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Low beneficial effects of short term antidiabetic diet treatment in streptozotocin – induced diabetic rats

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ABSTRACT: *Oxidative stress is currently suggested to play a role in the pathogenesis of Diabetes mellitus. The role of dietary management in diabetes mellitus is to provide a proper balance of total nutrients while meeting the special dietary needs of the patient. The present study was designated to evaluate the effect of special antidiabetic diet treatment upon oxidative stress parameters in the initial stages of the development of diabetes. Male Wistar strain rats were used as an experimental model, divided into five groups. A significant decrease in superoxide dismutase and total glutathione activities were observed in the liver of diabetic rats when compared with control animals. The plasma level of aminotransferases, creatine kinase, lactate dehydrogenase and urea were significantly increased after induction of diabetes, in all groups under treatment. In contrast, rats fed special diet food, have shown slight different, but not significant changes. The findings of the present study suggest that special diet formula useful for prevention of progressive hyperglycaemia in age induced diabetes in dogs, could not restore the imbalance of cellular defence mechanism provoked by streptozotocin.*

KEY WORDS: *oxidative stress, liver, hyperglycemia, diabetic rats, diet supplement*

I. INTRODUCTION

Diabetes mellitus is accepted to be the most commonest endocrine disease, which are multi-systemic disorders resulting from the deficiency in the secretion or action of the pancreatic hormone-insulin, which in turn produces profound abnormalities of metabolism [1]. Diabetes mellitus is known also in dogs, cats, rats and probably occurs in most mammals, although it is only likely to be diagnosed in laboratory animals [2]. At present, there is no internationally accepted criteria for the classification of canine diabetes. No laboratory test is readily available to identify the underlying cause of diabetes in dogs, and diagnosis is generally made late in the disease course. If the criteria established for human diabetes are applied to dogs, at least 50% of diabetic dogs would be classified as type 1, because this proportion has been shown to have antibodies against β cells. The reminder probably has other specific type of diabetes that results from pancreatic destruction or chronic insulin resistance [3].

The pathogenesis of diabetes mellitus and the possibility of its management by oral administration of hypoglycaemic agents have stimulated greater interest in recent years [4]. Nutrition is one of the most respective factors that had a great influence on maintenance of normal activity in the body determining his health condition. In diabetes mellitus, chronic hyperglycaemia produces multiple biochemical sequels, and diabetes-induced oxidative stress could play a role in the symptoms and progression of the disease [5]. Oxidative stress in cells and tissue results from the increased generation of reactive oxygen species and/or from decreases in antioxidant defence potential [6]. Several hypotheses have been put forth to explain the genesis of free radicals in diabetes. These include autoxidation processes of glucose, the non-enzymatic and progressive glycation of proteins with the consequently increased formation of glucose-derived advanced glycosylation end products, and enhanced glucose flux through the pathway [7, 8, 9]. According to the hypothesis of, normalizing the increased mitochondrial concentration of reactive oxygen species (ROS), prevents all major pathways for diabetes-induced damage.

The possible hypoglycaemic and antioxidant effect of special-designed animal feed was investigated in order to have new information about the nutrition and its influence on some biochemical parameters, body mass and activities of enzymatic and nonenzymatic antioxidants in the control and diabetic rats, that where feed special or commercial laboratory feed.

II. MATERIALS AND METHODS

2.1. Experimental Design. 30 White *Wistar* strain rats of male gender, 4 months old at the start of the experiment, were used. The animals were obtained from the animal facility of the Department of Physiology and Biochemistry of the Faculty of Natural Sciences and Mathematics, Skopje. Prior the experiment, they were given tap water and pellet diet (Filpaso, 52.11, Skopje, Republic of Macedonia). The components of the commercial feed as listed by manufacturer were: crude fat min. 5.7 % , crude proteins minimum 18 %, carbohydrates minimum 60 %, fibre maximum 4.5%, ash maximum 8 %.

Induction of diabetes in rats and Insulin treatment A single i.p. injection of 55 mg/kg streptozotocin (Sigma Aldrich Chemie, GmbH, Deutschland) dissolved immediately before administration in freshly prepared 50 mM citrate buffer (pH 4.0) was given to STZ, STZ+Ins. and STZ+Diet rats on day 0. The injections were given after 12 hours of food deprivation. Control animals received an equivalent volume of citrate buffer. The group under insulin treatment received subcutaneous initial dose of 4 to 8U (Novo Rapid, Flex Pen, 2880 Bagsvaerd, Denmark) followed by 1 to 2 U daily to obtain euglicemia. The diabetic and normal animals were kept in the cages separately and their body weight, the levels of serum glucose in all animals were measured and then these quantities were compared.

