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Resveratrol and resveratrol-hydroxypropyl-β-cyclodextrin complex recovered the changes of creatine kinase and Na^+, K^+-ATPase activities found in the spleen from streptozotocin-induced diabetic rats

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Abstract: Type 1 diabetes (T1D) is the result of the selective destruction of the pancreatic β-cells by T cells of the immune system. Although spleen is a secondary lymphoid organ, it is also involved in the T1D pathogenesis. However, the alterations in a variety of cellular processes of this disease need to be further understood. We aimed to analyze the benefits of resveratrol, and its complexed form on diabetic complications in the spleen of rats. To this end, we investigated important enzymes of phosphoryl transfer network, and Na^+, K^+-ATPase activity. Wistar rats were divided into non-diabetic groups: Control, Ethanol, Resveratrol, Hydroxypropyl-β-cyclodextrin, Resveratrol-hydroxypropyl-β-cyclodextrin, and diabetic groups with the same treatments. Diabetes was induced by a single dose of 60 mg/kg of streptozocin intraperitoneally, and treatments by intragastric gavage once daily for 60 days. Hyperglycemia reduced creatine kinase activity, which was reversed by the administration of resveratrol. Na^+, K^+-ATPase activity was greatly affected, but it was reversed by resveratrol and resveratrol-hydroxypropyl-β-cyclodextrin. This suggest an energetic imbalance in the spleen of diabetic rats, and in case this also occurs in the diabetic patients, it is possible that resveratrol supplementation could be beneficial to the better functioning of the spleen in diabetic patients.

Key words: Energy metabolism, Hydroxypropyl-β-cyclodextrin, Hyperglycemia, Nanotecnology.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease of high complexity, being responsible for health problems worldwide. The DM type 1 (DM1) is characterized by a decompensated energy metabolism, which causes total or partial autoimmune destruction of insulin-producing cells, resulting in high blood glucose levels (Bluestone et al. 2010, Nyaga et al. 2018). The spleen has a relevant participation in the metabolic changes of this disease since the splenocytes produce cytokines that contribute to the β-pancreatic cells destruction (Thorvaldson et al. 2005, García-Galicia et al. 2014, Tong et al. 2015). It is also reported that splenectomy may lead to diabetes (Kabelitz et al. 2008, Ley et al. 2012).

Streptozotocin (STZ) is usually used to induce type 1 diabetes in animal models (Eriksson et al. 2000, Lenzen 2008, Ghosh et al. 2018). 'Streptozotocin diabetes' is caused by specific necrosis in the pancreatic β-cells inducing glucotoxicity (Wu and Yan 2015). Some natural antioxidants have been reported to exhibit their therapeutic effects in 'streptozotocin diabetes' (Morishita 2004, Rugde et al. 2007, Ghosh et al. 2018). Resveratrol is a natural phytoalexin present in a wide variety of plants, grapes, and red wine has been linked with beneficial activities towards health because it acts as an antioxidant, preventing and treating metabolic diseases such as diabetes (Chen et al. 2011, Chang et al. 2012). Mice treated with resveratrol showed a significant reduction in the death of splenocytes. Resveratrol-induced autophagy of these cells, and the degradation of intracellular proteins and lipids provides energy (ATP) for the cell, preventing the activation of the apoptotic damage (Kim et al. 2007) and metabolic diseases such as DM (Gurusamy et al. 2010, Lee et al. 2011).

According to Baur and Sinclair (2006), resveratrol has a rapid metabolization due to its low bioavailability in the organism, being rapidly eliminated when administrated orally. So, an alternative for increasing the bioavailability is to complex it with cyclodextrin (CD), which is capable of forming inclusion complexes with hydrophobic substances (Teixeira et al. 2013, Torres et al. 2018). Besides, the bioavailability may be enhanced by increasing the solubility and stability, and dissolution rate (Trollope et al. 2014, Kumar et al. 2017).

Adenylate kinase (AK), pyruvate kinase (PK), and creatine kinase (CK) are the key enzymes participating in a network of production and cellular energy homeostasis (Dzeja and Terzic 2003, Matté et al. 2007, Alekseev et al. 2012). Another relevant enzyme for the organism is the Na^+, K^+-ATPase, responsible for generating and maintaining the ionic gradients through the cell membrane (Pressley et al. 2005, Jaitovich and Bertorello 2006). The nutrients and energy flow maintenance into the cell is essential for the homeostasis and cell work. The energy metabolism reduction can lead to a decrease in ATP synthesis. In this context, it has been shown that the energy metabolism deficiency may be implicated in the pathogenesis of several metabolic conditions (Mattevi et al. 1996, Alves-Filho and Pålsson-McDermott 2016, Xiao et al. 2018).

One of the pathways of cellular energy production is the glycolytic route. PK transfers phosphoryl group of the phospoenolpyruvate (PEP) to ADP resulting pyruvate and ATP. Aizawa et al. (2003) demonstrated apoptotic cells in the splenic red pulp of the PK-deficient patient. This indicates that PK may be considered a key enzyme for the cell metabolism. CK isoforms are part of a buffering system of cellular energy. It occurs between production and consumption of ATP in different compartments such as mitochondria and cytosol, contributing to homeostasis (Wallimann et al. 1992, 1998, Yan 2016). Among alternative phosphotransfer pathways, the AK-catalysed circuit, in normal conditions, maintains the concentrations

of ATP, ADP, and AMP to equilibrium. Impaired AK activity leads to an alteration in cellular functions. In addition, AK can take over the function of other kinases, such as CK, in pathological conditions (Dzeja et al. 2011, Wujak et al. 2015).

The Na^+, K^+-ATPase plays an important role in the maintenance of the Na^+ across the cell membrane. It generates an electrochemical gradient through the plasmatic membrane (Erecińska and Silver 1994, Jaitovich and Bertorello 2006) that is used for cellular volume regulation, osmotic balance, and to transport molecules (e.g. glucose) bonded to the co-transport of Na^+ (Kaplan 2002). This transport is essential for cellular functioning since more than one-third of hydrolyzed ATP is to pump ions across membranes. Shi et al. (2013) suggested that complications of type 1 diabetes may be partly due to the impaired Na^+, K^+-ATPase activity and that this dysfunction may be one of the mechanisms of type 1 diabetes-induced complications. The association between resveratrol with insulin may be an excellent treatment for type-1 diabetes. It was suggested, at least in part, by anti-hyperglycemic, and antioxidant potential of these molecules, preserving regulation of Na^+, K^+-ATPase (Bashir 2018). Thus, protecting the effective functioning of Na^+, K^+ -ATPase from the complications caused by type 1 diabetes could contribute to slow the progression of these injuries in this disease.

