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Protective effect of vitamin C on oxidative stress and DNA damage in rats with diabetes mellitus

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ABSTRACT

Diabetes mellitus (DM) is a metabolic disease of origin endocrine disease, whose main biochemical characteristic is chronic hyperglycemia. Triggers serious biochemical processes for the body such as oxidative stress (OE). The objective of this study was to evaluate the protective effect of vitamin C (VitC) on EO in rats Sprague-Dawley with Streptozotocin (STZ) -induced DM. 16 rats were used (100 ± 20 g), divided into 4 groups: 1) control, 2) STZ (diabetic), 3) VitC + STZ, 4) VitC. In homogenized from liver I know determined the concentration from malondiahaldehyde, dienes conjugated and protein totals. By other part I know they determined the activities from superoxide dismutase Y catalase. Too I know measured the hurt to the DNA hepatic by test Kite. The results I know they analyzed statistically through Program SPSS version 17.0 for Windows. Applied a Anova Y a test from comparison multiple DMS (p <0.05). It could show that in the group from animals treaties with VitC decreased the levels from glucose and the generation from EO; too there was less hurt of the DNA by radicals free. These data allow infer that the VitC exercises a effective action antioxidant and protective of the liver before the permanent production from radicals free; further from to be effective in the repair and conservation of the DNA.

Key words: rat, induced diabetes mellitus, vitamin C, DNA damage.

INTRODUCTION

Diabetes mellitus (DM) comprises a group of metabolic disorders characterized by a concentration elevated from glucose in blood. further from the hyperglycemia, clinical picture includes glycosuria, ketosis and acidosis, between others damage. Exist various types from DM the which I know must to a complex interaction between genetics, factors environmental and styles from lifetime. The from higher incidence They are the types I and II. In type I DM, destruction of cells occurs 13 beta of the pancreas, which leads to a relative or absolute insulin. This class is known as immune-mediated diabetes and represents 5 to 10% of the cases. Type II DM covers 90-95% of cases and is characterized by varying degrees of resistance to insulin,

insulin secretion disorders and increased glucose production. The risk of developing type II DM increases with 13 age, obesity, and lack of physical activity. The disease, being associated with an increase in risk of suffering from cardiovascular diseases, predisposes to a death early. Likewise, elevate the possibility from suffer blindness, insufficiency renal and amputations from members. Due to the great proliferation of this disorder, 2. 3 by 2025 the number of people with DM in the American continent will be approximately 64 million and 62% of cases will occur in America Latin and the Caribbean . In Venezuela, the number of people with diabetes it varies between 460,000 and 1,000,000 3. Due to its increasing prevalence, DM is considered a public health problem at the worldwide, both in developed and developing countries developmental. Its current prevalence is 3.5 to 4.5%. The highest incidence corresponds to Finland, where prevalence ranges between 35 and 40 people / 100,000 inhabitants, almost twice the rate of the United States, as indicated in 2003. If the sub-record of DM, she would reach 6%, of which 85% it would correspond to type II DM. In Venezuela, more than a million people suffer from it, of which half I know find without diagnosis. Oxidative stress (OE) has been linked to (9) pathogenesis of diabetes mellitus. The increase in free radicals (RL) aggravate the action of insulin to peripheral level, contributes to cell dysfunction pancreatic beta and is involved in the development of chronic complications(10, 19, 21). The body maintains an oxide-reduc-(11, 12) constant production, preserving the balance between production from pro-oxidants that I know generate as Outcome of the metabolism mobile and the systems from defending antioxidants. The lost from such balance carries yet state from stress oxidative, the which I know characterizes by a increase in the levels from RL and species reactive from oxygen, that no achieves to be compensated by the systems from defending antioxidant, causing hurt and death mobile. This occurs in infectious degenerative pathologies, immune and inflammatory, such as DM. Alteration of the balance between pro oxidants and antioxidants, may to have various degrees from magnitude. DNA, hurt to the conveyors from ions and proteins through from membranes and peroxidation from lipids. In diabetic patients there is an imbalance(8) between antioxidant and oxidative mechanisms, having proved a decrease from the levels plasmatic from enzymes antioxidants, from glutathione and from vitamins antioxidants. On the other hand, in these patients there is evidence of an increase in lipid peroxidation mediated by free radicals. Antioxidants in the diet play a important role in defense against aging and chronic diseases such as DM, cancer and cardiovascular disease. These substances inactivate the RLs involved in the EO and prevent its spread. Supplementation with antioxidants could have a beneficial effect by improving the morbidity and mortality of diabetic patients , in such a way that they could prevent and delay the development of complications chronic diabetes(5, 6, 7). In this longitudinal section investigation we we propose to determine the protective effect of the vitamin C (VitC) On the EO In the DM experimental.

