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The protective effects of vitamin D3 on histopathology of pancreas and liver in streptozotocin-induced diabetic rats

Asija ZAČIRAGIĆ [1]*, Višnja MUZIKA[3] Amina VALJEVAC [1], Amela DERVIŠEVIĆ [1], Maja MITRAŠINOVIĆ-BRULIĆ [2], Muhamed FOČAK [2], Esad ĆOSOVIĆ [3], Selma ALIČELEBIĆ [3], Samra ČUSTOVIĆ [3], Damir SULJEVIĆ [2]*

1 Department of Human Physiology, Faculty of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina.
2 Department of Biology, Faculty of Science, University of Sarajevo, Sarajevo, Bosnia and Herzegovina.
3 Department of Histology, Faculty of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina.
* Corresponding Author. E-mail: suljevic.damir@gmail.com.(D.S); Tel. +387-033-723-776.

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ABSTRACT: The purpose of this study was to explore possible protective effects of vitamin D3 on serum glucose concentration, body weight and histopathology of pancreas and liver. Animals were divided into 3 groups: Control group (n=6), streptozotocin (STZ) group (n=6) and streptozotocin + vitamin D3 (STZ+D3) group (n=6). Rats in the STZ+D3 group starting from the 7th day of experiment were given vitamin D3 for 14 days. Glucose levels and body weight were measured on the 1, 7, 14 and 21st day of experiment. Qualitative histological analysis of pancreas and liver was done using the light microscope with a digital camera. Differences between the groups were tested by one-way analysis of variance (ANOVA) followed by Dunnett's posttest. Differences in repeated measures were tested using paired t-test. On day 14 and 21, blood glucose level in STZ+D3 group was significantly higher compared to the control group of animals but significantly lower than the glucose level registered in STZ group of rats. On day 14 and day 21, body weight in STZ rats was significantly lower compared to weight in STZ+D3 and control groups of rats. Morphological changes, such as shrinkage of islets, vacuolation of both endocrine and exocrine cells, were observed in pancreas of STZ group of animals but were nearly absent in STZ+D3 rats. Similarly, STZ+D3 group of rats showed preserved liver histoarchitecture. Obtained results suggest that vitamin D3 treatment reduces hyperglycemia, exerts beneficial effects on body weight and alleviates histopathological changes in pancreas and liver in STZ-induced diabetic rats.

KEYWORDS: Diabetes mellitus; streptozotocin; vitamin D3; pancreas; liver.

1. INTRODUCTION

Diabetes mellitus (DM) is major health problem worldwide. The prognosis is that total number of patients with diabetes will increase from 171 million in 2000 to 366 million by 2030 [1]. Pancreas and liver are organs that play a pivotal role in the maintenance of blood glucose level. Hyperglycemia and hypoinsulinemia are main features of DM. Both pancreas and liver suffer significant adverse changes in DM with disturbed pancreatic and hepatic glucose homeostasis and metabolism [2].

Streptozotocin (STZ) has potent toxic effects on islet beta cells and is widely used in induction of insulin-dependent DM in experimental animals. It has various biological actions, including the production of acute and chronic cellular injury, teratogenesis mutagenesis and carcinogenesis. Apart from diabetogenic, STZ has also hepatotoxic and nephrotoxic effects [3]. Earlier study by Zafar et al. [4] has shown that STZ also causes a significant decrease in the body weight of STZ-induced diabetic albino rats.

Vitamin D through its active form the 1,25-dihydroxy vitamin D3 (1,25(OH)2D3) has numerous physiological functions in the human body. Best known are its effects on calcium and bone homeostasis. Novel research has proven other important roles of the vitamin D3 including its effects on immunoregulation, antiangiogenesis, apoptosis inhibition and blood pressure control [5, 6]. Moreover, vitamin D3 exerts anti-inflammatory and antioxidant actions [7-9].

Vitamin D3 deficiency is often present in patients with both type 1 and type 2 diabetes [10-12]. However, underlying mechanisms of observed deficiency are less clear. Animal model of STZ - induced diabetes is often