It is known that splenocytes are involved in the diabetes development, however, few reports are found about the activity of the AK, PK, CK, and Na^+, K^+-ATPase enzymes in the spleen in the presence and absence of resveratrol, which has been considered a hypoglycemic substance. The current study aimed to investigate the role of resveratrol-hydroxypropyl-β-cyclodextrin, and its free form on the activities of important enzymes of the energy metabolism in the spleen of normal and hyperglycemic rats.

MATERIALS AND METHODS

INCLUSION COMPLEX

Resveratrol ($C_{14}H_{12}O_3$; molecular weight = 228.25 g/mol; purity > 98%) and 2-hydroxypropyl-β-cyclodextrin (HPβCD) were obtained from Sigma-Aldrich. 0.1 mM HPβCD solution in water (8 mL) at 40 °C was prepared by vigorous stirring in ULTRA-TURRAX High-Speed Homogenizer T 25 ºC (IKA, UK) at 3,200 rpm and an equimolar amount of RSV (0.1 mM) was directly added to the suspension. The RSV was previously dissolved in ethanol (2 mL). After stirring for one minute, the suspension was filtered through 0.45 µm cellulose acetate membrane filter to removed undissolved particles. The solvent was removed by evaporation. The evaporation was performed using rotary evaporator at 40 °C for about 10 min under vacuum for 8 h. Since resveratrol is a photosensitive substance, the procedure was performed in a dark environment (Nishihira et al. 2017).

CHARACTERIZATION OF THE INCLUSION COMPLEX

Fourier Transformed Infra-Red Spectroscopy (FT-IR) analyses were performed Perkin Elmer (Spectrum One), R, HP and the complex spectra were collected using an FT-IR in a spectral region between 4,000 and 450 cm^{-1}. Samples were mixed in a pestle with potassium bromide (KBr) (1:100) and pressed in a hydraulic press (10 tons for 2 min) to small tablet and were placed in the infrared beam. Nuclear magnetic resonance (NMR) spectra were performed in BRUKER DPX-400 (Bruker, Germany). Fifteen grams of samples were dissolved in DMSO-d6 using tetramethylsilane (TMS) as internal reference standard. The characterization was carried out in the Laboratório de Química of the Universidade Federal de Santa Maria (UFSM).

ANIMAL MODEL

Seventy male Wistar rats from the Central Animal House of UFSM were submitted to a period of 15 days for adaptation. The animals with 60 days old and weighing an average and standard deviation of 216 ± 28 g were used in this experiment. The rats were kept in cages, four animals each, housed at a constant temperature and controlled humidity (23 ± 1 ºC and 70%, respectively) on a light/ dark cycle of 12 h. They had free access to commercial ration and water. All animal procedures were approved by the Animal Ethics Committee from Franciscan University (protocol under number: 14/2012).

EXPERIMENTAL INDUCTION OF DIABETES

Hyperglycemia was provoked by a single intraperitoneal injection of 60 mg streptozotocin (STZ)/kg body mass of rat, dissolved in 0.1 M sodium citrate buffer (pH 4.5), after fasting period of 12 h. The animals to normoglycemic group received an equivalent volume of sodium citrate buffer. STZ-treated rats received 5% glucose solution instead of water for 24 h after diabetes induction to avoid death due to hypoglycemic shock (Avila-Acevedo et al. 2012). Blood samples were taken from the tail vein five days after STZ or vehicle administration to measure glucose levels. Glycemia was determined by using a commercial glucometer (Accu-Chek Performa, Roche®, USA). Animals with fasting glycemia higher than 200 mg/ dL (Barbalho et al. 2011) were used for the diabetic group (Fig. 1).

TREATMENT WITH RESVERATROL

After measuring glycemia, seventy rats were primarily divided into two groups (normal and hyperglycemic). The normoglycemic rats were divided into five groups, with seven animals each: Control (C); Ethanol (E); Resveratrol (R); Resveratrol-hydroxypropyl-β-cyclodextrin (RHP) and Hydroxypropyl-β-cyclodextrin (HP). The hyperglycemic rats were divided into five groups, with seven animals each: Diabetic Control (DC); Diabetic Ethanol (DE); Diabetic Resveratrol (DR); Diabetic Resveratrol-hydroxypropyl-β-cyclodextrin (DRHP) and Diabetic Hydroxypropyl-β-cyclodextrin (DHP). Still on the fifth day was initiated the sixty-day chronic treatment with 1 daily administration, by intragastric gavage of water to the controls C and D (Figure 1). Resveratrol was dissolved in ethanol at 15% of aquose solution. A solution of 1 mg R/kg body mass was administered to R and DR groups and 15% ethanol solution to E and DE groups. 1 mg of R in the complex/ kg body mass to RHP and DRHP groups and the equivalent concentration of HP to the one found in the complex to HP and DHP groups. RSV dosage choice of 1 mg/kg was based on previous studies, which authors observed hypoglycemic effect even in low dose (Chang et al. 2011, 2012).

TISSUE PREPARATION

The rats were killed by decapitation without anesthesia, and the spleen was rapidly dissected, weighted and kept chilled until homogenization, which was performed using a ground glass type Potter-Elvejhem homogenizer (1:10 w/v) in buffer (pH 7.4) containing 0.32 M sucrose, 1 mM EGTA, 10 mM Tris-HCl. The homogenized was centrifuged at 800 × *g* for 10 min at 4 °C, and part of the supernatant was aliquotted to determine the AK and CK total activity. The other part of the supernatant was centrifuged at 10,000 × *g* for 15 min at 4 °C, and the supernatant was separated to determine the PK activity. Another spleen fraction was homogenized in buffer medium, pH 7.5 and centrifuged at 800 x *g* for 10 min at 4 °C. The supernatant was used for the determination of the Na^+, K^+-ATPase enzymatic activity. The supernatants were stored for no more than 1 week at -80 °C when the assay was not carried out immediately.

