samples of each group were collected for detection and subsequent analysis of glucose and insulin levels.

It is evident from the tab.1, that blood glucose level in the alloxan group was markedly higher (9,64±0,27 mmol/l) compared with the control group (4,93±0,21 mmol/l). In organism takes place a deterioration of cells ability to assimilate glucose and to use it for physiological needs.

**Table 1** – Blood glucose level (mmol/l) in alloxan induced diabetic rats with administration of resveratrol extracted from *Vitis vinifera* (*p<0,05; **p>0,05)

| **Blood glucose level (mmol/l)** | | | |
|---|---|---|---|
| **Group I** n=10 | **Group II** n=10 | **Group III** n=10 | **Group IV** n=10 |
| Control | Alloxan | Resveratrol | Alloxan+ Resveratrol |
| 4,93±0,21 | 9,64±0,27* | 5,34±0,32** | 6,21±0,18* |

Resveratrol extracted from *Vitis vinifera* has been shown an relative improvement on glycemic index (6,21±0,18 mmol/l) in the mixed group (Fig.1).

The decreased expression of blood glucose level in diabetic rats treated with resveratrol can be argued through the antioxidant property of this biological active substance which results in stoppage of free radicals' eliberation by alloxan. This natural compound also proved a stimulatory effect on the glucose absorption by hepatocytes and adypocytes (3).

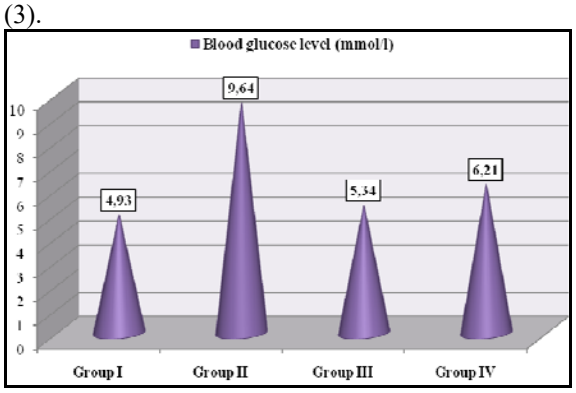


**Fig. 1 –** Influence of resveratrol on blood glucose level (mmol/l) in experimental diabetes

No statistically significant differences were identified between the resveratrol group and control group ($p>0,05$).

The present study also examined the levels of hormone insulin in alloxan-treated rats based on resveratrol administration (Tab.2). The results obtained by ELISA method, showed a decreased expression (0,68±0,11 pmol/l) of plasma insulin concentration in the group II. Insufficient insulin secretion has not only a negative metabolic consequence but also tends to ulterior pancreas β-cell exhaustion (2).

The interdependent relation between high glucose level and low insulin concentration in diabetes can be seen through the contribution of altered β-cell glucose metabolism to dysregulate insulin secretion (1).

**Table 2** – Plasma insulin concentration (pmol/l) in alloxan induced diabetic rats with administration of resveratrol extracted from *Vitis vinifera* (*p<0,05; **p>0,05)

| **Plasma insulin concentration (pmol/l)** | | | |
|---|---|---|---|
| **Group I** n=10 | **Group II** n=10 | **Group III** n=10 | **Group IV** n=10 |
| Control | Alloxan | Resveratrol | Alloxan+ Resveratrol |
| 2,23±0,18 | 0,68±0,11* | 1,95±0,17** | 1,51±0,14* |

Diabetic rats from the group IV, following the respective phytotherapy, demonstrated a partial recovery of pancreatic β-cells function by regulating the level of hormone insulin in plasma until 1,51±0,14 pmol/l compared with the control group ($p<0,05$).

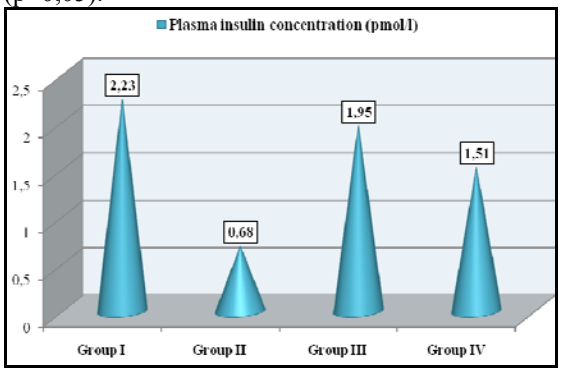


**Fig. 2** – Influence of resveratrol on plasma insulin concentration (pmol/l) in experimental diabetes

These data suggested that resveratrol may significantly contribute in prevention of beta-cell apoptosis due to its antidiabetic activity and associated metabolic effects.

As illustrated in fig.2, the group III of investigated animals reached a normal plasma insulin concentration (1,95±0,17 pmol/l) due to the well-tolerable property of this natural compound.

## Conclusions

The fast growth of population morbidity affected by diabetes mellitus enforces identification of optimal solutions in creation and diversification of new pharmaceutical drugs with predetermined functional properties. In this way, achievements by modern biotechnologies lead the attention of today's researchers to use resveratrol as biological active substance from natural source due to high qualities that he owns.

Based on the analysis of the obtained results in rat experimental model, can be concluded that resveratrol possesses protective effects against hyperglycemia and insulin resistance, which are the most interconnected processes with pancreatic β-cells degeneration. Respectively, this natural compound could be appreciated as a possible well-tolerable adjuvant in diabetes therapy.

## Rezumat

Actualmente se consideră că diabetul cunoaște o evoluție stadială, în care forma clinică manifestată este de fapt ultima etapă din cele patru stadii de expresivitate a acestei boli. În etapa finală, tulburarea metabolică se află în gradul cel mai avansat, iar distrugerea celulelor β-pancreatice

depășește 80%. Creșterea rapidă a morbidității populației afectată de diabet zaharat impune identificarea unor soluții optime în crearea și diversificarea de noi preparate farmaceutice cu proprietăți funcționale predeterminate. În acest sens, realizările biotehnologiilor moderne îndreaptă atenția cercetătorilor din prezent spre utilizarea resveratrolului ca substanță biologic activă, de origine naturală, datorită calităților deosebite pe care acesta le posedă.

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