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PROTECTIVE EFFECT OF 1-DEOXYNOJIRIMYCIN AND RESVERATROL AGAINST DIABETIC OXIDATIVE STRESS AND INFLAMMATION

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SUMMARY:Diabetes mellitus (DM) was induced in groups II, V, and VI. Animals III experimental group was administered 1-deoxynojirimycin (DNJ) in dose 0,75 mgKg-1 body weight/day for 14 days. *Mice IV experimental group was administered resveratrol (RSV)* in dose 30 mgKg-1 body weight/day for 14 days. Animals group V: diabetic group receiving *DNJ*, group VI: diabetic group receiving *RSV. Thirty minutes after the last injection animals were anaesthetized and decapitated. Serum level of interleukin-6 (IL-6) was assessed as surrogate markers of inflammation. The livers levels of reduced glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) activity were measured as surrogate markers of oxidative stress. Serum level of insulin and plasma glucose levels were assessed as surrogate markers of diabetes. The administration of DNJ or RSV significantly restored glucose, insulin, IL-6, GSH levels and SOD, CAT, GPx activity in the diabetic mice to near control. The results demonstrated that DNJ and RSV with its antidiabetic, antiinflammation and antioxidant properties could be a potential herbal medicine in treating diabetes and hepatic problems.*

Keywords: *oxidative stress, inflammation, 1-deoxynojirimycin, resveratrol, diabetes.*

INTRODUCTION

Diabetes mellitus (DM) is characterized by chronic hyperglycemia, resulting from defects in insulin secretion, action, or both, leading to disturbance in carbohydrate, lipid and protein metabolism. This state induces immediate oxidative stress (Jakus, 2000;

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King and Loeken, 2004). One of the therapeutic approaches for decreasing postprandial hyperglycemia is to retard absorption of glucose by the inhibition of carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidases, in the digestive system organs (Deshpande et al., 2009). Renewed attention in recent decades to alternative medicines and natural therapies has stimulated a new way of research interest in traditional practices. The plant kingdom has become a target for the search for new drugs and biologically active compounds. Many plant extracts and plant products have been shown to possess significant antioxidant activity, which may be an important property of medicinal plants. The mulberry leaves have high nutritional and medical values, and they contain active ingredients, such as 1-deoxynojirimycin and resveratrol (Yagi et al., 1976; Zhao et al., 2005).

The 1-deoxynojirimycin (DNJ) was first isolated from its roots by Yagi *et al.* in 1976. DNJ is a glucose analogue with a secondary amine group instead of an oxygen atom in the pyranose ring of glucose. DNJ potently inhibits α-glucosidase in the small intestine by binding to the active center of α-glucosidase (Junge et al., 1996). More DNJ has also been found in the leaves and fruits of *Morus alba* L. (Asano et al., 1994; Asano et al., 2001). DNJ inhibits postprandial hyperglycemia (Junge et al., 1996), by inhibiting α-glucosidase in the small intestine. There have been findings from animal studies supporting the hypothesis that mulberry leaves delay the onset of diabetes, as indicated by the fact that a rapid increase in the postprandial blood glucose level was inhibited (Hikino et al., 1985; Kong et al., 2008; Kimura et al., 1995).

Resveratrol (RSV; 3,5,4'-trihydroxystilbene) is a phenolic phytoalexin found in grapes, grape juice, red wines, peanuts, berries of *Vaccinum* species, including blueberries, bilberries, cranberries and *Morus alba.* Resveratrol has intracellular antioxidant activity and activates SIRT1, a NAD+-dependent histone deacetylase involved in mitochondrial biogenesis and the enhancement of peroxisome proliferator-γ-activated receptor coactivator-1α (PGC-1α) and FOXO activity. The anti-diabetic, neuroprotective and anti-adipogenic actions of resveratrol may be mediated via SIRT1 activation (McAnulty et al., 20913).

The present study was designed to evaluate the antihyperglycemic, antiinflammation and antioxidant action of 1-deoxynojirimycin (DNJ) and resveratrol (RSV) on diabetic mice.

MATERIAL AND METHODS

The experiments were carried out 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. All the experiments were performed with the acceptance (No. 122/2010) of the Local Ethical Committee, Cracow, Poland.