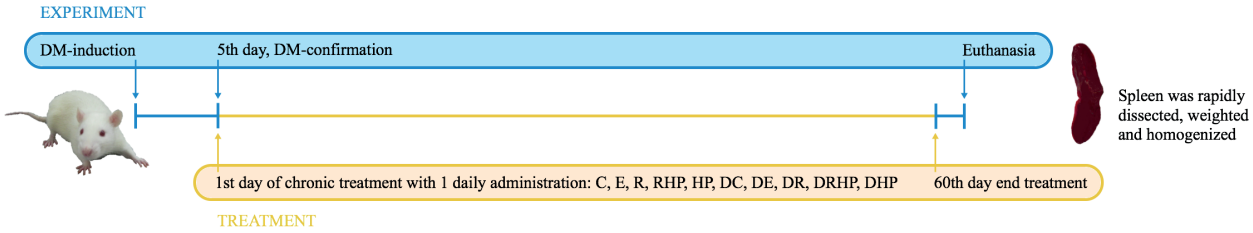

Figure 1 - Schematic view of the experimental protocol. Experimental induction of diabetics was provoked by a single intraperitoneal injection of 60 mg streptozotocin (STZ)/kg body mass of rat. Treatment refers to the C (Control), E (Ethanol), R (Resveratrol), RHP (Resveratrol-hydroxypropyl-β-cyclodextrin), HP (Hydroxypropyl-β-cyclodextrin), DC (Diabetic control), DE (Diabetic ethanol), DR (Diabetic resveratrol), DRHP (Diabetic resveratrol-hydroxypropyl-β-cyclodextrin) and DHP (Diabetic Hydroxypropyl-β-cyclodextrin).

ADENYLATE KINASE ACTIVITY

AK activity was measured with an assay containing hexokinase (HK) and glucose 6-phosphate dehydrogenase (G6PD), according to Dzeja et al. (1999). The reaction mixture contained 20 mM glucose, 100 mM KCl, 20 mM HEPES, 1 mM EDTA, 4 mM $MgCl_2$, 2 mM $NADP^+$, 4.5 U/mL of HK, 2 U/mL of G6PD, and 1 µg of protein homogenate. The reaction was initiated by adding 2 mM ADP. The reduction of $NADP^+$ was followed at 340 nm for 3 min in a spectrophotometer. ADP, $NADP^+$, G6PD, and HK were dissolved in water. Reagents concentration and assay time (3 min) were chosen to assure the linearity of the reaction. The results were expressed in nanomol of ATP formed per min per mg of protein.

PYRUVATE KINASE ACTIVITY

PK activity was assayed according to Feksa et al. (2003). The assay consisted of 0.1 M Tris/HCl buffer, pH 7.5, 0.16 mM NADH, 10 mM $MgCl_2$, 75 mM KCl, 5.0 mM ADP, 0.1% (v/v) Triton X-100, 7 U of L-lactate dehydrogenase, and 10 µL of the mitochondria-free supernatant in a final volume of 0.5 mL. After 10 min of pre-incubation at 37 ºC, the reaction was started by the addition of 1 mM phosphoenol pyruvate. Results were expressed as µmol of pyruvate per min per mg of protein.

CREATINE KINASE ACTIVITY

CK activity was assayed in the mixture containing the following final concentrations: 65 mM Tris–HCl buffer, pH 7.5, 9 mM $MgSO_4$,7 mM phosphocreatine, and 1 µg of protein in a final volume of 0.1 mL. After 10 min of pre-incubation at 37 ºC, the reaction was started by the addition of 0.3 µmol of ADP. The reaction was stopped after 10 min by the addition of 1 µmol of ρ-hydroxymercuribenzoic acid. The incubation time and the reagent concentrations were chosen to assure linearity of the enzymatic reaction. Appropriate controls were carried out to measure chemical hydrolysis of phosphocreatine. The creatine formed was measured according to the method of Hughes (1962). The color was formed by the addition of 0.1 mL 2% α-naphthol and 0.1 mL 0.05% diacetyl in a final volume of 1 mL and read after 20 min at 540 nm. Results were expressed as nanomol of creatine formed per min per mg of protein.

NA^+, K^+-ATPase ACTIVITY

The reaction mixture for Na^+, K^+-ATPase activity assay contained 5.0 mM $MgCl_2$, 80.0 mM NaCl, 20.0 mM KCl, and 40.0 mM Tris–HCl, pH 7.4, in a final volume of 200 mL. After 10 min of pre-incubation at 37 ºC, the reaction was started by the addition of ATP to a final concentration of 3.0 mM and was incubated for 20 min. Controls were carried

out under the same conditions with the addition of 1.0 mM ouabain. Na^+, K^+-ATPase activity was calculated by the difference between the two assays (Wyse et al. 1999). Released inorganic phosphate (Pi) was determined by the method of Chan et al. (1986), and enzyme specific activity was expressed as nmol of Pi released per min per mg of protein.

PROTEIN CONTENT

Protein content of spleen homogenates was determined by the methods of Lowry et al. (1951) to AK, PK and CK and Bradford (1976) to Na^+, K^+-ATPase, using serum bovine albumin as the standard.

BLOOD GLUCOSE LEVELS

Before the experiment, there were no significant differences between blood glucose of groups. Sixty days after to start the treatments, all rats were weighed, killed, and the blood was collected for serum levels glucose analysis. This was determined in an automated analyzer Vitros 250 (Ortho-Clinical Diagnostics), using kits Johnson & Johnson® by the method chemical dried.

STATISTICAL ANALYSIS

Results were presented as the mean ± standard deviation (SD). Assays were performed in duplicate or triplicate, and the mean or median was used for statistical analysis. Data were analyzed using one-way analysis of variance (ANOVA) followed by the post-hoc Tukey's multiple comparison tests when F values were significant. Differences between groups were rated significantly at $p < 0.05$. All analyses were carried out using the GraphPad software.

RESULTS

EFFECTS OF TREATMENTS ON BLOOD GLUCOSE LEVELS

Streptozotocin diabetes showed a significant increase in the blood glucose compared to normal levels [$F_{9,43} = 288.4$], ($p = 0.000$). In relation to treatments, neither resveratrol free form, nor resveratrol-hydroxypropyl-β-cyclodextrin was able to revert hyperglycemia (Figure 2).

EFFECTS OF TREATMENTS ON ENZYME ACTIVITIES

The AK activity was neither changed by streptozotocin diabetes, nor treatments in the spleen when compared to controls [$F_{9,60} = 1.5$], ($p = 0.153$) (Figure 3a). In the PK activity, ANOVA shows that there was no significant difference ($p > 0.05$) between the non-diabetic and the diabetic control. All treatments reduced PK activity, when compared to control (Figure 3b). The CK activity was reduced by hyperglycemia when compared to the control group ($p < 0.05$). The administration of the ethanol, and resveratrol prevented it. Already, the administration of resveratrol-hydroxypropyl-β-cyclodextrin (RHP), as free cyclodextrin (HP)

