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BIOCHEMICAL AND HISTOLOGICAL PARAMETERS IN INDUCED TYPE 2 DIABETIC RATS FED WITH HIGH FAT DIET WITHOUT EXOGENOUS CHOLESTEROL AND LOW-DOSE STREPTOZOTOCIN

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ABSTRACT

The objective of the present study is to establish a rat model for diabetic type 2 (T2DM) fed with high fat diet without addition of exogenous cholesterol and low streptozotocin (STZ) dose aiming higher survival rate under the local experimental conditions for evaluating sources of biodiversity to supply alternative energy for T2DM individuals mainly from the Pantanal (wet land) and the Savanna-like area (Cerrado) of the Mato Grosso do Sul State. Male Wistar rats were dividided into five groups and fed with normal pellet diet or with high-fat diet HFD), after 21 days the animals received a single intraperitoneal injection of STZ (0, 15, 25 and 35 mg/kg body weight). Animals with fasting blood glucose over 250 mg/dL, seven days after STZ administration were considered T2DM. The HFD + STZ (35 mg/kg bw) group produced significantly hyperglycemia which clearlly shows insulin resistance confirmed by insulin levels and HOMA values. The biochemical profile showed an increase in the concentration of triacylglycerydes, VLDL-C, AST, ALT and lower levels of HDL-C, LDL-C, total cholesterol in the group HFD + STZ (35mg/kg bw). Morphological differences were detected in the liver, kidney and pancreas between the HFD and the HFD + STZ (15, 25 and 35 mg/kg bw) groups.

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INTRODUCTION

Diabetes mellitus (DM) is a group of multifactorial metabolic diseases, characterized by high blood glucose; defect of insulin secretion and/or in its signal transduction pathways. Several pathogenic processes are involved such as destruction of pancreatic β cells, which impairs insulin secretion and alteration in the peripheral tissues leading to insulin resistance (Malecki, 2005; Kumar *et al*., 2012; ADA, 2014; Subramanian, 2014).

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The prevalence of worldwide DM (type 1 and 2) in the adult population (20-79 years) was estimated to be 382 millions in the year of 2013 corresponding to 8.3%. By the year of 2035 the DM population might reach 592 millions corresponding to 10.0% of the estimated population (https://www.idf.org/ sites/default/files/EN_6E_Atlas_Full_0.pdf); (https:// www.idf.org/ sites/default/ files/DA-regional-factsheets-2014_FINAL.pdf). The augment of DM incidence is related to different factors such as population aging, growing urbanization, sedentary lifestyle, inadequate diet and obesity. The majority of the diabetic population carries type 2 diabetes mellitus (T2DM), 90-95% of the patients (ADA, 2015;

http://www. diabetes.org.br/ images/pdf/conduta-terapeutica-no-dm2-algorit mo-sbd-2014-versa o-final-impressao.pdf) . In this respect Brazil is internationally ranked in the fourth place and in South and Central America it is the country with the greatest number of diabetic patients (https://www.idf.org/sites/ default/files/ EN_6E_Atlas_Full_0.pdf.; https://www.idf.org/ sites/default/ files/DA-regional-factsheets-2014_FINAL.pdf. T2DM is related to the reduction of insulin secretion by pancreas and resistance to insulin action in the peripheral tissues, diminishing glucose uptake and leading to a chronic hyperglycemia. Primary pathogenesis probably involves genetic threats and environmental conditions such as hyperglycemia and hyperlipidemia which can damage the β cells, an event known as glycolipotoxicity (Poitout, 2008).

To treat T2DM patients several approaches have been used in the medical clinic such as adequate diet, physical exercises, and healthy lifestyle and in the more severe cases the use of hypoglycemic drugs. There are several synthetic commercial hypoglycemic medicines as well as those extracted from natural sources. Biodiversity has provide sources for folk´s medicines which have demonstrated potential for the treatment of diabetes, thus, there is an increasing need to search for more natural antidiabetic agents from the traditional medicine. Some were efficient as an antihyperglycemic and antihyperlipidemic on HFD + STZ induced diabetic rats, such as quercetin extracted from *Dodonaea viscosa* (L). Jacq. (Sapindaceae) (Veerapur *et al*., 2010), bergerin extracted from *Ficus racemosa* Linn (Veerapur *et al*., 2012). naringenin, a flavonone found in *Citrus sp* mainly grapefruit (Priscilla *et al*., 2015), trigonelline is, a alkaloid extracted from fenugreek seeds *Trigonella foenum graecum* (Subramanian, 2014).