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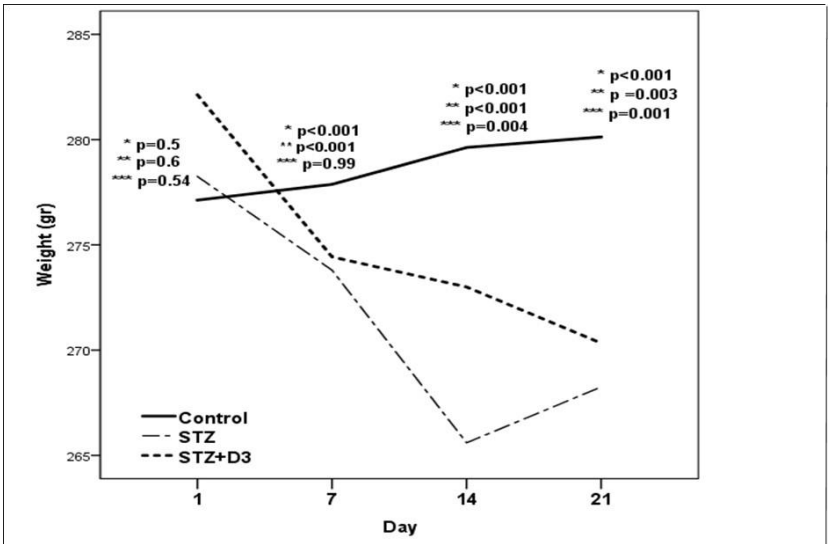
used in studies of liver and pancreas diabetic complications [2, 13] and disturbed glucose homeostasis. Recent study by Derakhshanian et al. [14] has showed that administration of vitamin D3 ameliorates complications of diabetes by improving blood glucose levels among other mechanisms. Furthermore, studies have shown that treatment with vitamin D3 results in reduced diabetes complications such as diabetic retinopathy and nephropathy [1, 15, 16].

Bearing in mind global incidence and prevalence of diabetes and vitamin D3 deficiency that are related to numerous health complications, extensive studies of both of these detrimental conditions are of paramount importance. Hence, the purpose of this study was to explore possible protective effects of vitamin D3 on serum glucose concentration and histopathology of pancreas and liver.

2. RESULTS

In STZ and STZ+D3 groups of rats, body weight significantly decreased from day 1 to day 21, while in the control group of rats no significant changes were observed. On day 1, no significant difference in weight between the groups of rats was observed. On day 7, body weight was significantly lower in STZ and STZ+D3 group of rats compared to controls. On day 14 and day 21, weight in STZ rats was significantly lower compared to weight in STZ+D3 and control groups of rats (Figure 1).



	1	7	14	21	p-value¶
Control	277.1±29.0	278.0±29.0	280.0±28.4	280.1±26.4	0.17
STZ	278.3±24.2	274.4±22.1	266.0±22.0	268.3±22.0	0.01
STZ+D3	282.1±20.3	274.0±22.2	273.0±20.2	270.3±19.7	0.009

Figure 1. Body weight changes in control, diabetic and diabetic treated with vitamin D3 group of rats during the 21 days of follow up. Data are expressed as mean±SD. STZ - streptozotocin group; STZ+D3 - streptozotocin with vitamin D3 group; *- control vs. STZ group; **- control vs. STZ+D3 group; ***- STZ vs. STZ+D3 group; ¶ - between day 1 and day 21 within the group.

When blood glucose levels were compared within the groups, results have shown that in STZ group of rats blood glucose level significantly increased from day 1 to day 21, while in STZ+D3 and control group of rats no significant changes during the same period were observed. In STZ+D3 group, after the initial increase in blood glucose from day 1 to day 7, blood glucose started decreasing onwards to day 21, but the difference was not statistically significant. There were no statistically significant differences in the blood glucose levels between the groups on day 1 of the experiment. On day 7, blood glucose was significantly higher in STZ and STZ+D3 group of rats compared to controls. On the same day, no significant difference in blood glucose between STZ and STZ+D3 was observed. On day 14 and 21, blood glucose level in STZ+D3 group was significantly higher compared to the control group of animals but significantly lower than the glucose level registered in STZ group of rats (Figure 2).

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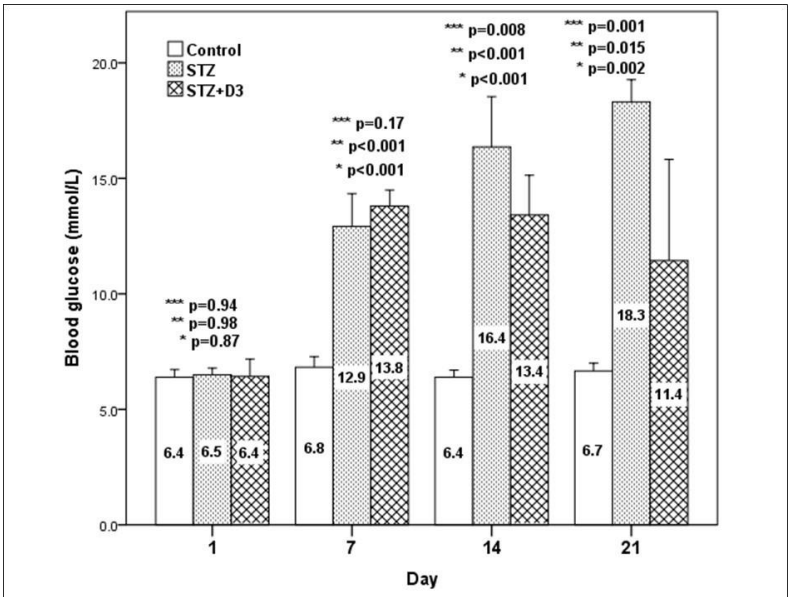