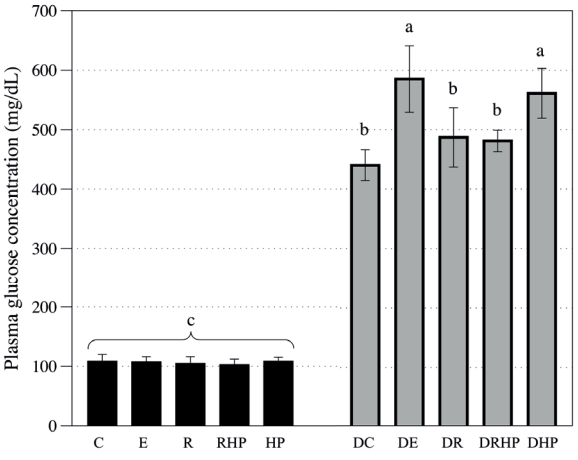

Figure 2 - Effects of free and resveratrol-hydroxypropyl-β-cyclodextrin administration on plasma glucose concentration of non-diabetic and diabetic rats. Data are mean ± standard deviation (SD) for 5-7 animals in each group. Data from blood glucose are expressed as mg/dL. C (Control), E (Ethanol), R (Resveratrol), RHP (Resveratrol-hydroxypropyl-β-cyclodextrin), HP (Hydroxypropyl-β-cyclodextrin), DC (Diabetic control), DE (Diabetic ethanol), DR (Diabetic resveratrol), DRHP (Diabetic resveratrol-hydroxypropyl-β-cyclodextrin) and DHP (Diabetic Hydroxypropyl-β-cyclodextrin). Groups with different letters have significant differences ($p < 0.05$) (one-way ANOVA followed by Tukey test).

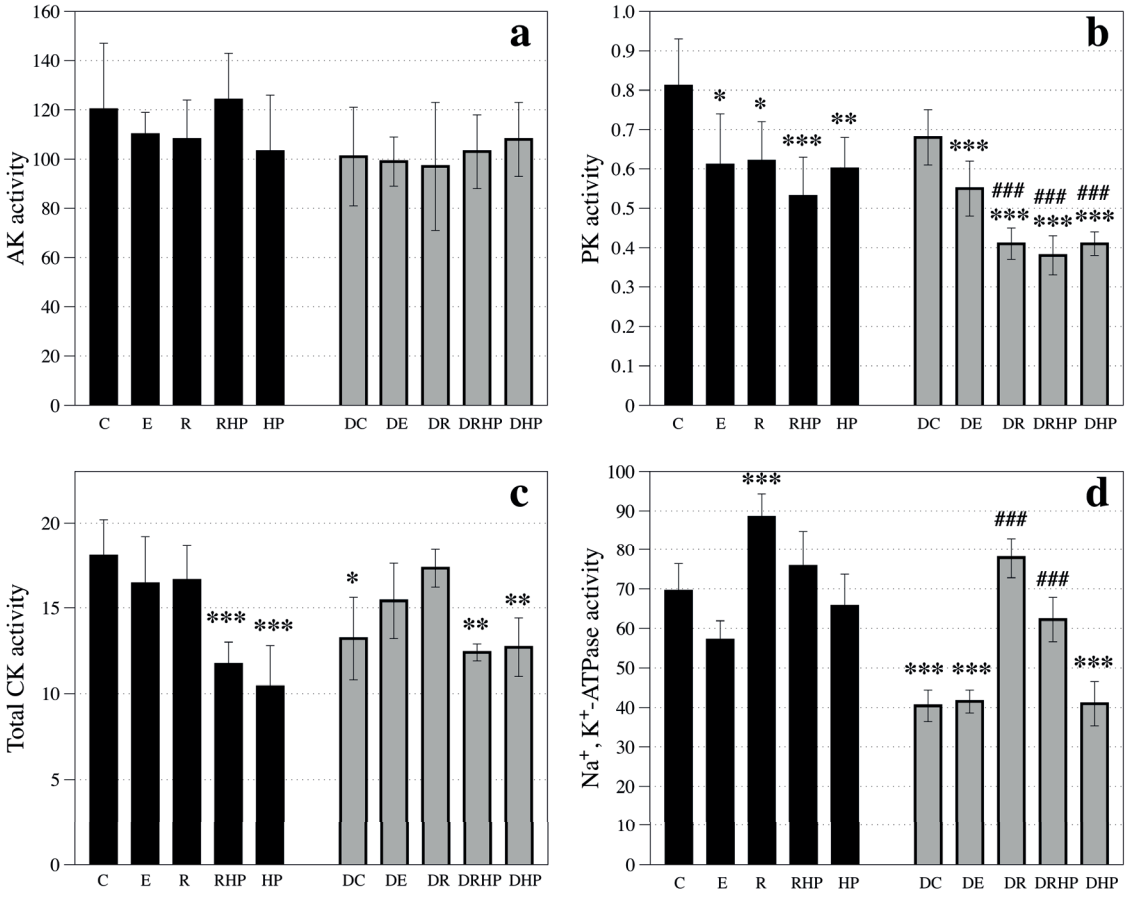

Figure 3 - Effects of free and resveratrol-hydroxypropyl-β-cyclodextrin administration on enzyme activities in spleen of non-diabetic and diabetic rats. (**a**) AK activity (**b**) PK activity (**c**) CK activity (**d**) Na^+, K^+-ATPase activity. Data are mean ± standard deviation (SD) for 5-7 animals in each group. C (Control), E (Ethanol), R (Resveratrol), RHP (Resveratrol-hydroxypropyl-β-cyclodextrin), HP (Hydroxypropyl-β-cyclodextrin), DC (Diabetic control), DE (Diabetic ethanol), DR (Diabetic resveratrol), DRHP (Diabetic resveratrol-hydroxypropyl-β-cyclodextrin) and DHP (Diabetic Hydroxypropyl-β-cyclodextrin). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ compared to non-diabetic control and $^{\#\#\#}p < 0.001$ when compared to diabetic control (one-way ANOVA followed by Tukey test).

reduced enzyme activity in normoglycemic rats ($p < 0.001$) (Figure 3c). Na^+, K^+-ATPase activity increased with administration of free resveratrol in normoglycemic animals ($p < 0.001$). Hyperglycemia caused an expressive reduction of the activity of the Na^+, K^+-ATPase activity ($p < 0.001$), which was prevented by free and resveratrol-hydroxypropyl-β-cyclodextrin (DR and DRHP groups) administration. Ethanol, and HPβCD failed to prevent it, indicating that only treatments with the presence of resveratrol prevented this change (Figure 3d).

BODY AND SPLEEN WEIGHT

All diabetic groups reduced body weight when compared to non-diabetic groups ($p < 0.001$). There was no difference between treatments (Figure 4a). Ethanol, resveratrol-hydroxypropyl-β-cyclodextrin, and HPβCD treatment reduced the weight of diabetic spleen (Figure 4b).