The animals were divided into six groups. Control group (C) (I group) received an intraperitoneal (i.p.) injection of 0.1 mol/L sodium citrate buffer (pH 4.5).

Diabetes mellitus (DM) was induced in groups II, V, and VI and received a single i.p. injection of sreptozotocin - STZ, 60 $mgKg^{-1}$ body weight, freshly dissolved in 0.1 M citrate buffer (pH 4.5). DM was verified by measuring blood glucose in tail nick blood samples. Mice with non fasting blood glucose levels of ≥20 mmol/L after 48 h of STZ injection or greater and symptoms of polyuria, polyphagia, and polydipsia were considered diabetic. Animals III experimental group was administered 1-deoxynojirimycin

(DNJ) in dose 0,75 $mgKg^{-1}$ body weight/day for 14 days. Mice IV experimental group was administered resveratrol (RSV) in dose 30 $mgKg^{-1}$ body weight/day for 14 days. Animals group V: diabetic group receiving DNJ in dose 0,75 $mgKg^{-1}$ body weight/day for 14 days. Group VI: diabetic group receiving RSV in dose 30 $mgKg^{-1}$ body weight/day for 14 days. Animals were orally fed by gastric intubation with 1-deoxynojirimycin and resveratrol.

Synthetic 1-Deoxynojirimycin hydrochloride, synonym: 1,5-Dideoxy-1,5-imino-D-sorbitol hydrochloride (DNJ), resveratrol (RSV), synonym: 3,4′,5-Trihydroxy-*trans*-stilbene, 5-[(1E)-2-(4-Hydroxyphenyl)ethenyl]-1,3-benzenediol and streptozotocin (STZ) were purchased from Sigma-Aldrich Corp. St. Louis, MO, USA. STZ-induced diabetic (STZ-DM) mice were used as the severe insulin-deficient diabetic model. STZ is widely used as a strong inducer of diabetes in the experimental animals (Rerup, 1970). STZ selectively destroys the pancreatic cells that secrete insulin, inhibits syntheses and the release of insulin, and produces DM (Gilman et al., 1990). STZ-induced hyperglycemia has been described as a useful experimental model to study the activity of hypoglycemic agents (Ledoux et al. 1986).

Thirty minutes after the last injection animals were anaesthetized and decapitated. The blood samples were collected from the carotid artery. All blood samples were processed immediately and frozen at minus 70 degrees centigrade until assay. Serum level of interleukin-6 (IL-6) was assessed as surrogate markers of inflammation. The livers were homogenized with a homogenizer (Ultra Turrax T25, Rose Scientific Ltd., Edmonton, Canada) in 10 volumes of a 50 mM sodium phosphate buffer (pH 7.4) at 4 °C. Homogenates were centrifuged (Beckman, U.S.A.) at 15000*g* for 10 min, and the supernatant obtained was used for the following antioxidant enzyme measurements. The supernatant were collected and stored at −70°C for further analysis. Levels of reduced glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) activity were measured as surrogate markers of oxidative stress. Serum level of insulin and plasma glucose levels were assessed as surrogate markers of diabetes.

Total GPx (EC 1.11.1.9) activity in liver tissues was determined according to Paglia and Valentine's method (Paglia and Valentine, 1967). The enzyme solution was added to a mixture containing hydrogen peroxide and glutathione in 0.1 mM Tris buffer (pH 7.2) and the absorbance at 340 nm was measured. Activity was evaluated from a calibration curve, and the enzyme activity was defined as nmoles of NADPH oxidized per mg protein per min. Total SOD (EC 1.15.1.1) activity was determined by the inhibition of cytochrome c reduction, according to Flohe and Otting method (Flohe and Otting, 1984). The reduction of cytochrome c was mediated by superoxide anions generated by xanthine/xanthine oxidase system and monitored at 550 nm. One unit of SOD was defined as the amount of enzyme required to inhibit the rate of cytochrome c reduction by 50%. Total CAT activity was based on that of Aebi (Aebi, 1984). In brief, the reduction of 10 mM H_2O_2 in 20 mM of phosphate buffer (pH 7.0) was monitored by measuring the absorbance at 240 nm. The activity was calculated using a molar absorption coefficient, and the enzyme activity was defined as nmoles of dissipating hydrogen peroxide per mg protein per min. The concentration of GSH in hepatic tissues was estimated by evaluating free-SH groups, using the 5, 5-dithiobis-2-nitrobenzoic acid (DTNB) method as described by Sedlak and Lindsay (Sedlak and Lindsay, 1968).