Dietary characteristics of Purina Veterinary Diets® DCO® brand Canine formula provides complete and balanced nutrition for maintenance of the adult dog and has been formulated to achieve the following characteristics: High level of complex carbohydrates; Increased fibre including soluble fibre; Moderate total dietary fat and calories and high omega 6:3 ratio. The components of the special Purina feed as listed by manufacturer were: crude protein minimum 21%; crude fat minimum 10%; crude fibre maximum. 10%; moisture maximum 12%; Carbohydrate maximum 46%. Chemical composition of the special feed: whole grain corn, dried beet pulp, poultry by-product meal, corn gluten meal, pearled barley, animal fat preserved with mixed-tocopherols (form of Vitamin E), pea fibre, animal digest, calcium phosphate, dried whey, potassium chloride, fish oil, salt, calcium carbonate, L-Lysine monohydrochloride, choline chloride, Vitamin E supplement, zinc sulphate, ferrous sulphate, niacin, manganese sulphate, Vitamin A supplement, calcium pantothenate, thiamine mononitrate, copper sulphate, riboflavin supplement, Vitamin B-12 supplement, pyridoxine hydrochloride, folic acid, Vitamin D-3 supplement, calcium iodide, biotin, menadione sodium bisulphite complex (source of Vitamin K activity), sodium selenite. *15% - a source of fibre.

Animals were divided into 5 groups as follows:
C -control group: control group of rats (n=6)
Diet - group: healthy rats with diet food *ad libitum* (6);
STZ - group: rats with induced diabetes mellitus – diabetic control (6);
STZ+Diet - group: rats with induced diabetes mellitus and diet food (6);
STZ+Ins. - group: rats with induced diabetes mellitus and insulin (6).

All procedure was in accordance with National Institutes of Health guidelines for the care and use of experimental animals. At overnight fast of 12 hours, the animals were sacrificed by exsaquination under ketamine: xylazine anaesthesia (90 mg/kg i.p. and 10 mg/kg, i.p. respectively). For plasma separation, blood samples were collected into tubes with anticoagulant solution. Tissue samples were removed immediately, flash frozen, measured and stored in –80ºC until further analysis. Liver tissue, heart, aorta and testes were harvested between 10-12 AM, except for those animals, which died during the experiment.

2.1.1 Plasma biochemical assay. Biochemical parameters were measured in heparinised plasma of rats at the start and the end of the experiment by a photometric clinical chemistry analyser (Olympus, AU 400) using routine clinical chemical assays. Blood samples were collected into tubes after an overnight fast of 12 hours, and centrifuged at 1450 × g at 4ºC for 10 minutes.

2.1.2 Tissue antioxidant analysis. Superoxide dismutase activity Liver SOD (EC 1.15.1.1) activity was assayed with a method of Winterbourn and co workers (1975) [10], with SOD determination kit (RA20408, Fluka, Biochemika, Steinheim, Germany). Glutathione reductase activity Liver glutathione reductase (GSSG-Red; EC 1.6.4.2) activity was assayed with a glutathione reductase assay kit (GRSA 114K4000, Sigma-Aldrich, Steinheim, Germany) according to the method of Dolphin et al. (1989) adopted by Garcia-Alfonso et al. (1993). Tissue glutathione content Liver glutathione (GSH) content was measured with a glutathione assay kit (CS0260, Sigma-Aldrich, Steinheim, Germany) according to method described by Akerboom et al. (1981).

2.2. Protein quantification. The protein content in different tissues was measured according to Lowry et al (1951).

2.3. Statistical analysis. Statgrafics (version No 5.0) was used for determination of statistical significance using analysis of variance and post-hoc analysis as appropriate. A $p<0.05$ value was considered significant.

III. RESULTS

Eighteen hours after STZ administration, and daily thereafter, the animals were weighed, urinalysis were performed for glucose and ketones using Forty-eight hours after STZ administration, all animals that had been treated with STZ displayed glucosourea, hyperglycemia, hypoinsulinemia (data not shown) and a moderate loss of body weight.