	1	7	14	21	p-value¶
Control	6.4±0.3	6.8±0.5	6.4±0.3	6.7±0.3	0.16
STZ	6.5±0.3	12.9±1.4	16.4±2.2	18.3±1.0	<0.001
STZ+D3	6.4±0.8	13.8±0.7	13.4±1.7	11.4±4.4	0.074

Figure 2. Blood glucose changes in control, diabetic and diabetic treated with vitamin D3 group of rats during the 21 days of follow up. Data are expressed as mean±SD. STZ - streptozotocin group; STZ+D3 - streptozotocin with vitamin D3 group *- control vs. STZ group; **- control vs. STZ+D3 group; ***- STZ vs. STZ+D3 group; ¶ - between day 1 and day 21 within the group.

Control group of animals reveals regular architecture of both, exocrine and endocrine pancreas (Fig 3A1-3). In the STZ group, both components of the pancreas show alterations, and the stromal compartment is reduced (Fig. 3B1). Acinar cells are enlarged with several well-defined vacuoles that are uneven in size and usually found in the basal part of the cell. Cytoplasm is well stained, with the typical distribution of basophilia and acidophilia (Fig. 3B2). The islets appear ill-defined and shrunken with less numerous cells. The cytoplasm of endocrine cells is poorly stained and sporadically vacuolated. Central region of islets is occupied with the homogenous, acidophilic material (Fig. 3B3). Pancreas in the STZ+D3 group of animals reveals nearly regular structure (Fig. 3C1). Acinar cells appear moderately swollen with only a few vacuoles present in the basal region of the cytoplasm (Fig. 3C2). Langerhans islets, nearly regular in size, show enlarged poorly stained endocrine cells and inconspicuous capillaries (Fig. 3C3).

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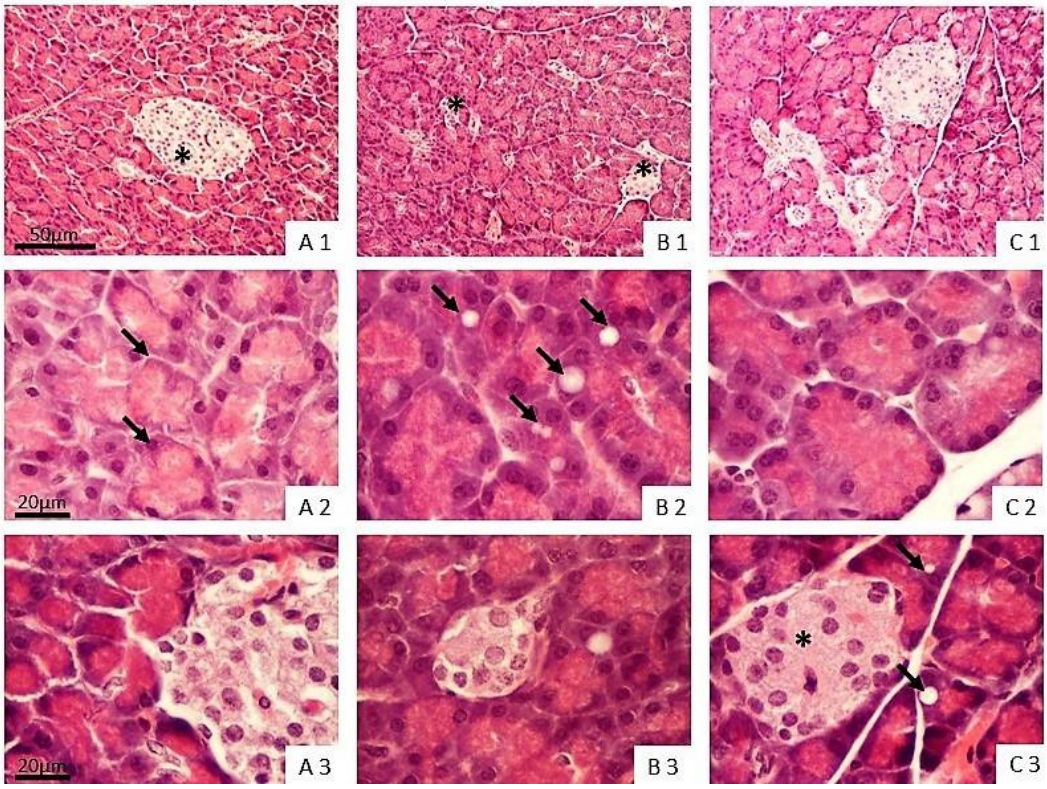