The protein content of hepatic tissue extract was analyzed using the Bradford pro-

tein assay (Bradford, 1976) using the protein-dye kit (Bio-Rad, Richmond, CA, USA). A commercially available bovine serum albumin (Sigma Chemical, St Louis, MO, USA) was used as a standard solution. Changes in optical density were monitored at 595nm.

Plasma insulin was detected by an ELISA kit (Mercodia AB, Sylveniusgatan 8A, SE-754 50 Uppsala, Sweden). The level IL-6 was measured by ELISA using cytoscreen immunoassay kits (Peprotech, Rocky Hill, NJ, USA).

The results were expressed as means ± standard deviation. The statistical analysis of the results was carried out with Statistica program version 9. The distribution was tested using Shapiro-Wilk test. Differences between consecutive groups were analyzed using one-way ANOVA followed by post hoc analysis with Tukey test. Statistical significance was defined at $P<0.05$.

RESULT AND DISCUSSION

Under the diabetic condition, increase glucose levels are significant sources of free radicals and inducers of oxidative stress. The administration of 1-deoxynojirimycin at dose 0,75 $mgKg^{-1}$ body weight/day and resveratrol at dose 30 $mgKg^{-1}$ body weight/day to STZ-induced diabetic mice led to significant decreases in the levels of glucose versus DM group. The untreated diabetic animals showed significantly raised serum glucose levels and reduced serum insulin concentration as compared to the control group. The administration of DNJ or RSV however, significantly restored glucose and insulin levels in the diabetic mice to near control ($P < 0.05$),(Table 1).

Table 1. Effect of oral administration of DNJ and RSV on serum glucose and insulin concentration in STZ- induced diabetic mice (mean ± SD)

Groups	Glucose (mg/dl)	Insulin (ng/ml)
Control group (C)	115.4 ± 4.2[a]	O.79 ± 0.08[a]
Diabetes mellitus (DM)	462.4 ± 17.3	0.37 ± 0.06
1-deoxynojrimycin (DNJ)	98.2 ± 3.6[a]	0.87 ± 0.03[a]
Resveratrol (RSV)	103.6 ± 1.3[a]	0.81 ± 0.02[a]
Diabetes mellitus+1deoxynojrimycin (DM+DNJ)	303.7 ± 11.5[a]	0.69 ± 0.04[a]
Diabetes mellitus+resveratrol (DM+RSV)	329.83 ± 9.51[a]	0.51 ± 0.02[b]

Significance: $^{a}P < 0.001$; $^{b}P < 0.01$ versus DM.

DNJ-0,75 $mgKg^{-1}$ body weight/day for 14 days, RSV-30 $mgKg^{-1}$ body weight/day for 14 days.

Since redox imbalance is causally linked to inflammatory processes, we evaluated the expression levels of some major factors implicated in inflammation in the diabetic mice model. Throughout exercise, in the DM group, IL-6 mean concentrations were quantitatively greater than in the control group. As shown in figure 1, mice in diabetic group showed significant increase in serum concentrations of IL-6 as compared with control group. Administration of RSV or DNJ once daily for 14 days a significant decrease in IL-6 level compared to the diabetic group ($P<0.05$), (Fig.1).