After streptozotocin injection, glucose concentration was measured using tail vein blood samples obtained from rats after overnight food deprivation. A glucose level >14mmol/L was considered indicative of diabetes, approximately 48 hours after the experimental groups start with insulin administration (group STZ+Ins.), and fed with diet feed (STZ+Diet.). Blood glucose concentrations were determined from blood samples obtained from the tail vein from all animals (MediSense, Bedford, Mass., USA). The blood glucose concentrations and body weights were monitored weekly throughout the course of the study.

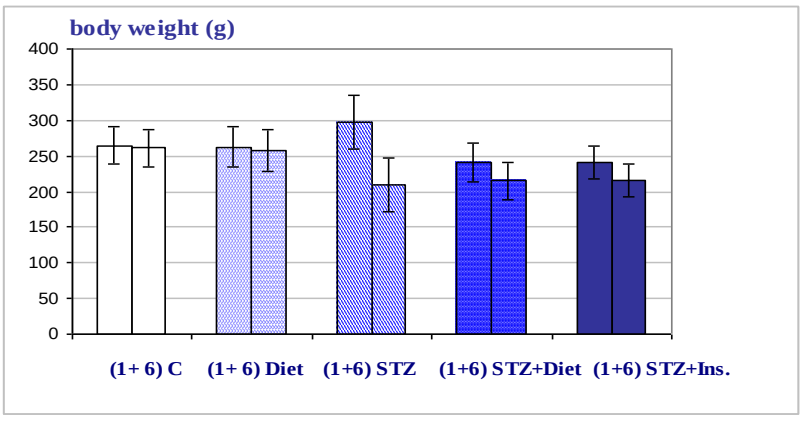
A single intravenous injection of 55 mg/kg dose of Streptozotocin in adult Wistar rats, made pancreas swell and at the end of the experiment it caused degeneration in Langerhans islet beta cells and provoked experimental diabetes mellitus within 3-4 days. Streptozotocin induced one type of diabetes which is similar to diabetes mellitus with non-ketosis hyperglycaemia in some animal species. Three days after degeneration of beta cells, diabetes was induced in all animals.

The effects of a Purina Veterinars Diets Colitis Canine Formula DCO are presented:

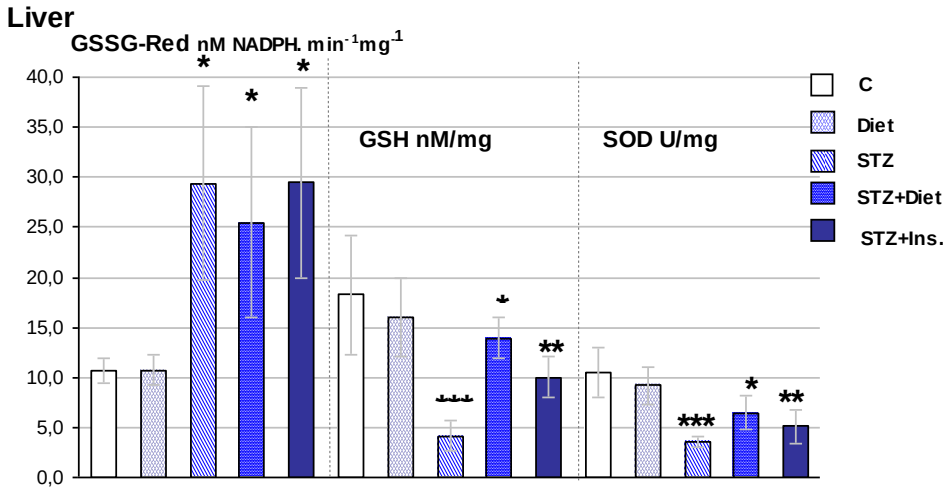
Table 1 Biochemical data in plasma of control and diabetic rats