In our research group we have been looking for alternative sources for supplying energy for individuals with T2DM syndrome. The richness of food sources in both Pantanal (wet lands) and Savanna-like area of Central Brazil (the Cerrados) in the State of Mato Grosso do Sul provide alternative energy sources to carbohydrate. One species with great potential for that is macauba (macaw) palm (*Acrocomia aculeata* (Jacq) Lood. Ex Mart.), which is a traditional staple food for native Indians and for people living in rural areas. The fatty acid composition and its physicochemical properties of macaw oil is well characterized by Nunes *et al*. (2015). To investigate the potential of the local biodiversity to supply alternative energy sources for individuals carrying T2DM we have established an animal model mimicking the human syndrome, which involved a HFD associated with the injection of low-dose STZ.

This model has been used because the HFD leads to increased adiposity and reduced effectiveness of insulin mainly in the peripheral tissues (Veerapur *et al*., 2012). STZ is widely used to induce diabetes in rats. High doses are used to induce T1DM, and low doses to induce T2DM (Reed *et al*., 2000). STZ is a nitrosourea analogue which induces the breakage of DNA strands in β cells, cell death related to alkylation of DNA, and, consequently an increase in the blood sugar level in experimental animals (Delaney *et al*., 1995; Elsner *et al*., 2000). The objective of the present study is to establish a rat model for T2DM fed with high fat diet without addition of exogenous cholesterol and low STZ dose aiming higher survival rate under the local experimental conditions for evaluating biodiversity sources to supply alternative energy for T2DM individuals.

MATERIALS AND METHODS

Experimental Animal

Male albino Wistar rats, weighting 200-220 g were used. The animals were obtained from the Central Laboratory for animals, Dom Bosco Catholic University (UCDB), Campo Grande, MS, Brazil. The animals were housed in standard sanitized polypropylene cages containing paddy husk as bedding (2 − 3 rats/cage) and maintained under controlled conditions, temperature (22 ± 2 °C) and light-dark cycles (12 h). The animals were acclimatized for a week and had free access to standard pellets as basal diet (commercial standard chow, Nuvilab® CR-1, Nuvital, PR, Brazil) and water *ad libitum*. The experiments with rats were approved by Dom Bosco Catholic University Committee for Ethics in Animal Experimentation under protocol N° 003/2015. We selected only male animals, since females are shown to be protected from changes in lipid-induced insulin action (Hevener *et al*., 2012). Body weight, food and water intake were measured every two days during 28 days using a semi-analytical balance.

Induction T2DM by feeding high fat diet (HFD) and low dose streptozotocin (STZ)

A total of 35 rats were used in our study. The animals were divided into five groups of seven animals each. Group I: STD Control; Group II: HFD Control; Group III: HFD + STZ (15 mg/kg bw); Group IV: HFD + STZ (25 mg/kg bw); Group V: HFD + STZ (35 mg/kg bw). The rats were allocated into two dietary regimens and were fed *ad libitum* with standard pellet diet - STD or high fat diet - HFD (58% fat - lard, 25% protein and 17% carbohydrate, as a percentage of total kcal), for three weeks. The composition of the HFD has been earlier described by Srinivasan *et al*. (2005) and was produced by the PragSolucões® Co. Company, Brazil, without the addition of exogenous cholesterol.

After three weeks of feeding high fat diet rats were submitted to 12 hours of fasting, followed by an administration of a single intraperitoneal dose of streptozotocin - STZ (Sigma-Aldrich, St. Louis, MO, USA. Ref S0130) dissolved in sodium citrate buffer (0.1 M, pH 4.5) (Sigma-Aldrich, St. Louis, MO, USA. Ref. W302600). Three doses of STZ (15, 25 and 35 mg/kg bw) in combination with the diet (HFD) were tested to define the optimum dose for induction of T2DM. The control groups (STD and HFD) received only the vehicle intraperitoneally. After 6 hours of STZ injection the rats were fed again on their respective diet and to prevent fatal hypoglycemia it was offered a solution of 5% dextrose (Sigma-Aldrich, St. Louis, MO, USA. Ref. D9434) for a period of 24 h. After this period the dextrose solution was removed and the animals were kept on their diet for one more week, totalizing 4 weeks. At the end of the fourth week, blood glucose levels were measured in fasted animals (12 hours), by the enzymatic glucose oxidase method using a commercial glucometer (Accu-Chek®Active, Roche Diagnostic, Mannheim, Germany); animals with blood glucose ≥ 250 mg/dL were considered diabetic. Fasting blood glucose (FBG) levels were monitored on days 0, 7, 14, 21 and 28 using a Glucometer Accu-Chek® Active (Roche Diagnostics, Mannheim, Germany). The animals were fasted for 12 hours and a blood drop collected from the distal part of the rat tail through a small incision and placed on the reagent strip.