MATERIAL AND METHODS

16 female Sprague-Dawley rats were used belonging to the population of the Central animals of the Al-Qadisiyah University, college of veterinary medicine (Iraq). The weight average from the rats was from 100 g. I know they kept in cages individual, with free access to the Water and one diet commercial standard, low cycles from light from 12 hours. The rats were divided into four groups: 1 = control, group 2 = diabetic (injected with streptozotocin (STZ) to dose from 60 mg / kg from weight, group 3= VitC + STZ (treaties with VitC 700 mg / kg via oral+STZ) and group 4 = treated with VitC). On day 1 of the experiment, all animals a blood sample was drawn (without subjecting them to fasting), by means of a cardiac puncture. The blood centrifuged at 3500 revolutions per minute, for 15 minutes, at 15 ° C in an Eppendorf 5402 centrifuge (Westburry, NY, USA). In plasma, glucose level was determined by Trinder's enzymatic method (1969) by kit Qualitest. These blood tests were repeated the day of the sacrifice. Rats in groups 2 and 3 were induced diabetes by administering an injection intraperitoneal STZ (60 mg / kg per 1000 g of body weight) diluted in sodium citrate buffer. Twenty Experimental groups 3 and 4 were administered the treatment with VitC (700 mg / kg in the Water from drink, During 6 days), starting of the fifth day from the induction from diabetes. Then from a fast from 12 h the rats I know they sacrificed low light etherization and I know dissected the liver for obtain a homogenized of the tissue, checking in his appearance macroscopic. The homogenate was kept on ice and in the supernatant I know they determined the levels from concentrations malondialdehyde (MDA) by the test for reactants with 2-thiobarbituric acid (Ohkawa 1979 technique), conjugated dienes (DC) by the method described by Wallin (1993) and total protein through the kit BioRad Richmond, based in the method from Bradford (1976)(4).

A stock solution of 1.41 mg / ml bovine serum albumin and then a piece of the liver was homogenized with 0.25 M sucrose and diluted to 10% w / v to be centrifuged at 12,000 rpm at 4 ° C for 10 minutes. The supernatant was separated with Pasteur pipette and in it the activity of the superoxide dismutase (SOD) using a commercial kit (Calbiochem). Another piece of liver tissue was homogenized with the buffer solution provided by the Calbiochem kit and catalase activity was determined in the supernatant (CAT) through the protocol from Aebi. For to size the hurt to the DNA hepatic in rats occasioned by the DM, I know performed a electrophoresis in cells individual("test Kite"), technique based in the capacity from the fragments from DNA for to be embedded in a gel from agarose and respond yet countryside electric. The run of the extended from DNA It depends directly of the hurt of the DNA Present in the cells. It is important to mention that DNA damage consist of chain breakage after treatment with alkali or without East, and even from the combination with certain enzymes, the which increase the migration from DNA. Thus, the cells under study are placed in agarose, lysed and then electrophoresed under conditions specific. In lysis, cells lose their proteins and are unable to repair damaged DNA. During electrophoresis, damaged and fragmented DNA migrates inside of the gel since the core toward the direction of the anode. The quantity from DNA my degree It constitutes a measure from the extension of the hurt. DNA observation was carried

out through the staining with the etidium bromide dye, being examined for emission fluorescence with a filter excitation of 515 nm and emission of 560 nm. The cells that they contain DNA damaged appear then from the electrophoresis with appearance from Kite, with a head sparkly and one tail. By the contrary, the cells that they contain DNA without injury, appear with a core intact and without tail. The results were statistically analyzed(18) Using the SPSS version 17.0 program for Windows, applying an Anova test and a comparison test multiple with least significant difference-DMS ($p < 0.05$).

RESULTS AND DISCUSSION

The study carried out showed an increase in blood glucose levels in rats treated with STZ. The data confirmed the diabetogenic effect caused by this compound, which manifests itself up to 7 days post-induction (Figure 1). This result match with the obtained by others researchers, who point out that STZ generates a diabetogenic effect in the animals from laboratory.