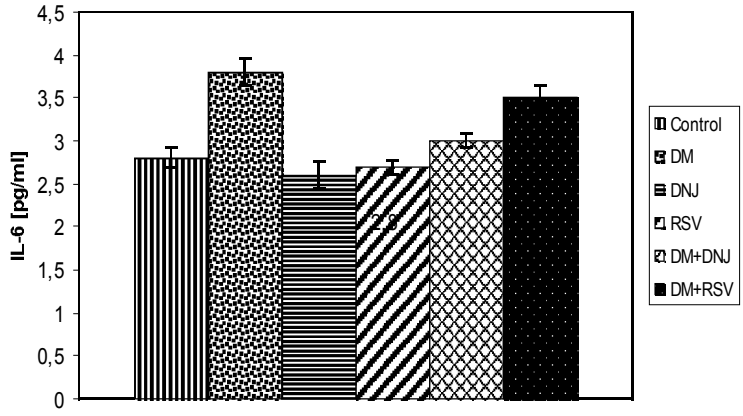

Fig. 1. Effect of 1-deoxynojirimycin and resveratrol on inflammatory cytokine IL-6 in STZ-induced diabetic mice. Values are given as means ± SD of at least ten values.

We also found that the activities of enzymatic antioxidants (GPx, SOD and CAT) were significantly reduced in the liver tissue of the diabetic animals, but they were significantly improved following treatment with the DNJ and RSV for 14 days ($P<0.05$), (Fig. 2-4).

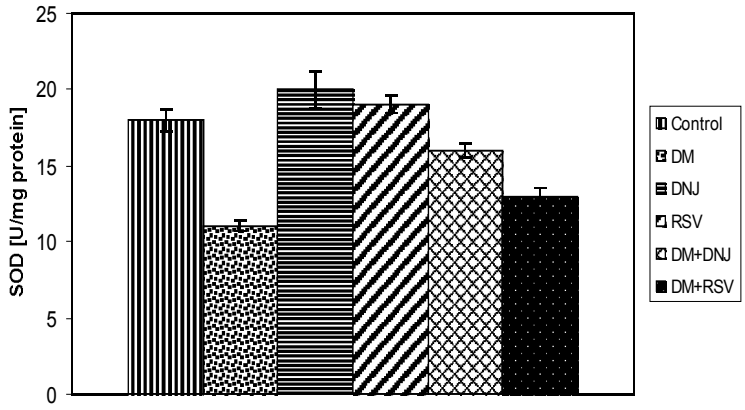

Fig. 2. Effect of 1-deoxynojirimycin and resveratrol on superoxide dismutase (SOD) activities in the liver tissue of normal and streptozotocin induced diabetic mice.
Values are given as means ± SD of at least ten values.

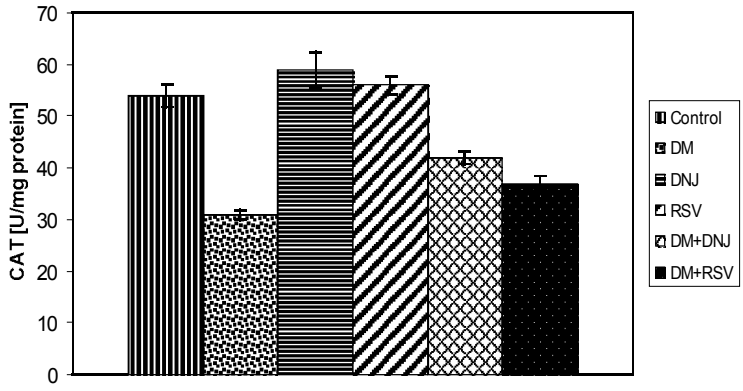

Fig. 3. Effect of 1-deoxynojirimycin and resveratrol on catalase (CAT) activities in the liver tissue of normal and streptozotocin induced diabetic mice. Values are given as means ± SD of at least ten values.

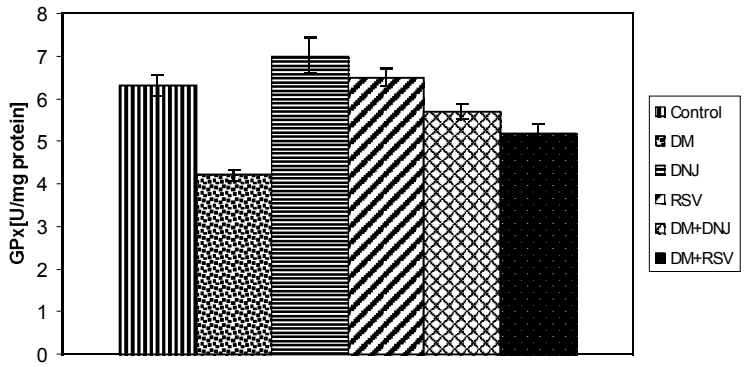

Fig. 4. Effect of 1-deoxynojirimycin and resveratrol on glutathione peroxidase (GPx) activities in the liver tissue of normal and streptozotocin induced diabetic mice. Values are given as means ± SD of at least ten values.