Biochemical parameters

On the 28th day the animals were deprived of food for 12 h and euthanized by inhalational anesthesia (isoflurane). Blood was collected in tubes with and without anticoagulants (Vacuplast®collect line) and then centrifuged at 1900 *g* for 15 min and the plasma and serum were separated, stored at -20 °C and used for biochemical analysis. Epididymal adipose tissue, liver, kidney, heart and pancreas were removed, washed immediately rinsed in cold saline, dried and weighted. Blood glucose determined in the plasma, and total cholesterol (TC), triglycerides, high density lipoprotein cholesterol (HDL-C), creatinine, urea, albumin, total protein (TP), aspartate amino transaminase (AST) and alanine amino transaminase (ALT), were estimated in serum using diagnostic kit (Labtest Diagnostica SA, Lagoa Santa, MG, Brazil). Low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) were calculated based on the equation previously described (Friedewald *et al*., 1972). Serum insulin levels were analyzed using a commercially available ultra sensitive rat insulin ELISA kit (Crystal Chem. Inc., Downers Grove, IL 60515, USA). This assay had 100% cross-reactivity to rat insulin. The insulin resistance was evaluated by homeostasis model assessment of insulin resistance (HOMA-IR) as follows (Matthews *et al*., 1985).

Histological analysis

Liver, kidney and pancreas were rinsed with cold saline and tissues were fixed in buffered formalin 10% and stored. The tissues were embedded in paraffin wax, sectioned about 5 µm and then mounted on glass slides. The slides were deparaffinated and stained with hematoxylin and eosin. The stained sections were observed under the microscope (ZEISS, Axio Scope. A1, Germany) and the images were captured. The histological analyses were based on the following parameters: atrophy and cell vacuolization of the pancreatic islets, apoptotic bodies in the pancreas exocrine portion; liver steatosis and hepatocytes necrosis; kidney steatosis and hyaline degeneration of the renal tubules of cortical region. For each parameter there were established score: grade zero (absent), grade 01 (+/mild), grade 02 (++/moderate) and grade 03 (+++/intense).

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the Tukey test as post-test. The results were expressed as mean ± standard error of the mean (SEM), using software GraphPad Prism® version 5.00. $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Body weight, food and water intake

Figure 1A, shows the steady weight gain up to STZ injection, followed by a decline related to the amount of the drug administered. The final weight gain after 28 days was higher in the HFD group ($p<0.05$) although the STD group had higher food intake ($p<0.05$). This finding was also reported in studies in which wistar rats were used for induction of T2DM with HFD + STZ (Veerapur *et al*., 2014). Furthermore, there were no statistical differences in water consumption among the groups ($p>0.05$) (Table 1).