Figure 3. Representative photomicrographs of HE stained pancreas section, magnification x 10 and x 40 (A-control group; B- STZ group; C-STZ + D3 group)
A1 - 3 The control group reveals regular histoarchitecture of exocrine (arrows) and endocrine pancreas (asterisk);
B1 - 3 In the STZ group, endocrine islets appear ill-defined and shrunken with less numerous cells (asterisk) while acinar cells are enlarged with well-defined vacuoles in the basal part (arrows);
C1 - 3 Pancreas in the STZ+D3 group shows Langerhans islets that are relatively preserved (asterisk) and moderately swollen acinar cells with few vacuoles found in the basal region of the cytoplasm (arrows).

In the control group of animals, normal structure of the liver lobules and the portal area is present (Fig. 4A1). Hepatocytes are arranged in plates, divided by sinusoids (Fig. 4A3) that converge to the central vein (Fig. 4A2). Their cytoplasm appears acidophilic without apparent vacuolation. In the STZ group, portal area and lobular architecture show no remarkable changes (Fig. 4B1) while sinusoids and central vein appear relatively narrowed and hyperemic (Fig. 4B2). Enlarged hepatocytes with the coarse vacuolation of the cytoplasm (Fig. 4B3) can be found, more prominently in centrilobular area (Fig. 4B2). In the STZ + D3 group, there is no evident alterations in the portal area (Fig. 4C1). Sinusoids are readily observed, running between well-defined plates of hepatocytes to the slightly hyperemic central vein (Fig. 4C2). The cytoplasm of hepatocytes has acidophilic properties with mild vacuolar changes (Fig. 4C2-3).

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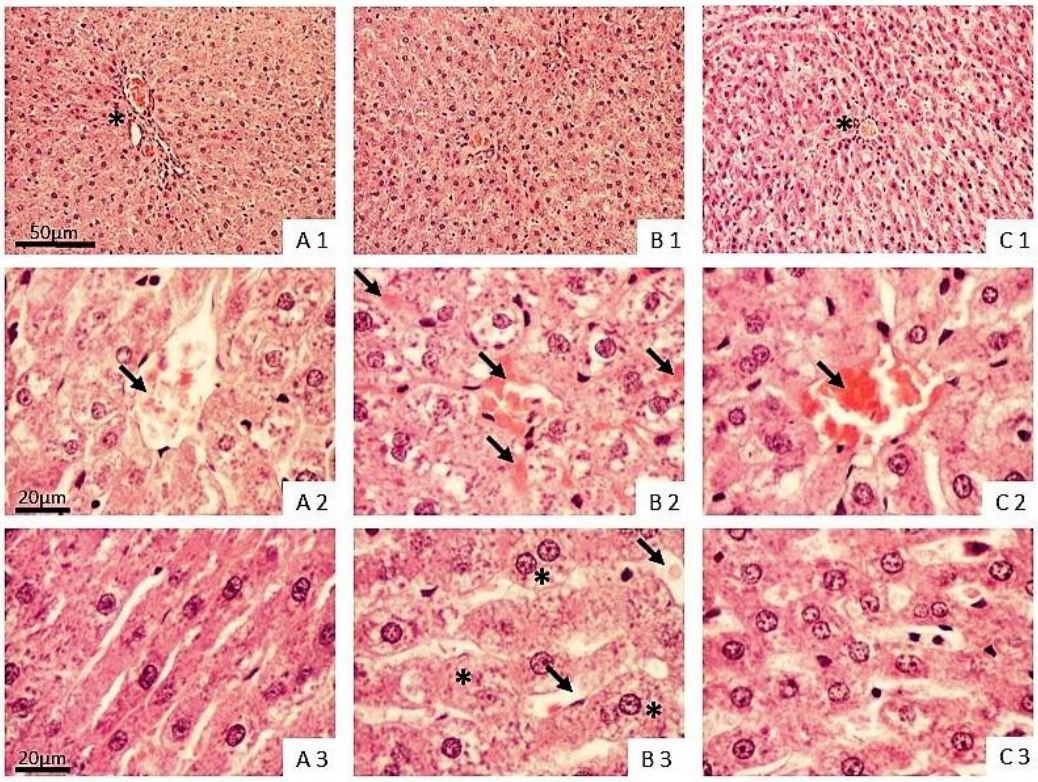