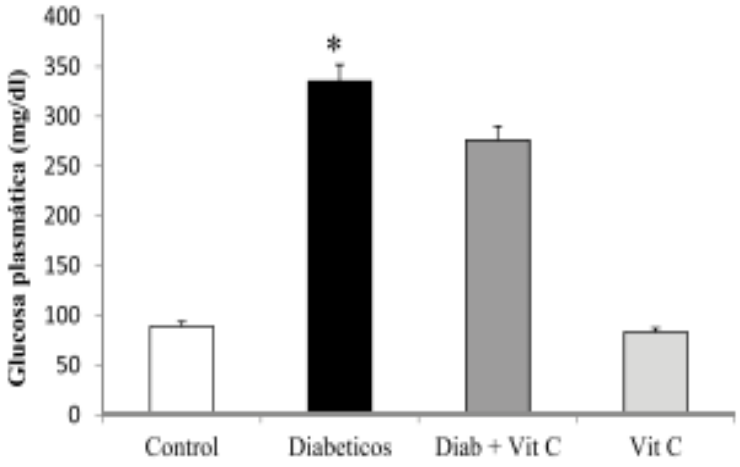


Figure 1. Effect of vitamin C on blood glucose of control and experimental rats with diabetes (X ± DS). Asterisk indicates significant difference (* $p < 0.05$).

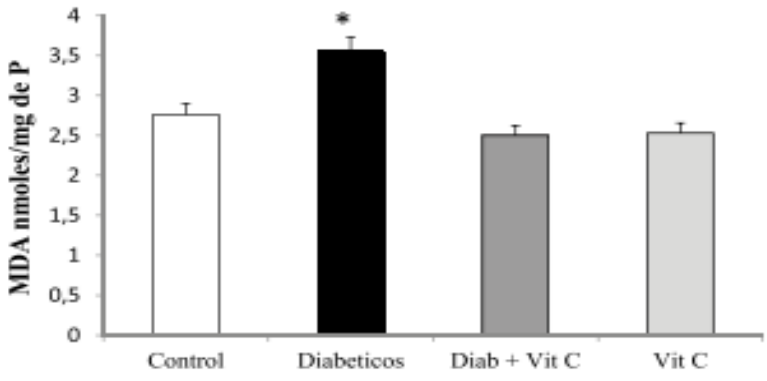


Figure 2. Effect of vitamin C on concentration liver from malondialdehyde in rats controls And experimental (X ± DS). Asterisk indicates* p <0.05.

It has also been shown that the administration(10, 16) high doses of STZ in laboratory animals causes the death of beta cells of the islets of Langerhans of the pancreas in 24 h and his administration in dose low It allows perform the study cytotoxic from East compound. The increase in glucose concentration causes (14) proportional increase in glycosylation products no enzymatic, that may to be scarcely harmful yes I know achieves a good control metabolic, but in case contrary generate products endings from the glycosylation advanced, with degradation oxidative and release from RL that damage the structure and functionality from the protein. The imbalance between RL production and anti-oxidants generate oxidative stress(17), mild EO can lead the cell to have more resistance to injuries later in a good regulation of the systems of antioxidant defense. On the contrary, if the EO is very intense, would affect all components of the cell (DNA, lipids and proteins). In addition to the characteristic hyperglycemic picture, (15) chronic disease, DM is responsible for these types of complications, by the that to what long from the years I know have wanted alternatives therapeutic for to get better the quality from lifetime from patients diabetics, as it is the case from the

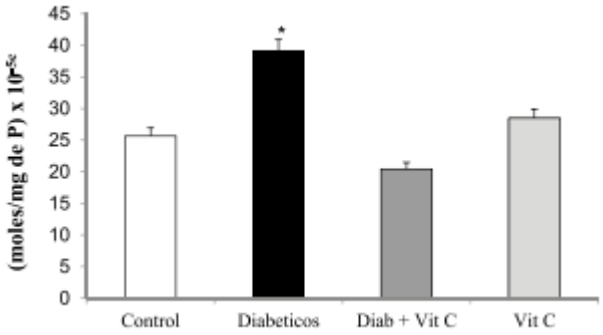


Figure 3. Effect of vitamin C on concentration Liver from dienes conjugated in rats controls and experimental (X ± DS). Asterisk indicates* p <0.05.