DISCUSSION

The present study shows a reduction in the activity of some important spleen enzymes caused by

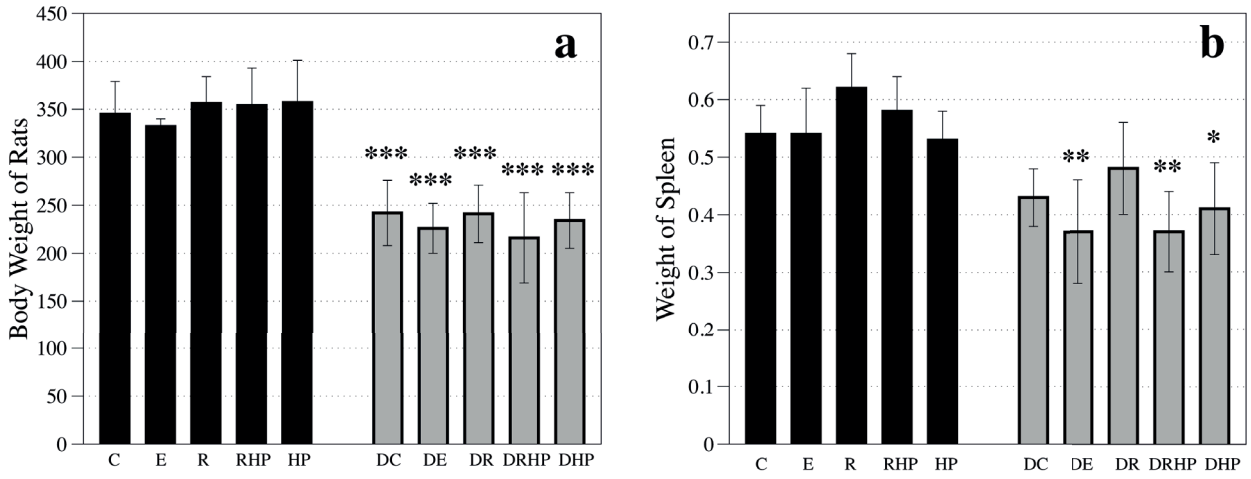

Figure 4 - Weight of non-diabetic and diabetic rats treated with free and resveratrol-hydroxypropyl-β-cyclodextrin administration. (**a**) Body weight (**b**) Weight of Spleen. Data are mean ± standard deviation (SD) for 5-7 animals in each group. Data from body weight are expressed as g. C (Control), E (Ethanol), R (Resveratrol), RHP (Resveratrol- hydroxypropyl-β-cyclodextrin), HP (Hydroxypropyl-β-cyclodextrin), DC (Diabetic control), DE (Diabetic ethanol), DR (Diabetic resveratrol), DRHP (Diabetic Resveratrol- hydroxypropyl-β-cyclodextrin) and DHP (Diabetic Hydroxypropyl-β-cyclodextrin). *$p < 0.5$; **$p < 0.01$; *** $p < 0.001$ compared to non-diabetic control (one-way ANOVA followed by Tukey test).

hyperglycemia, which may alter the energetic homeostasis of this tissue in type 1 diabetes. CK, and Na^+, K^+-ATPase activities were recovered by resveratrol, and resveratrol-hydroxypropyl-β-cyclodextrin, and its free form, respectively.

The spleen is a secondary lymphoid organ involved in the type 1 diabetes pathogenesis (Ghosh et al. 2018). This disease is marked by alterations in a variety of cellular processes that need to be further understood (Badr et al. 2015). For the first time, we measured activities of the phosphotransfer network, and Na^+, K^+-ATPase in the spleen of hyperglycemic rats, in the presence of the resveratrol complexed with HPβCD, or its free form. Here, streptozotocin increased blood glucose, and reduced body and spleen weight, as expected. Unfortunately, resveratrol treatment (complexed or not) not reverted hyperglycemia. Curiously, hyperglycemia was more severe with ethanol and HPβCD treatment. Persistent hyperglycemia can lead to ketoacidosis, a serious condition associated with very high blood glucose levels in type 1 diabetes. Besides, it can happen associated with alcohol abuse in diabetics (Bradford et al. 2017, Umpierrez and Freire 2002), suggesting that ethanol treatment could have developed this condition in our diabetic animals.

Moreover, a positive effect of resveratrol, against possible death of splenocytes, was observed. Even though the treatments did not affect the weight of spleens in the normoglycemic rats, hyperglycemic spleen had a reduction in the weight with all treatments, except resveratrol. Similar protection was shown by Szkudelski and Szkudelska (2011), which reported a significant reduction in the death of splenocytes from mice treated with resveratrol. Besides, weight loss of the spleen may be related to body weight reduced of the diabetic animals, once 'streptozotocin diabetes' significantly decreases body weight of the rats (Zafar and Naeem-ul-Hassan Naqvi 2010).

Intracellular processes regulate the mechanisms involved in energy homeostasis. For a correct functioning of the cell energy system is necessary that energy compounds, such as ATP, are synthesized and delivered to the consumption spots in an appropriate speed for its use rate (Dzeja et al. 2002). Coupling of spatially separated intracellular ATP producing,

and ATP consuming processes is fundamental to maintenance of cellular activities. AK, CK, and PK are key enzymes arranged to maintain energy homeostasis. In particular, PK support high energy phosphoryl transfer and signal communication between ATP generating, and ATP consuming/ATP sensing processes (Franceschi et al. 2013). As these systems operate together, a reduced enzymatic activity may be compensated by increased activity of the others enzymes. However, a change in the activity of two or more enzymes can lead to a cumulative impairment in communication between sites of production and consumption of ATP (Dzeja and Terzic 2003). In this study, neither hyperglycemia, nor treatments were able to alter AK and PK activities. It is crucial in homeostasis of the adenine nucleotides metabolism, as well in regulating intracellular ATP, and to produce processes through complex phosphoryl transfer networks (Dzeja and Terzic 2003).

PK is a critical enzyme to energy metabolism in the mammalian tissues. It is a crucial enzyme of the glucose metabolism, the main pathway that provides energy for cell function (Hall and Cottam 1978). In this study, all treatments reduced PK activity in all groups. We observed that RHP did not show the expected effects, once the administration of this complex decrease, even more, the enzyme activity, when compared to resveratrol and HP separately. Deficiency of this enzyme causes a decline in ATP production, leading to the destruction of red blood cells present in the spleen (Rüfer and Wuillemin 2013).