DM resulted in significant decrease in the level of reduced glutathione (GSH) when compared to normal control. Administration of RSV or DNJ once daily for 14 days a significant increase in GSH level compared to the diabetic group ($P<0.05$), (Fig. 5).

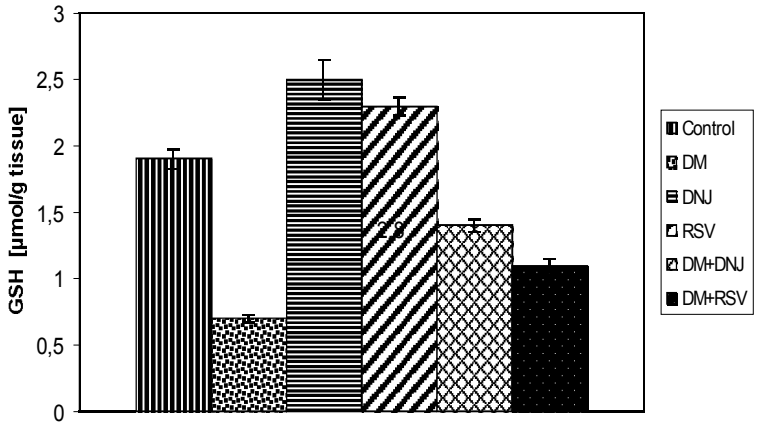

Fig. 5. Effect of 1-deoxynojirimycin and resveratrol on concentration of reduced glutathione (GSH) in the liver tissue of normal and streptozotocin induced diabetic mice. Values are given as means ± SD of at least ten values.

The many studies indicate that hyperglycemia-induced diabetic complications are likely from oxidative dysfunction and inflammatory effect (Murugan and Pari, 2006; Dandona et al., 2004). In prevention and complementary therapy in early stages and of type 1 diabetes phytotherapy may be beneficial. For several plant materials hypoglycaemic effect had been found both in animal studies and in clinical trials (Greń and Formicki, 2012; Greń et al., 2013). Although some of them have not been tested clinically but have been used for a long time in traditional medicine. Among medicinal plants that may be efficient in insulin – resistance and in treatment and prevention of type 1 diabetes mellitus the most popular is Morus alba L. Among the biologically active ingredients contained in the morus, most important therapeutic attributed to 1-deoxynojirimycin (DNJ) and resveratrol (RSV) (Zdrojewicz Z, Belowska-Bień, 2005). Hyperglycemia increases the inflammatory markers tumor necrosis factor TNF-α, interleukin IL-1 and IL-6 (Dhindsa et al., 2004; Pickup, 2004). Overproduced proinflammatory cytokines enhances inflammatory stress in diabetes and diabetic complications. A great deal of evidence indicates close ties between inflammatory and metabolic pathways (Pickup and Mattock, 2003; Wellen and Hotamisligil, 2005; Hotamisligil et al., 1996). Several studies suggested that TNF-a, , IL-6 and other inflammatory mediators may activate the intracellular pathways, such as the I-kappa, I-kappa-B, kinase-b, nuclear factor-kappa B and the protein c-Jun N-terminal kinase axes, amplify and aggravate low-grade inflammation, and these processes may become self-perpetuating through a positive feedback loop created by the proinflammatory cytokines, and lead to diabetes (Shoelson et al., 2006; Ishihara and Hirano, 2002). In diabetes, the suppressed proliferation potential of diabetic T lymphocytes with mitogen stimulated was achieved by a decreased expression of adenosine kinase (Sakowicz-Burkiewicz et al., 2006). The increased production of IL-6 and IFN-γ by STZ-induced diabetic rats was identified as autoimmune diabetes (Shoelson et al., 2006). These results our research support the fact that biologically active components of mulberry are a potent agent against diabetes-associated inflammatory injury via inhibiting inflammatory cytokines production. Both inflammation

and oxidative stress play a major role in the development of tissue insulin resistance (Dandona et al., 2004). Oxidative stress may be an important factor in the pathogenesis of different diabetic complications (Sakowicz-Burkiewicz et al., 2006).