Figure 4. Representative photomicrographs of HE stained liver sections, magnification x 10 and x 40 (A-control group; B- STZ group; C- STZ +D3 group)
A1 – 3 In the control group, portal area appears preserved (asterisk) as well as the hepatocytes, regularly arranged in plates that converge to the central vein (arrow) of the liver lobule;
B1 – 3 In the STZ group, hepatocytes appear enlarged (asterisk), sinusoids and central vein of the liver lobule are relatively narrowed and hyperemic (arrows).
C1 – 3 In the STZ + D3 group, portal area is preserved (asterisk), sinusoids are readily observed, while the central vein is slightly hyperemic (arrow).

3. DISCUSSION

The results of the present study have shown that vitamin D3 treatment reduces hyperglycemia in STZ-induced diabetic rats and alleviates histopathological changes in pancreas and liver. We also report significant decrease in body weight in both STZ and STZ+D3 groups of rats compared to the control group. However, our results have shown that on the day 14 and day 21, weight in STZ rats was significantly lower compared to weight in STZ+D3 and control groups of rats. Observed reduction of body weight in STZ group might be explained by both detrimental effects of diabetes and toxic actions of STZ. These findings are in compliance with previous studies in which decrease of body weight was regarded as a consequence of deficient insulin secretion that results in increased lipolysis in adipose tissue alongside with protein breakdown caused by reduced the amino acid uptake of tissues [17, 18]. On the other hand, our results have shown increase in body weight in STZ+D3 rats from the 14th to the 21st day of experiment that could suggest beneficial effects of vitamin D3 treatment on body weight in STZ-induced diabetes mellitus.

Results of Alatawi et al. [19] have shown that single intraperitoneal injection of STZ in a dosage of 45 mg/kg of body weight induced elevated blood glucose levels at the third day post-injection in rats. In their study, authors assessed the antioxidative effect of vitamin D and calcium administration for 28 consecutive days. Contrary to their study, we were able to induce diabetes with the lower dosage of STZ (35 mg/kg). We were also able to show decrease of glucose concentration in STZ+D3 group of animals after 14 days of vitamin D3 administration.

Protective effects of vitamin D3 on glucose homeostasis are in the accordance with similar recent studies [14, 20]. Earlier study by Kumar et al. [13] has also shown that vitamin D3 treatment reduces blood glucose levels in STZ-induced diabetic rats. Also, similar protective effects are obtained in alloxan-induced DM animal

model treated with vitamin D3. The effect was even more pronounced when a vitamin D3 is applied before the induction of disease as a preventive measure [21].

According to novel research, protective effects of vitamin D3 on glucose homeostasis might be explained by reduction of serum levels of advanced glycation end products [22]. During hyperglycemia glucose transporters allow influx of glucose into pancreatic β-cells, thus controlling insulin release. Process that follows is glycolysis that is accompanied by rise in intracellular calcium. Studies have shown that this process is enhanced by vitamin D through regulation of extracellular calcium concentration and consequent flux into β-cells but also intracellular calcium content via calbindin, a cytosolic calcium-protein [23]. As a result of rise in calcium levels in pancreatic β-cells insulin is released from granules [24]. In addition, it is suggested that vitamin D stimulates the expression of insulin receptors and affects insulin sensitivity [23]. Protective effects of vitamin D3 treatment on blood glucose levels observed in our study might be explained by rise in intracellular calcium levels in pancreatic β-cells that resulted in the release of insulin, which reduced blood glucose levels in STZ+D3 diabetic rats. However, we did not measure serum insulin levels in our study that should be regarded as a study limitation.

Previous studies [23, 25] demonstrated alterations of endocrine pancreas in STZ-induced diabetic rats and mice similar to our findings. STZ is widely used in experimental studies since it has been proved to cause insulitis, pancreatic islet inflammation and insulin deficiency [26]. Wang Y et al. [27] reported conspicuous inflammation in and around islets that was not noted in the present study. Even though experimental design was similar, Wang et al. [27] applied higher dose of STZ, which can be a possible explanation for the observed differences. Also, the authors of the aforementioned studies reported protective role of vitamin D3, based on the histological findings in endocrine pancreas of diabetic rats treated with vitamin D3, such as regular size and shape of islets, markedly reduced β-cells degeneration, decreased atrophy and vacuolation of their cytoplasm. Those reports are in accordance with our results.