We also tested the effects of hyperglycemia and treatments on the total CK activity in the spleen. We found that RHP, and HP administration decreased the CK activity, independent of the hyperglycemia. However, resveratrol, and ethanol reverted this effect in hyperglycemic animals. The reduction of CK activity by hyperglycemia was not compensated by other enzymes evaluated in this work, such as PK, AK and Na^+, K^+-ATPase, which leads us to believe that there is an injury to the spleen tissue. Na^+, K^+-ATPase activity was significantly inhibited by hyperglycemia. Resveratrol complexed or not, reversed it, indicating that resveratrol can recover the changes of CK, and Na^+, K^+-ATPase activities in the spleen provoked by diabetes. Resveratrol is suggested as a preventive agent for cerebral ischemia. The administration of resveratrol before cerebral ischemia, brought Na^+, K^+-ATPase activity in the cortex, and hippocampus back to normal levels (Simão et al. 2011). Furthermore, it is suggested that the elimination of ROS contributes, in part, to the resveratrol-induced neuroprotection. However, more tests are necessary to elucidate the activity of Na^+, K^+-ATPase in the spleen, once these enzymatic dosages performed could have been distorted by interferents, like another cell line (erythrocytes), due to its function of the blood filtering.

Although the formulations were tested in a particular organ in this work, the measurements of enzymatic activities evaluated in this study are unique in the literature. The presence of PK, AK, and CK already been confirmed in different animal species including human (Böckelmann and Ritter 1968, Brock 1970, Fauquier et al. 2008), however, more studies are required for a better understanding about splenic bioenergetics. Furthermore, additional data are necessary to reinforce the efficacy of the performed analysis and to certify that the data obtained truly belongs to the spleen enzymes, once it is a path organ for other cells that could influence the results.

CONCLUSION

Based on this study, we concluded that resveratrol, and resveratrol-hydroxypropyl-β-cyclodextrin can recover the enzymatic changes in the spleen provoked by hyperglycemia in rats. Therefore, resveratrol, as a potential therapeutic agent, may hold promise auxiliary supplementation to the better functioning of the spleen in diabetic patients.

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AUTHOR CONTRIBUTIONS

V.C. Rech, V.S.K. Nishihira and J. Kolling designed the research. V.S.K. Nishihira, L.S. Fernandes, L.R. Feksa, and J.L. Giongo developed the characterization of the inclusion complex. V.S.K. Nishihira, J. Kolling, C.G. Pinto, N.J. Mezzomo, M.D. Baldissera and G.M. do Carmo were involved in rat care and treatment. J. Kolling, J. Kolling, I.D. de Franceschi, L.R. Feksa, and A.T.S. Wyse performed the measurements of enzymes of phosphoryl transfer network. J. Kolling, and A.T.S. Wyse executed the measurement of Na^+, K^+-ATPase activity. G. Orengo performed the statistics analysis and graphic displays. V.C. Rech, L.R. Feksa, A.T.S. Wyse and C.M.D. Wannmacher collected and analyzed the data. V.C. Rech and J. Kolling wrote the paper. V.C. Rech, N.J. Mezzomo, R.A. Vaucher, L.S. Fernandes, J.L. Giongo, and C.M.D. Wannmacher revised it critically the paper.

REFERENCES

ALEKSEEV AE, REYES S, SELIVANOV VA, DZEJA PP AND TERZIC A. 2012. Compartmentation of membrane processes and nucleotide dynamics in diffusion-restricted cardiac cell microenvironment. J Mol Cell Cardiol 52(2): 401-409.

ALVES-FILHO JC AND PÅLSSON-MCDERMOTT EM. 2016. Pyruvate Kinase M2: A Potential Target for Regulating Inflammation Front Immunol 21(7): 145.

AIZAWA S, KOHDERA U, HIRAMOTO M, KAWAKAMI Y, AISAKI K, KOBAYASHI Y, MIWA S, FUJII H AND KANNO H. 2003. Ineffective erythropoiesis in the spleen of a patient with pyruvate kinase deficiency. Am J Hematol 74(1): 68-72.

AVILA-ACEVEDO JG, GARCÍA-BORES AM, MARTÍNEZ-RAMÍREZ F, HERNÁNDEZ-DELGADO CT, IBARRA-BARAJAS M, VIVAR ARD, FLORES-MAYA S, VELASCO-LARA P AND CESPEDES CL. 2012. Antihyperglycemic effect and genotoxicity of *Psittacanthus calyculatus* extract in streptozotocin-induced diabetic rats. Bol Latinoam Caribe Plantas Med Aromáticas 11: 345-353.

BADR BM, MOUSTAFA NA, ELDIEN HMS, MOHAMED AO, IBRAHIM HM, EL-ELAIMY IA, MAHMOUD MH AND BADR G. 2015. Increased levels of type 1 interferon in a type 1 diabetic mouse model induce the elimination of B cells from the periphery by apoptosis and increase their retention in the spleen.Cell Physiol Biochem 35(1): 137-147.

BARBALHO SM, DAMASCENO DC, SPADA APM, LIMA IE DOS RN, ARAÚJO AC, GUIGUER EL, MARTUCHI KA, OSHIIWA M AND MENDES CG. 2011. Effects of *Passiflora edulis* on the Metabolic Profile of Diabetic Wistar Rat Offspring. J Med Food 14(12): 1490-1495.

BASHIR SO. 2018. Concomitant administration of resveratrol and insulin protects against diabetes mellitus type-1-induced renal damage and impaired function via an antioxidant-mediated mechanism and up-regulation of Na+/K+-ATPase. Arch Physiol Biochem 125(2): 104-113.

BAUR JA AND SINCLAIR DA. 2006. Therapeutic potential of resveratrol: the *in vivo* evidence. Nat Rev Drug Discov 5(6): 493-506.

BLUESTONE JA, HEROLD K AND EISENBARTH G. 2010. Genetics, pathogenesis and clinical interventions in type 1 diabetes. Nature 29(464): 1293-1300.

BÖCKELMANN W AND RITTER H. 1968. Tissue variability of the phosphotransferases adenylate kinase (EC: 2.7.4.3.) and pyruvate kinase (EC: 2.7.1.40.). Humangenetik 6(4): 373-376.

BRADFORD AL, CRIDER CC, XU X AND NAQVI SH. 2017. Predictors of Recurrent Hospital Admission for Patients Presenting with Diabetic Ketoacidosis and Hyperglycemic Hyperosmolar State. J Clin Med Res 9(1): 35-39.

BRADFORD MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 7(72): 248-254.

BROCK DJ. 1970. Adenylate kinase isoenzyme patterns in tissues of people of different phenotype. Biochem Genet 4(5): 617-625.

CHAN KM, DELFERT D AND JUNGER KD. 1986. A direct colorimetric assay for Ca2+ -stimulated ATPase activity. Anal Biochem 157(2): 375-380.