In biological systems, antioxidants such as GSH, a major nonenzymatic antioxidant involved in the maintenance of the redox balance, ameliorate cellular oxidative damage. At cellular and molecular levels, redox imbalance causes the activation of redox-sensitive transcription factors that lead to inflammation (Chung et al., 2001). Therefore, enhanced oxidative stress due to uncontrolled ROS is a major factor in both acute and chronic inflammation and inflammatory-related diseases including diabetes (Lin et al., 2005). The decrease in markers of oxidative stress in diabetes was attenuated by 1-deoxynojirimycin and resveratrol. This suggests that DNJ and RSV would probably ameliorate diabetic oxidative stress. In diabetes mellitus, oxidative stress may be attributed to a combination of hyperglycemia-induced glycoxidation, sorbitol system activation, and reduced GSH synthesis. Reduced activities of GPx, CAT, and SOD in hepatic tissues of diabetic group mice were observed in our study. Treatment with DNJ and RSV reversed this change. According to the results, *Morus alba L.* may have an antioxidative effect against the pathological alterations caused by ROS.

CONCLUSION

The pathogenesis of diabetes and diabetes complications is complex. 1-deoxynojirimycin and resveratrol has a beneficial effect in the STZ-induced diabetic animal model. The present investigation shows that DNJ or RSV possesses several treatment-oriented properties, including the control of hyperglycemia, antioxidant effects, and anti-inflammatory effects. Considering these observations, it appears that biologically active components of mulberry may be a useful adjunct supplement to delay the development of diabetes and diabetes complications. However, more work is necessary to further elucidate the role of 1-deoxynojirimycin and resveratrol, particularly looking at the underlying mechanism of treatment.

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ZAŠTITINI EFEKT 1-DEOXYNOJIRIMYCINA I RESVERATROLAPROTIV DIABETIČKOG OKSIDATIVNOG STRESA I INFLAMACIJE

AGNIESZKA GREŃ, GRZEGORZ FORMICKI, NORBERT LUKAČ, PETER MASSÁNYI, ROBERT STAWARZ

Izvod

Dijabetes melitus (DM) je indukovana kod miševa grupe II, V, i VI. Kod životinje III eksperimentalne grupe je primenjen 1-deokinojirimicin (DNJ) u dozi 0,75 mg/kg^{-1} telesne težine dnevno, tokom 14 dana. Kod miševi IV eksperimentalne grupe primenjen je resveratrol (RSV) u dozi 30 mg/kg^{-1} telesne težine dnevno, toko 14 dana. Životinje grupa V: dijabetična grupa, primala je DNJ, a VI dijabetična grupa je primala RSV. Trideset minuta nakon poslednje injekcije, životinje su anesteziranе i dekapitovane. Se-

rumski nivo interleukin - 6 (IL - 6) je ocenjen kao surogat marker inflamacije. Nivoi redukovanog glutationa u jetre (GSH), aktivnost glutation peroksidaze (GPk), katalaze (CAT) i superoksid dismutaze (SOD) mereni su kao surogat marker oksidativnog stresa. Serumski nivo insulina u plazmi i nivoa glukoze su ocenjeni kao surogat marker dijabetesa. Administracija DNJ ili RSV značajno utiče na obnavljanje glukoze, insulina, IL-6, kao i nivo aktivnosti GSH i SOD, CAT, GPKS u dijabetičnih miševa, do nivoa vrlo sličnih onom u kontrolne grupe. Rezultati su pokazali da DNJ i RSV sa svojim antidijabetičnim, antizapaljenskim i antioksidantnim svojstvima, može biti potencijalni biljni lek u lečenju dijabetesa i problema jetre.

Ključne reči: *oxidativni stres, inflammacija, 1-deoxynojirimycin, resveratrol, diabetes.*

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