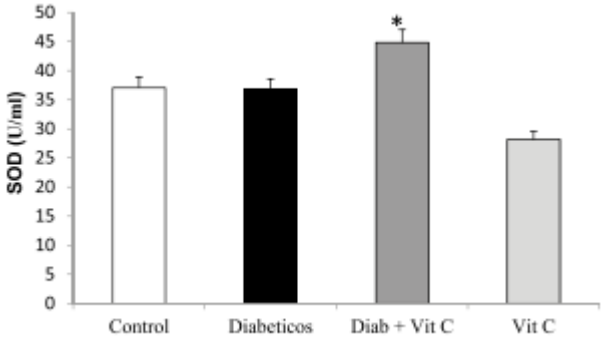


Figure 4. Effect of vitamin C on concentration Liver from superoxide dismutase in rats controls and experimental (X ± DS). Asterisk indicates* p <0.05.

VitC, which -for its benefits- has been used as antioxidant. In this research the protective effect was measured of VitC before the generation of oxidative stress and damage to DNA in rats with experimental diabetes. As soon as to the degree of EO, a hepatoprotective effect could be observed from the VitC, evidenced by a decrease very significant (p <0.05) from the levels from MDA (nmoles /mg from proteins) in the group from diabetics treaties with VitC, to the compare it with the group control (Figure 2). In the case from the DC, products initials of the process from lipoperoxidation, I know I observe a decrease from the themselves in the animals treaties with the antioxidant compared to the control (Figure 3). When measuring the SOD enzyme observed (Figure 4) a significant increase of its activity (p <0.05) expressed in U / ml in the group of animals treated with VitC, when comparing it with the group control. In shape Similary, I know got a increase significant in the exercise from CAT (Figure 5). There is experimental evidence which indicates that the EO can determine the Start and the progression

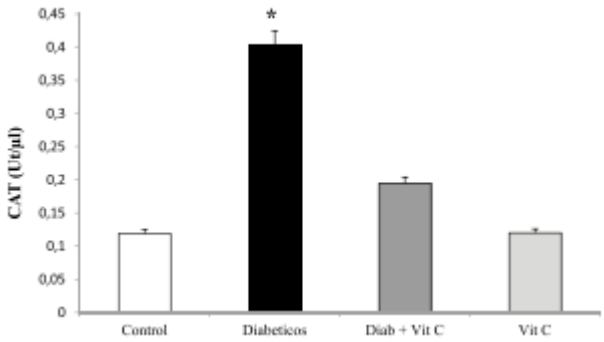
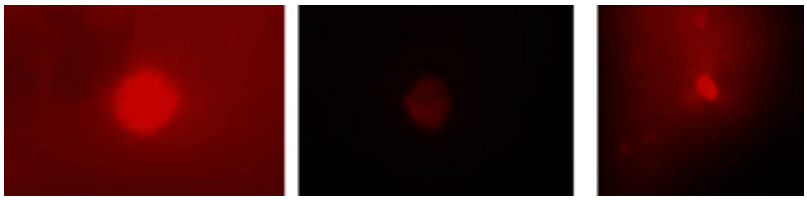


Figure 5. Effect of vitamin C on concentration Liver from catalase in rats controls and experimental (X ± DS). Asterisk indicates* p <0.05.

late complications of DM; Nevertheless there is controversy as to whether its increase is associative or causal from is disease. It has been proved that exists increase from RL accompanied from a decrease from agents antioxidants in patients diabetics. Free radicals are atoms or molecules that in their last orbital have an unpaired electron; when trying from obtain his stability, may affect the physiology from the cells to the oxidize to the lipids from membrane, to the carbohydrates, at protein or to DNA, the which would be a hurt caused by the EO. In this investigation the effect of VitC on the possible alteration in DNA caused by EO.(8) Figure 6 shows the migration of the DNA, by forming a clear image of the "tail of the come t a ", obtained in diabetic animals. Conversely, the image coming from of the group control no reveals migration, neither training from the tail, While that in the coming from of the group treaty with VitC I know I observe a mild migration and-in Some cases- images very

Similar to the group control. It allows infer that the VitC prevent damage in the DNA, characteristic in the patients with diabetes.

a-control b-DM c-DM



d-DM+VitC e- DM+VitC f- DM+VitC

In conclusion, it appears that vitamin C constitutes an effective antioxidant and an important protector of liver, due to the permanent production of free radicals sources from various sources, in addition to being effective in DNA repair and preservation.

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