CHANG CC, CHANG CY, HUANG JP AND HUNG LM. 2012. Effect of resveratrol on oxidative and inflammatory

stress in liver and spleen of streptozotocin-induced type 1 diabetic rats. Chin J Physiol 30(55): 192-201.

CHANG CC, CHANG CY, WU YT, HUANG JP, YEN TH AND HUNG LM. 2011. Resveratrol retards progression of diabetic nephropathy through modulations of oxidative stress, proinflammatory cytokines, and AMP-activated protein kinase. J Biomed Sci 23(18): 47.

CHEN KH, CHENG ML, JING YH, CHIU DTY, SHIAO MS AND CHEN JK. 2011. Resveratrol ameliorates metabolic disorders and muscle wasting in streptozotocin-induced diabetic rats. Am J Physiol Endocrinol Metab 301(5): E853-E863.

DZEJA PP, BORTOLON R, PEREZ-TERZIC C, HOLMUHAMEDOV EL AND TERZIC A. 2002. Energetic communication between mitochondria and nucleus directed by catalyzed phosphotransfer. Proc Natl Acad Sci 23(99): 10156-10161.

DZEJA PP, HOYER K, TIAN R, ZHANG S, NEMUTLU E, SPINDLER M AND INGWALL JS. 2011. Rearrangement of energetic and substrate utilization networks compensate for chronic myocardial creatine kinase deficiency. J Physiol 1(589) (Pt 21): 5193-5211.

DZEJA PP AND TERZIC A. 2003. Phosphotransfer networks and cellular energetics. J Exp Biol 206(Pt 12): 2039-2047.

DZEJA PP, VITKEVICIUS KT, REDFIELD MM, BURNETT JC AND TERZIC A. 1999. Adenylate Kinase–Catalyzed Phosphotransfer in the Myocardium. Circ Res 28(84): 1137-1143.

ERECIŃSKA M AND SILVER IA. 1994. Ions and energy in mammalian brain. Prog Neurobiol 43(1): 37-71.

ERIKSSON UJ, BORG LAH, CEDERBERG J, NORDSTRAND H, SIMÁN CM, WENTZEL C AND WENTZEL P. 2000. Pathogenesis of diabetes-induced congenital malformations. Ups J Med Sci 105(2): 53-84.

FAUQUIER DA, MAZET JAK, GULLAND FMD, SPRAKER TR AND CHRISTOPHER MM. 2008. Distribution of tissue enzymes in three species of pinnipeds. J Zoo Wildl Med Off Publ Am Assoc Zoo Vet 39(1): 1-5.

FEKSA LR, CORNELIO AR, DUTRA-FILHO CS, DE SOUZA WYSE AT, WAJNER M AND WANNMACHER CMD. 2003. Characterization of the inhibition of pyruvate kinase caused by phenylalanine and phenylpyruvate in rat brain cortex. Brain Res 11(968): 199-205.

FRANCESCHI ID DE, RIEGER E, VARGAS AP, ROJAS DB, CAMPOS AG, RECH VC, FEKSA LR AND WANNMACHER CMD. 2013. Effect of Leucine Administration to Female Rats During Pregnancy and Lactation on Oxidative Stress and Enzymes Activities of Phosphoryltransfer Network in Cerebral Cortex and Hippocampus of the Offspring. Neurochem Res 38(3): 632-643.

GARCÍA-GALICIA MC, BURGUEÑO-TAPIA E, ROMERO-ROJAS A, GARCÍA-ZEBADÚA JC, CORNEJO-GARRIDO J AND ORDAZ-PICHARDO C. 2014. Anti-hyperglycemic effect, inhibition of inflammatory cytokines expression, and histopathology profile in streptozotocin-induced diabetic rats treated with *Arracacia tolucensis* aerial-parts extracts. J Ethnopharmacol 27(152): 91-98.

GHOSH S, CHOWDHURY S, SARKAR P AND SIL PC. 2018. Ameliorative role of ferulic acid against diabetes associated oxidative stress induced spleen damage. Food Chem Toxicol 118: 272-286.

GURUSAMY N, LEKLI I, MUKHERJEE S, RAY D, AHSAN MK, GHERGHICEANU M, POPESCU LM AND DAS DK. 2010. Cardioprotection by resveratrol: a novel mechanism via autophagy involving the mTORC2 pathway. Cardiovasc Res 1(86): 103-112.

HALL ER AND COTTAM GL. 1978. Isozymes of pyruvate kinase in vertebrates: their physical, chemical, kinetic and immunological properties. Int J Biochem 9(11): 785-793.

HUGHES BP. 1962. A method for the estimation of serum creatine kinase and its use in comparing creatine kinase and aldolase activity in normal and pathological sera. Clin Chim Acta Int J Clin Chem 7: 597-603.

JAITOVICH AA AND BERTORELLO AM. 2006. Na^+, K^+ -ATPase: an indispensable ion pumping-signaling mechanism across mammalian cell membranes. Semin Nephrol 26(5): 386-392.

KABELITZ D, GEISSLER EK, SORIA B, SCHROEDER IS, FÄNDRICH F AND CHATENOUD L. 2008. Toward cell-based therapy of type I diabetes. Trends Immunol 29(2): 68-74.

KAPLAN JH. 2002. Biochemistry of Na^+, K^+-ATPase. Annu Rev Biochem 71: 511-535.

KIM I, RODRIGUEZ-ENRIQUEZ S AND LEMASTERS JJ. 2007. Selective degradation of mitochondria by mitophagy. Arch Biochem Biophys 15(462): 245-253.

KUMAR R., KAUR K, UPPAL S AND MEHTA SK. 2017. Ultrasound processed nanoemulsion: A comparative approach between resveratrol and resveratrol cyclodextrin inclusion complex to study its binding interactions, antioxidant activity and UV light stability. Ultrason Sonochem 37: 478-489.

LEE SM, YANG H, TARTAR DM, GAO B, LUO X, YE SQ, ZAGHOUANI H AND FANG D. 2011. Prevention and treatment of diabetes with resveratrol in a non-obese mouse model of type 1 diabetes. Diabetologia 54(5): 1136-1146.

LENZEN S. 2008. The mechanisms of alloxan-and streptozotocin-induced diabetes. Diabetologia 51(2): 216-226.

LEY EJ, SINGER MB, CLOND MA, JOHNSON T, BUKUR M, CHUNG R, MARGULIES DR AND SALIM A. 2012. Long-term effect of trauma splenectomy on blood glucose. J Surg Res 177(1): 152-156.

LOWRY OH, ROSEBROUGH NJ, FARR AL AND RANDALL RJ. 1951. Protein measurement with the Folin phenol reagent. J Biol Chem 193(1): 265-275.