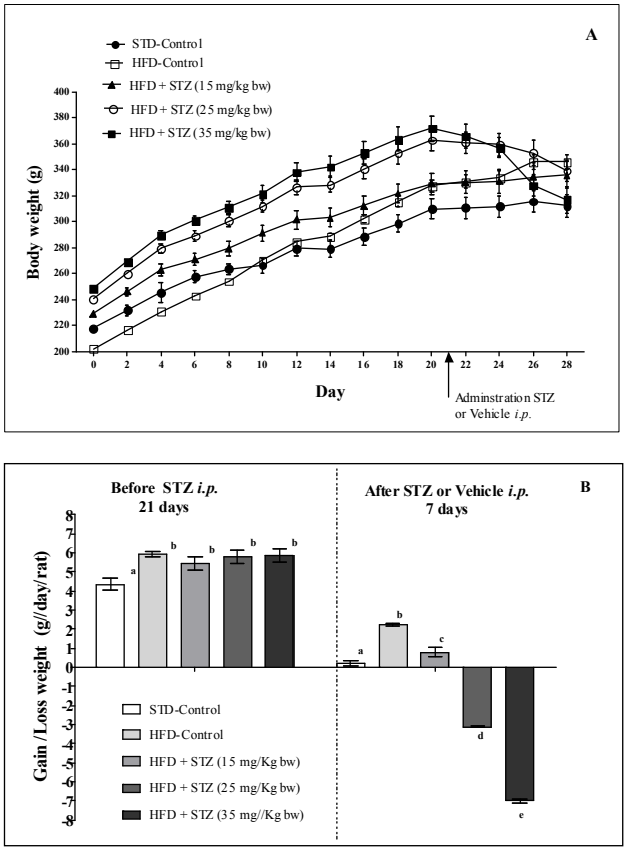

Figure 1. Effect of diet and STZ on rats body weight in the experimentals groups (A). The weight gain or loss before and after STZ in wistar rats (B). Data are expressed as mean ± SEM (n=7). Different letters between columns indicate significant differences ($p<0.05$). One-way ANOVA followed by the Tukey post-hoc test

The weight loss of the animals in the HFD + STZ treatment was related to the STZ dosage (15, 25 and 35 mg/kg bw) and it was accentuated in the HFD + STZ 35 mg STZ/kg bw treatment (Fig. 1 A, B). This profile was also described by Correia-Santos *et al*. (2012), using the same dose of STZ in a study conducted with female wistar rats. The weight loss could be explained based on a higher protein catabolism, lipolysis and lipid mobilization due to the lower plasma insulin levels (Sundaram *et al*., 2013) and to the observed animal dehydration. In diabetes, it is commonly observed symptoms such as hyperglycemia, reduction in body weight, polyuria, polydipsia and polyphagia (Ramachandran *et al*., 2012). In our study, besides the weight loss, the groups treated with HFD + STZ presented symptoms typical of diabetes type 2 such as polyuria in comparison with the control groups (STD and HFD). In the 28 days of experiment it was not observed any difference ($p>0.05$) among the weight of the organs measured (liver, heart and pancreas), however, there was increase ($p<0.05$) in the weight of the epididymal adipose tissue in the HFD control in comparison with the STD control group, the increased epididymal adipose tissue in HFD control group could be explained by high intake of caloric diet supplied with saturated fat. Furthermore, the epididymal adipose tissue in the HFD + (35 mg STZ/kg bw) was decreased ($p<0.05$) in comparison with the HFD control group, after STZ injection (Table 1). Figure 1B, shows the weight gain or loss before and after STZ treatment. During the period of 0 - 21 days representing the phase before STZ injection, all animals had a net daily weight gain.

Table 1. Body and organs weight, food and water intake in wistar rats

	STD (Control)	HFD (Control)	HFD + STZ (15 mg/kg bw)	HFD + STZ (25 mg/kg bw)	HFD + STZ (35 mg/kg bw)
Body weight (g)					
Initial	218.14 ± 1.14^{d}	202.29 ± 2.02^{e}	229.29 ± 1.52^{c}	240.71 ± 0.97^{b}	248.71 ± 2.13^{a}
Final	312.23 ± 8.84^{b}	346.24 ± 5.59^{a}	335.76 ± 10.35^{ab}	339.03 ± 8.06^{ab}	316.82 ± 10.43^{ab}
Food intake (g/rat/day)	24.49 ± 0.60^{a}	17.79 ± 0.33^{b}	17.08 ± 0.58^{b}	18.74 ± 0.64^{b}	18.44 ± 0.82^{b}
Water intake (mL/rat/day)	38.06 ± 1.63^{a}	39.67 ± 1.33^{a}	36.78 ± 1.28^{a}	37.25 ± 1.68^{a}	40.89 ± 1.93^{a}
Organs weight (g/rat)					
Epididymal adipose tissue	3.59 ± 0.44^{c}	10.12 ± 0.40^{a}	9.12 ± 0.83^{ab}	8.27 ± 0.40^{ab}	7.84 ± 0.31^{b}
Liver	9.19 ± 0.47^{a}	9.79 ± 0.27^{a}	9.40 ± 0.54^{a}	10.24 ± 0.17^{a}	9.89 ± 0.59^{a}
Pancreas	0.60 ± 0.07^{a}	0.54 ± 0.04^{a}	0.59 ± 0.05^{a}	0.55 ± 0.02^{a}	0.60 ± 0.01^{a}
Heart	1.15 ± 0.05^{a}	1.24 ± 0.04^{a}	1.17 ± 0.05^{a}	1.23 ± 0.05^{a}	1.11 ± 0.03^{a}