Variables	(1th week)	(6 th week)	(6 th week)	(6 th week)	(6 th week)
	Control	**Diet**	**STZ**	**STZ+Diet**	**STZ+Ins.**
Glucose mM/L	4,4 ± 0,7	4,8 ± 0,3	**14 ± 4,4****	**12,0 ± 0,6****	**6,0 ± 0,4***
Urea mM/L	4,8 ± 1,6	6,6 ± 2,4	**9,9 ± 0,4****	**8,8± 2,3****	**7,0 ± 1,2***
Creatinin µM/L	62,8 ± 8,0	58,2 ± 4,8	**77,1 ± 1,6 ****	62,8 ± 8,0	64,8 ± 9,7
AST U/L	73,7 ± 15,3	111,4 ± 24,3	**142,1±18,6***	**210,4 ± 73,6 ****	**120,8±11,2***
ALT U/L	44,6 ± 4,3	58,8 ± 18,3	**158,9 ± 8,6****	**130,0 ± 33,4****	**98,8 ± 13,9***
CK U/L	321, 7 ± 139,9	456,0 ± 153,8	**890,2 ± 143,1****	**808,1 ± 160,1****	**508,3 ± 157,0***
LDH U/L	1263,1 ± 444,8	1985,2 ± 620,0	**4o21,8 ± 1096,4****	**3838,8 ± 1058,1****	**1941,6 ± 587,2***
tot. Proteins g/L	75,5 ± 3,9	76,9 ± 3,5	78,6±4,3	78,5±5,9	76,3±5,4
Albumins g/L	33,1 ± 2,2	32,1 ± 1,8	26,9±0,4	28,8±3,1	35,7±1,5
Uric acid µM/L	119,2 ± 21,0	103,1 ± 21,3	117,3 ± 19,8	125,8 ± 47,0	72,6 ± 25,8

The biochemical parameters were also analyzed in the normal, diabetic untreated rats, rats with diet food, diabetic treated rats with diet food and diabetic treated rats with insulin. The streptozotocin injected rats developed diabetes as indicated by increased fasting blood glucose values, which were 3 to 3.5 fold higher in STZ groups: 4,4 ± 0,7 in control, 14 ± 4.4 in STZ rats and 12.0 ± 0.6 in group STZ + diet fed. Significant reduction of glucose level, were registered in STZ group treated with insulin. The activities of serum AST, ALT and LDH were increased in treated animals compared to control animals, which are AST 210.4 ± 73,6; ALT 130.0 ± 33.4; CK 808.1±160.1 and LDH 3838,8 ± 1058,1 in treated group compared with AST 73,7 ± 15,3; ALT 44,6 ± 4,3; CK 321.7 ± 139.9 and LDH 1263.1 ± 444,8 in control group.



The weights of diabetic rats were significantly lower as compared with those in the control group: diabetic animals weighed 210±31g at the end of experiment, while control animals weight 261.03±29 g at the end of 8th week. Consumption of water, food and serum glucose increases in diabetic animals in comparison with normal rats, followed by decreased in the body weight.



LIVER TISSUE

GSSG-red (nM NADPH.min $^{-1}$mg^{-1})		SOD (U/mg)	GSH (nM/mg)
STZ:C	p<0.05 (177%)	p<0,001(-65,67%)	p<0.001(-77,21%)
STZ+Diet.:C	p<0.05 (139%)	p<0,05 (-38.17%)	p<0.05 (-23,76%)
STZ+Ins.:C	p<0.05 (177%)	p<0,01(-51,48%)	p<0.01 (-45,34%)

Figure 2 and Table 2 Oxidation parameters in liver tissue of control and diabetic male rats *significant changes in total glutathione content, activity of glutathione reductase and superoxide dismutase compared with control group, *p< 0.050; ** p<0.01; *** p<0.001

Fig.2 demonstrates a significant interaction between glutathione level and glutathione reductase activity in liver tissue. The analyzed results marked that the glutathione content in liver tissue of diabetic animals, was decreased for 77,21 % for group STZ and 24% for STZ+Diet, and decreased for 45,34% for STZ+Ins. Significant enhancements in glutathione reductase activity were achieved in all diabetic groups. In parallel, tissue activity of superoxide dismutase (SOD) within diabetic group tended to decline up to the sixth week compared with respective control.

IV. DISCUSSION

STZ is widely used in studies of experimental diabetes because it selectively destroys the pancreatic β-cell. Researchers around the world have used STZ to create experimental diabetes because it is a simple, inexpensive and available method. Streptozotocin induces one type of diabetes which is similar to diabetes mellitus with non-ketosis hyperglycaemia in some animal species [11].