MATSUMOTO N, ISHIHARA T, NAKASHIMA K, MIWA S AND UCHINO F. 1972. Sequestration and destruction

of reticulocyte in the spleen in pyruvate kinase deficiency hereditary nonspherocytic hemolytic anemia. Nihon Ketsueki Gakkai Zasshi J Jpn Haematol Soc 35(4): 525-537.

MATTÉ C, SCHERER EBS, STEFANELLO FM, BARSCHAK AG, VARGAS CR, NETTO CA AND WYSE ATS. 2007. Concurrent folate treatment prevents Na^+, K^+-ATPase activity inhibition and memory impairments caused by chronic hyperhomocysteinemia during rat development. Int J Dev Neurosci Off J Int Soc Dev Neurosci 25(8): 545-552.

MATTEVI A, BOLOGNESI M AND VALENTINI G. 1996. The allosteric regulation of pyruvate kinase. FEBS Lett 24(389): 15-19.

MORISHITA H. 2004. Antioxidant activity and hypoglycemic effect of ferulic acid in STZ-induced diabetic mice and KK-Ay mice. Biofactors 21(1-4): 315-319.

NISHIHIRA VSK ET AL. 2017. Resveratrol inclusion complex with β-cyclodextrin (RCD): characterization and evaluation of toxicity in wistar rats. Int J Pharm Pharm Sci 9(1): 27-33.

NYAGA DM, VICKERS MH, JEFFERIES C, PERRY JK AND O'SULLIVAN JM. 2018. The genetic architecture of type 1 diabetes mellitus. Mol Cell Endocrinol 5(477): 70-80

PRESSLEY TA, DURAN MJ AND PIERRE SV. 2005. Regions conferring isoform-specific function in the catalytic subunit of the Na, K-pump. Front Biosci 1(10): 2018-2026.

RUDGE MVC, DAMASCENO DC, VOLPATO GT, ALMEIDA FCG, CALDERON IMP AND LEMONICA IP. 2007. Effect of *Ginkgo biloba* on the reproductive outcome and oxidative stress biomarkers of streptozotocin-induced diabetic rats. Braz J Med Biol Res 40(8): 1095-1099.

RÜFER A AND WUILLEMIN WA. 2013. The spleen in non-malignant haematological disorders. Ther Umsch Rev Ther 70(3): 157-162.

SHI Q, ZENG J, DONG Y AND XU KY. 2013. Concurrent impairment of (Na^+, K^+)-ATPase activity in multi-organ of type-1 diabetic NOD mice. J Diabetes Complications 27(1): 29-033.

SIMÃO F, MATTÉ A, MATTÉ C, SOARES FMS, WYSE ATS, NETTO CA AND SALBEGO CG. 2011. Resveratrol prevents oxidative stress and inhibition of Na(+) K(+)-ATPase activity induced by transient global cerebral ischemia in rats. J Nutr Biochem 22(10): 921-928.

SZKUDELSKI T AND SZKUDELSKA K. 2011. Anti-diabetic effects of resveratrol. Ann N Y Acad Sci 1215: 34-39.

TEIXEIRA BN, OZDEMIR N, HILL LE AND GOMES CL. 2013. Synthesis and characterization of nano-encapsulated black pepper oleoresin using hydroxypropyl beta-cyclodextrin for antioxidant and antimicrobial applications. J Food Sci 78(12): 1913-1920.

THORVALDSON L, JOHANSSON SE, HÖGLUND P AND SANDLER S. 2005. Impact of plastic adhesion *in vitro* on analysis of Th1 and Th2 cytokines and immune cell distribution from mice with multiple low-dose streptozotocin-induced diabetes. J Immunol Methods 20(307): 73-81.

TONG Z, LIU W, YAN H AND DONG C. 2015. Interleukin-17A deficiency ameliorates streptozotocin-induced diabetes. Immunology 146(2): 339-46.

TORRES V, HAMDI M, MILLÁN DE LA BLANCA MG, URREGO R, ECHEVERRI J, LÓPEZ-HERRERA A, RIZOS D, GUTIÉRREZ-ADÁN A AND SÁNCHEZ-CALABUIG MJ. 2018. Reprod Domest Anim 53(4): 850-858.

TROLLOPE L, CRUICKSHANK DL, NOONAN T, BOURNE SA, SORRENT M, CATENACCI L AND CAIRA MR. 2014. Inclusion of trans-resveratrol in methylated cyclodextrins: synthesis and solid-state structures. Beilstein J Org Chem 29(10): 3136-3151.

UMPIERREZ G AND FREIRE AX. 2002. Abdominal pain in patients with hyperglycemic crises. J Crit Care 17(1): 63-67.

WALLIMANN T, DOLDER M, SCHLATTNER U, EDER M, HORNEMANN T, O'GORMAN E, RÜCK A AND BRDICZKA D. 1998. Some new aspects of creatine kinase (CK): compartmentation, structure, function and regulation for cellular and mitochondrial bioenergetics and physiology. Biofactors 8(3-4): 229-234.

WALLIMANN T, WYSS M, BRDICZKA D, NICOLAY K AND EPPENBERGER HM. 1992. Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the "phosphocreatine circuit" for cellular energy homeostasis. Biochem J 1(281): 21-40.

WU J AND YAN LJ. 2015. Streptozotocin-induced type 1 diabetes in rodents as a model for studying mitochondrial mechanisms of diabetic β cell glucotoxicity. Diabetes Metab Syndr Obes 2(8): 181-188.

WUJAK M, CZARNECKA J, GORCZYCKA M AND HETMANN A. 2015. Human adenylate kinases - classification, structure, physiological and pathological importance. Postepy Hig Med Dosw 2(69): 933-945.

WYSE AT, NORILER ME, BORGES LF, FLORIANO PJ, SILVA CG, WAJNER M AND WANNMACHER CM. 1999. Alanine prevents the decrease of Na+, K+-ATPase activity in experimental phenylketonuria. Metab Brain Dis 14(2): 95-101.

XIAO W, WANG R, HANDY DE AND LOSCALZO J. 2018. NAD(H) and NADP(H) Redox Couples and Cellular Energy Metabolism. Antioxid Redox Signal 20(28): 251-272.

YAN YB. 2016. Creatine kinase in cell cycle regulation and cancer. Amino Acids 48(8): 1775-1784.

ZAFAR M AND NAEEM-UL-HASSAN NAQVI S. 2010. Effects of STZ-Induced Diabetes on the Relative Weights of Kidney, Liver and Pancreas in Albino Rats: A Comparative Study. Int J Morphol 28(1): 135-142.