Data are expressed as mean ± SEM (n = 7). Different letters between columns indicate significant differences ($p < 0.05$). One-way ANOVA followed by the Tukey post-hoc test.

Table 2. Blood glucose fasting levels, determined by commercial glucometer during 28 days

	Blood glucose fasting level (mg/dL)				
Period	STD (Control)	HFD (Control)	HFD + STZ (15 mg/kg bw)	HFD + STZ (25 mg/kg bw)	HFD + STZ (35 mg/kg bw)
Day 0	101.86 ± 3.74^{a}	94.43 ± 2.54^{a}	96.86 ± 7.54^{a}	102.71 ± 2.24^{a}	105.71 ± 2.36^{a}
Day 7	97.71 ± 3.52^{a}	108.14 ± 1.58^{a}	105.86 ± 6.16^{a}	107.00 ± 2.65^{a}	113.43 ± 4.36^{a}
Day 14	91.57 ± 2.30^{a}	96.57 ± 2.36^{a}	95.43 ± 3.33^{a}	101.57 ± 4.96^{a}	102.29 ± 2.49^{a}
Day 21	90.57 ± 2.29^{a}	96.71 ± 1.02^{a}	96.86 ± 1.98^{a}	94.43 ± 1.86^{a}	94.29 ± 1.23^{a}
Day 28	96.86 ± 1.34^{b}	100.57 ± 2.61^{b}	101.00 ± 6.54^{b}	129.71 ± 16.56^{b}	369.14 ± 4.01^{a}

Data are expressed as mean ± SEM (n=7). Different letters between columns indicate significant differences ($p<0.05$). One-way ANOVA followed by Tuk ey post-hoc test.

Table 3. Effects of HFD and low doses of STZ on biochemical parameters in wistar rats

Parameters	Groups				
	STD (Control)	HFD (Control)	HFD + STZ (15 mg/kg bw)	HFD + STZ (25 mg/kg bw)	HFD + STZ (35 mg/kg bw)
Glucose (mg/dL)	140.76 ± 6.48^{b}	164.92 ± 7.85^{b}	152.46 ± 11.6^{b}	159.87 ± 6.79^{b}	412.70 ± 5.12^{a}
Serum insulin (μU/mL)	9.89 ± 0.01^{d}	13.83 ± 0.04^{bc}	13.35 ± 0.04^{c}	18.27 ± 0.04^{a}	8.43 ± 0.01^{ed}
HOMA-IR value	3.47 ± 0.06^{d}	5.88 ± 0.05^{b}	5.40 ± 0.10^{c}	8.52 ± 0.17^{a}	8.77 ± 0.14^{a}
Total cholesterol (TC)	89.96 ± 2.77^{b}	90.36 ± 2.10^{b}	90.12 ± 1.43^{b}	90.67 ± 2.26^{b}	78.90 ± 0.91^{a}
Triacylglycerol (TG)	107.69 ± 4.55^{b}	97.46 ± 2.29^{b}	106.30 ± 3.94^{b}	114.60 ± 5.36^{b}	164.64 ± 6.10^{a}
HDL-C	22.79 ± 1.88^{b}	20.67 ± 1.31^{b}	21.50 ± 1.32^{b}	22.50 ± 1.04^{b}	12.93 ± 0.95^{a}
LDL-C	49.11 ± 2.73^{b}	50.20 ± 1.57^{b}	49.70 ± 1.92^{b}	43.90 ± 1.00^{b}	29.80 ± 2.53^{a}
VLDL-C	20.78 ± 0.59^{b}	19.49 ± 0.46^{b}	21.24 ± 0.79^{b}	22.03 ± 0.73^{b}	33.88 ± 0.88^{a}
AST (U/L)	87.06 ± 7.22^{b}	88.44 ± 2.92^{b}	82.03 ± 2.88^{b}	89.09 ± 2.29^{b}	134.98 ± 7.09^{a}
ALT (U/L)	57.90 ± 3.37^{b}	45.84 ± 1.82^{b}	49.16 ± 4.62^{b}	59.40 ± 4.23^{b}	92.17 ± 4.21^{a}
Creatinine (mg/dL)	0.46 ± 0.04^{a}	0.53 ± 0.03^{a}	0.55 ± 0.07^{a}	0.53 ± 0.05^{a}	0.52 ± 0.05^{a}
Urea (mg/dL)	40.37 ± 1.79^{b}	37.54 ± 1.63^{b}	35.24 ± 1.71^{b}	40.83 ± 1.98^{b}	66.07 ± 4.74^{a}
Total Protein (g/dL)	5.49 ± 0.09^{a}	5.01 ± 0.34^{a}	5.23 ± 0.32^{a}	5.14 ± 0.07^{a}	5.25 ± 0.21^{a}
Albumin (g/dL)	3.03 ± 0.06^{a}	2.90 ± 0.07^{a}	2.83 ± 0.08^{a}	2.97 ± 0.06^{a}	2.42 ± 0.05^{b}