The changes in exocrine pancreas associated with diabetes and STZ application, described in the present study, are not frequently reported in the literature. Vacuolation and swelling of acinar cells can be in part explained by disturbance of autophagy, the process that is important for the cell homeostasis in general, and is linked to diabetes development when present in endocrine pancreas [27]. These changes were ameliorated with vitamin D3 treatment. However, the major limitation of the presented morphological analysis is the lack of more detailed approach such as electron microscopy and immunohistochemical techniques.

Liver is the primary site of glucose metabolism. Vitamin D3 binds to vitamin D receptor (VDR), which has been shown to be present in numerous tissues including liver [28]. Diabetic patients often suffer from liver failure accompanied with vitamin D deficiency [23]. It is possible that in combination of diabetes and vitamin D deficiency VDR is downregulated, which results in hepatic complications and disturbed glucose metabolism with consequent hyperglycemia. However, roles and functions of VDR remain largely unknown, not only in the liver but also in other tissues, and further research is warranted in order to attain more in-depth knowledge of VDR functions in human body.

Amelioration of hyperglycemia observed in STZ+D3 rats in our study might be due to upregulation of liver VDR receptor caused by vitamin D treatment. However, we did not measure hepatic expression of VDR and this should be regarded as another limitation of the present study. Our results, supported by the histological findings in liver of STZ+D3 group of rats, suggest hepatoprotective effects of vitamin D3 in diabetes and are in compliance with previous studies [2, 20, 21]. The liver histoarchitecture is nearly preserved and changes as narrow sinusoids, hepatocytes swelling and vacuolations, found in STZ rats, are minimal or completely absent after D3 treatment. Similar hepatoprotective effects of vitamin D3 were reported by Risha et al. [29]. In their study, as in ours, 35mg/kg of STZ was used for the induction of diabetes. However, administration of vitamin D3 lasted for four weeks, whereas we were able to show hepatoprotective effects of vitamin D3 after only 14 days of its administration.

4. CONCLUSION

Results of the present study have shown that vitamin D3 treatment reduces hyperglycemia, exerts beneficial effects on body weight and alleviates histopathological changes in pancreas and liver in STZ-induced diabetic rats. Attained results have clinical implications and suggest that vitamin D3 administration may be used as a successful treatment in a prevention and reduction of the pathological complications of diabetes. However, more elaborative studies using electron microscopy and immunohistochemical techniques are required to corroborate obtained findings.

5. MATERIALS AND METHODS

5.1. Animals and experimental design

Study included Wistar rats, both sexes, of 180 - 240 g body weight. Rats had free access to water ad libitum and standard dry pellet diet. Study was approved by Institutional Ethical Committee; ethical code: 02-3-4-4638/14. All animal care and procedures were carried out in the accordance with the National and Institutional Guide for the Care and Use of Laboratory Animals, and the ethical treatment of all experimental animals was conformed to the guidelines provided by the Institutional Animal Care and Use Committee. All efforts were made to minimize the suffering of animals and to use minimal number of animals. Reagents used in the present study were purchased from Sigma-Aldrich Chemical Company. Animals were housed in separate cages for 2 weeks to allow their acclimatization under standard laboratory conditions (humidity 50% ± 10%, temperature 23 °C ± 3 °C and under 12 h light - 12 h dark periods). Study was experimental and prospective.

5.2. Induction of diabetes

Animals were divided into 3 groups: Control group (n=6) that were intraperitoneally (i.p.) injected with citrate buffer (pH 4.5; 0.1M) on the first day of the experiment. Streptozotocin (STZ) group included rats (n=6) that were injected by a single i.p. dose (35 mg/kg) of STZ, which was freshly dissolved in citrate buffer (pH 4,5; 0,1M) on the first day of the experiment. Streptozotocin + vitamin D3 (STZ+D3) group included rats (n=6) that were injected by a single i.p. dose (35 mg/kg) of STZ, freshly dissolved in citrate buffer (pH 4.5; 0.1M) on the first day of the experiment. Rats in the STZ+D3 group starting from the 7th day of experiment were given vitamin D3 (12 micrograms of cholecalciferol/kg dissolved in 0,3ml of coconut oil) via oral gavage for 14 days [2].

5.3. Biochemical analysis and body weight measurement

Diabetes was confirmed on the 7th day of experiment in animals with fasting glucose level ≥ 16,7 mmol/L measured via test strips from the blood obtained by incision of tail vein. Glucose levels and body weight were measured on the 1, 7, 14 and 21st day of experiment. Glucose levels were determined to confirm the induction of diabetes using a test strips of One Touch Basic Glucometer (Accu-Chek Active, Roche, Germany). Body weight was measured using an electronic scale (Acculab V-1200®, IL, United States). On 21st day of experiment, animals were sacrificed by decapitation after being anaesthetized by ether.