We performed our experiments 3 days after STZ treatment, considering that this is an adequate interval to address the time point of cell necrosis when STZ is used. In this sense, Doi K., (1975) [12] has determined that 48 h after injection, rats were completely diabetic and microscopic examinations showed pyknosis, degranulation and marked degeneration of β-cells. Hyperglycaemia, hypoinsulinemia, polyphagia, polyuria and polydipsia accompanied by weight loss were seen in adults rats within three days of Streptozotocin treatment and, within one week to ten days, the amounts of the relevant factors were almost stable, which indicates irreversible destruction of Langerhans islets cells moreover. The glucose level was 3 to 3.5 fold higher in the diabetic groups according to baseline during the 6 weeks treatment.

The biochemical parameters were also analyzed in the normal, diabetic untreated rats, diabetic treated rats with diet food and diabetic rats treated with insulin. The streptozotocin injected rats developed diabetes as indicated by increased fasting blood glucose values, followed with decrease in body weight. We marked a slight

but significant increase in the level of glucose in group under STZ + insulin administration. This observation is in agreement with the studies reported by Stephen and Ezekiel (2006)[13].

Since muscle and liver dysfunction is frequently associated with diabetes mellitus, many clinical reports have indicated that serum enzymes activities derived from the muscle and liver such as lactate dehydrogenase (LDH), creatine kinase (CK) and γ glytamil transferase (GGT), are elevated. The plasma level of ALT, AST, CK, LDH and urea were significantly increased after induction of diabetes, in all groups under treatment, in contrast, rats fed special diet food, have shown slight, but not significant changes. The decrease in total protein and albumin fraction may be due to microproteinuria and albuminuria, which are important clinical markers of diabetic nephropathy, and/or may be due to increased protein catabolism (Table 1). This result is consistent with results obtained by Bakris G.L. (1997) [14]. Parameters of kidney function, such as urea and creatinine, were slightly reduced after fed with special diet food. This seems to be in accordance with many publishing data [15], describing the effects of different types of mostly antioxidants on markers of artificially induced diabetes. In parallel, the weights of diabetic rats were significantly lower as compared with those in the control group (Fig.1), which are consistent with results of Kakkar et al. (1998) [16].

In both type 1 and type 2 diabetes mellitus the late diabetic pathological complications are mostly due to excessive elevated production of reactive oxygen species over the capacity of their removal by internal enzymatic and non-enzymatic mechanisms [17]. Therefore, additional numerous dietary artificial or natural antioxidants may be of great importance in such cases [18]. Fig.2 shows the activity of enzymatic antioxidants such as SOD, GSH and GSSG-Red in the liver of normal, STZ-diabetic animals, Diet, STZ+Diet, and STZ+Ins. group of animals. There were significant reductions in the activity of SOD and GSH in the liver of diabetic rats while the activity of GSSG-Red increased in the liver in diabetic rats. GSH is known to protect the cellular system against the toxic effects of lipid peroxidation. There is also sample evidence that elevation in glucose concentration may depress natural antioxidant defense such as SOD and GSH, SOD scavenges the superoxide radical by converting it to H_2O_2 and molecular oxygen. The activity of SOD was found to be lower in diabetic control rats [19]. The observed decrease in SOD activity could result from inactivation by H_2O_2 or by glycation of the enzyme, which have been reported to occur in diabetes [20]. The possible source of oxidative stress in diabetes includes shifts in redox balance resulting from altered carbohydrate and lipid metabolism, increased generation of reactive oxygen species, and decreased level of antioxidant defences such as GSH and SOD.

V. CONCLUSION

Hyperglycemia, hypoinsulinemia, polyphagia, polyuria and polydipsia accompanied by weight loss were seen in adults rats within three days of Streptozotocin treatment and, within one week to ten days, the amounts of the relevant factors were almost stable, which indicates irreversible destruction of Langerhans islets cells moreover. Researchers around the world have used STZ to create experimental diabetes because it is a simple, inexpensive and available method.

The role of dietary management in diabetes mellitus is to provide a proper balance of total nutrients while meeting the special dietary needs of the patient. Complex carbohydrates and dietary fiber help to delay the absorption of glucose from the intestinal tract and minimize postprandial fluctuation of glucose. Soluble fiber in the diet may also prolong gastrointestinal transit time, allow greater water absorption, and promote the production of short chain fatty acids which nourish the intestinal mucosa.

The findings of the present study suggest that special diet formula useful for prevention of progressive hyperglycaemia in aged depended diabetes in dogs, could not restore the imbalance of cellular defence mechanism provoked by streptozotocin. However, these diabetic induced alteration in ROS production, might be prevented if longer period of supplementation of this hypoglycaemic and antioxidant diet is applied.

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