Data are expressed as mean ± SEM (n=7). Different letters between columns indicate significant differences ($p<0.05$). One-way ANOVA followed by the Tukey post-hoc test.

Nevertheless, after higher STZ dosages (25 and 35 mg/kg bw) the animals presented a significant daily weight loss ($p<0.05$). This finding could be explained based on the loss of muscle protein and catabolism of lipids (Sundaram *et al*., 2013). Furthermore, the total weight gain is higher ($p<0.05$) in the HFD control (144.0 g) when compared with the STD group (94.0 g), whereas there was a clear decrease ($p<0.05$) in the total weight gain in the HFD + STZ doses of 15 mg/kg/bw (106.0 g), 25 mg/kg bw (98.0 g) and 35 mg/kg bw (68.0 g).

Monitoring glucose levels

Glucose fasting levels was monitored in all groups on days 0, 7, 14, 21 and 28. Before STZ injection, there was no difference in the glucose fasting level among groups, which had an average of 100 mg/dL. After STZ administration, hyperglycemia was detected only in the HFD + STZ (35 mg/kg bw) (Table 2). Streptozotocin is a nitrosourea deoxy-S [((metil-nitrosoamino) carbonil)-amino)]–D glucopyranose produced by *Streptomyces achromogenes* that mediates DNA alkylation causing a total or partial damage of the pancreatic β-cells, leading to insulin deficiency which in turn increases the blood glucose level (Szkudelski, 2001; Szkudelski, 2012). The experimental data showed in (Table 2 and 3), clearly points out to the establishment of hyperglycemia in the HFD + STZ (35 mg/kg bw) group characterized by glucose fasting levels ≥ 250 mg/dL which was considered T2DM. Similar animal models using wistar rats have been established in the current literature (Priscilla, 2015).

Fasting blood glucose, serum insulin and HOMA-IR

As shown in Table 3, the blood glucose level of HFD + STZ (35 mg/kg bw) group was increased, produced significantly hyperglycemia ($p<0.05$) after injection of STZ, when compared with other groups, but the levels of serum insulin was not significantly different from the STD group, on the other hand, a hyperisulinemia was observed in the HFD group and HFD + STZ (15 and 25 mg/kg bw) groups. The HOMA model is used to estimate insulin sensitivity and β-cell function based on the fasting plasma insulin and glucose concentration (Matthews *et al*., 1985). The HOMA-IR index clearly shows high degree of insulin resistance in the HFD + STZ (25 and 35 mg/kg bw) groups.

The HFD and HFD + STZ (15 mg/kg bw) groups lead to a mild degree of insulin resistance (Table 3). There were no statistical differences between the insulin levels in the STD and HFD + STZ (35 mg/kg bw) group, however the latter group developed hyperglycemia, which clearlly shwons insulin resistance, confirmed by the the HOMA values (Table 3). In contrast, the HFD-control, HFD + STZ (15 and 25 mg/kg bw) groups, did not produce significant hyperglycemia although the insulin levels were higher than the STD group, which indicates insulin resistance. Srinivasan *et al*. (Srinivasan *et al*., 2005), also demonstrated higher production of insulin in rats fed with high fat diet in comparison with the control group. This finding has been explained through Randle or glucose-fatty acid cycle (Randle *et al*., 1963).