5.4. Histological analysis

After sacrificing the animals, liver and pancreas were removed and fixed in 10% buffered neutral formalin. Tissue samples were processed in an automated tissue processor and embedded in paraffin following the standard procedure. Paraffin blocks were sectioned in 5µm thick sections and stained with hematoxylin and eosin (HE). Qualitative histological analysis was done using the light microscope (Nikon, Eclipse 400) with a digital camera (Nikon, DN 100). Researchers were blinded to the treatment group.

5.5. Statistical analysis

Statistical analysis was performed with SPSS software version 17.0 (SPSS, Inc., Chicago, Illinois). Results are expressed as the mean ± standard deviation (SD). Differences between the groups were tested by one-way analysis of variance (ANOVA) followed by Dunnett's posttest. Differences in repeated measures were tested using paired t-test. P values ≤ 0.05 were considered significant.

Author contributions: Concept – A.Z., A.V., V.M, D.S; Design – A.Z., M.MB., A.V., A.D.; Supervision – D.S., S.A.; Resources – M.F., E.Ć., S.A.; Materials – M.F., M.MB., D.S.; Data Collection and/or Processing – M.F., M.MB.; Analysis and/or Interpretation – A.V., V.M., S.A., S.Č., E.Ć.; Literature Search – S.Č., A.D., E.Ć.; Writing – A.Z., V.M, A.D.; Critical Reviews – A.Z., V.M., A.V., A.D., M.MB., M.F., E.Ć., S.A., S.Č., D.S.

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REFERENCES

[1] Lu L, Lu Q, Chen W, Li J, Li C, Zheng Z. Vitamin D3 protects against diabetic retinopathy by inhibiting high-glucose-induced activation of the ROS/TXNIP/NLRP3 inflammasome pathway. J Diabetes Res. 2018; 8193523. [CrossRef]

[2] George N, Kumar TP, Antony S, Jayanarayanan S, Paulose CS. Effect of vitamin D3 in reducing metabolic and oxidative stress in the liver of streptozotocin-induced diabetic rats. Br J Nutr. 2012; 108 (8): 1410-1418. [CrossRef]

[3] Mitrašinović-Brulić M, Dervišević A, Začiragić A, Fočak M, Valjevac A, Hadžović-Džuvo A, Suljević D. Vitamin D3 attenuates oxidative stress and regulates glucose level and leukocyte count in a semi-chronic streptozotocin-induced diabetes model. J Diabetes Metab Disord. 2021; 20 (1): 771-779. [CrossRef]

[4] Zafar M, Naeem-Ul-Hasan Naqui S. Effects of STZ-induced diabetes on the relative weights of kidney, liver and pancreas in albino rats: a comparative study. Int J Morphol. 2010; 28 (1): 135-142. [CrossRef]

[5] Plum LA, DeLuca HF. Vitamin D, disease and therapeutic opportunities. Nat Rev Drug Discov. 2010; 9 (12): 941-955. [CrossRef]

[6] Prietl B, Treiber G, Pieber TR, Amrein K. Vitamin D and immune function. Nutrients. 2013; 5 (7): 2502-2521.[CrossRef]

[7] Chagas CE, Borges MC, Martini LA, Rogero MM. Focus on vitamin D, inflammation and type 2 diabetes. Nutrients. 2012; 4 (1): 52-67. [CrossRef]

[8] Guillot X, Semerano L, Saidenberg-Kermanac'h N, Falgarone G, Boissier MC. Vitamin D and inflammation. Joint Bone Spine. 2010; 77 (6): 552-557. [CrossRef]

[9] Tarcin O, Yavuz DG, Ozben B, Telli A, Velioglu Ogunc A, Yuksel M, Toprak A, Yazici D, Sancak S, Deyneli O, Akalin S. Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. J Clin Endocrinol Metab. 2009; 94 (10): 4023-4030. [CrossRef]

[10] Payne JF, Ray R, Watson DG, Delille C, Rimler E, Cleveland J, Lynn MJ, Tangpricha V, Srivastava SK. Vitamin D insufficiency in diabetic retinopathy. Endocr Pract. 2012; 18 (2): 185-193. [CrossRef]

[11] Hyppönen E, Läärä E, Reunanen A, Järvelin M-R, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birthcohort study. Lancet. 2001; 358: 1500-1503. [CrossRef]

[12] Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. J Clin Endocrinol Metab. 2007; 92 (6): 2017-2029. [CrossRef]

[13] Kumar PT, Antony S, Nandhu MS, Sadanandan J, Naijil G, Paulose CS. Vitamin D3 restores altered cholinergic and insulin receptor expression in the cerebral cortex and muscarinic M3 receptor expression in pancreatic islets of streptozotocin induced diabetic rats. J Nutr Biochem. 2011; 22 (5): 418-425. [CrossRef]

[14] Derakhshanian H, Djalali M, Mohammad Hassan MH, Alvandi E, Eshraghianet MR, Mirshafiey A, Nadimi H, Jahanabadi S, Zarei M, Djazayery A. Vitamin D suppresses cellular pathways of diabetes complication in liver. Iran J Basic Med Sci. 2019; 22 (6): 690-694. [CrossRef]

[15] Kaur H, Donaghue KC, Chan AK, Benitez-Aguirre P, Hing S, Lloyd M, Cusumano J, Pryke A, Craig ME. Vitamin D deficiency is associated with retinopathy in children and adolescents with type 1 diabetes. Diabetes Care. 2011; 34 (6): 1400-1402. [CrossRef]

[16] Diaz VA, Mainous AG, Carek PJ, Wessell AM, Everett CJ. The association of vitamin D deficiency and insufficiency with diabetic nephropathy: implications for health disparities. J Am Board Fam Med. 2009; 22 (5): 521-527. [CrossRef]

[17] Hussein AG, Mohamed RH, Shalaby SM, Abd El Motteleb DM. Vitamin K2 alleviates type 2 diabetes in rats by induction of osteocalcin gene expression. Nutrition. 2018; 47: 33-38. [CrossRef]

[18] Kocaman N, Kuloğlu T. Expression of asprosin in rat hepatic, renal, heart, gastric, testicular and brain tissues and its changes in a streptozotocin-induced diabetes mellitus model. Tissue Cell. 2020; 66: 101397. [CrossRef]

[19] Alatawi FS, Faridi UA, AlatawiMS. Effect of treatment with vitamin D plus calcium on oxidative stress in streptozotocin-induced diabetic rats. Saudi Pharm J. 2018; 26 (8): 1208-1213. [CrossRef]

[20] Liu L, Lv G, Ning C, Yang YE, Zhu J. Therapeutic effects of 1,25-dihydroxyvitamin D3 on diabetes-induced liver complications in a rat model. Exp Ther Med. 2016; 11 (6): 2284-2292. [CrossRef]

[21] Hamden K, Carreau S, Jamoussi K, Miladi S, Lajmi S, Aloulou D, Ayadi F, Elfeki A.1 Alpha, 25 dihydroxyvitamin D3: therapeutic and preventive effects against oxidative stress, hepatic, pancreatic and renal injury in alloxan-induced diabetes in rats. J Nutr Sci Vitaminol. 2009; 55 (3): 215-222. [CrossRef]

[22] Omidian M, Djalali M, Javanbakht MH, Eshraghian MR, Abshirini A, Omidian P, Alvandi E, Mahmoudi M. Effects of vitamin D supplementation on advanced glycation end products signaling pathway in T2DM patients: a randomized, placebo-controlled, double blind clinical trial. Diabetol Metab Syndr. 2019; 11: 86. [CrossRef]

[23] Mitri J, Pittas AG. Vitamin D and diabetes. Endocrinol Metab Clin North Am. 2014; 43 (1): 205-232. [CrossRef]

[24] Neelankal John A, Jiang FX. An overview of type 2 diabetes and importance of vitamin D3-vitamin D receptor interaction in pancreatic β-cells. J Diabetes Complications. 2018; 32 (4): 429-443. [CrossRef]

[25] Lee HA, Lee E, Do GY, Moon EK, Quan FS, Kim I. Histone deacetylase inhibitor MGCD0103 protects the pancreas from streptozotocin-induced oxidative stress and β-cell death. Biomed Pharmacother. 2019; 109: 921-929. [CrossRef]

[26] Furman BL. Streptozotocin-induced diabetic models in mice and rats. Curr Protoc Pharmacol. 2015; 70: 5471-54720. [CrossRef]

[27] Wang Y, He D, Ni C, Zhou H, Wu S, Xue Z, Zhpu Z. Vitamin D induces autophagy of pancreatic β-cells and enhances insulin secretion. Mol Med Rep. 2016; 14 (3): 2644-2650. [CrossRef]

[28] Zmijewski MA. Vitamin D and human health. Int J Mol Sci. 2019; 20 (1):145. [CrossRef]

[29] Megahed A, Gadalla H, Abdelhamid F, Fawzy M, Risha E. Protective effects of Vitamin D and Metformin against hematological, biochemical and histopathological alterations in induced diabetic rats. Ann Vet Anim Sci. 2020; 7 (1): 1-14. [CrossRef]