Determination of biochemical parameters - Lipid profile

The concentration of total cholesterol (TC) was significantly lower in the HFD + STZ (35 mg/kg bw) in comparison with HFD control and the HFD + STZ (15 and 25 mg/kg bw) groups (Table 3). The lower TC concentration could be explained by the deleterious effect of STZ on hepatocytes metabolism, DNA methylation caused by STZ leading to low cholesterol biosynthesis in the liver and by the insulin resistance (Table 3). Gujjala *et al*. (Gujjala, 2016), demonstrated that HMG-CoA reductase levels were lower in rats fed with HFD than the control group. This could partially explain the results above. The death of liver and renal cells has been explained in the literature by the effect of STZ alkylating DNA (Szkudelski, 2001; Rerup *et al*., 1970; Weiss, 1982; Lenzen, 2008). In protocols for induction of T2DM with HFD + STZ in wistar rats, using diet supplemented with cholesterol, increased plasmatic TC concentration (Veerapur *et al*., 2010; Priscilla *et al*., 2015). This fact could be related to high absorption of dietary cholesterol in the small intestine when rats were fed with HFD supplemented with cholesterol[31]. Alternatively, high concentration of cholesterol in the plasma could be also explained because of low conversion to biliary salts in the condition of reduced hepatocytes metabolism in rats treated with STZ leading to T2DM. This assumption is supported by data observed in the metabolism of cholesterol in the humans (Jagannathan *et al*., 1974).

The lower concentration of TC in HFD + STZ (35 mg/kg bw) group in our experiment (Table 3), could be explained because the HFD was not supplemented with cholesterol and besides the low cholesterol biosynthesis in the liver according to the histological alteration shown (Table 3 and Fig. 2), which reduces net cellular metabolism. Our data indicate a significant increase ($p<0.05$) in the concentration of triglycerides and VLDL-C, and lower levels ($p<0.05$) of both HDL-C and LDL-C in the HFD + STZ (35 mg/kg bw) group in comparison with STD and HFD control, HFD + STZ (15 and 25 mg/kg bw) groups (Table 3). These results are consistent with those reported for induction of T2DM in wistar rats using the same protocol with HFD and low STZ dose, except the results for LDL-C (Veerapur *et al*., 2014; Priscilla *et al*., 2015; Srinivasan *et al*., 2005). The observed data could be explained by two hypotheses. Firstly, the metabolism of animals under T2DM condition leads to higher TG mobilization from the adipose tissue increasing the concentration of free fatty acids in the plasma, which are discharged in the liver. The fatty acids are then converted to TG leading to a higher serum concentration, responsible for the increase in the VLDL-C levels and lowering LDL-C in the serum.

This hypothesis is supported by the decrease in the weight of epididymal adipose tissue (Table 1). Secondly the higher concentration of TG in the plasma in the HFD + STZ (35 mg/kg bw) group could be explained due to the low insulin level lowering glycolytic metabolism in the adipose tissue reducing the biosynthesis of TG and maintaining its high blood concentration. The uptake of glucose in the adipose tissue is mediated by glucose transporter GLUT4, in the T2DM the internalization of glucose is reduced leading to low level of dihydroxyacetone phosphate diminishing TG biosynthesis (Charron *et al*., 1999; Gandhi *et al*., 2013).

Hepatic and renal function markers

The liver toxicity markers are altered in diabetic condition. It was observed a significant increase ($p< 0.05$) of 55% in the serum activities of aspartate aminotransferase (AST) and 59% of alanine aminotransferase (ALT) in the HFD + STZ (35 mg/kg bw) group, compared with both STD and HFD groups (Table 3). Probably these results indicate hepatocyte damage caused by the higher STZ concentration, resulting in aminotransferases leakage into the blood, consequently raising the serum levels of both aminotransferases (Ramachandran *et al*., 2012; Subramanian *et al*., 2015). It was observed hepatocytes necrosis on both HFD and HFD + STZ treatments (Fig. 2 B, C, D) in comparison with the STD group (Fig. 2 A). On the other hand, there were no statistical differences in the serum concentration of both total protein and creatinine (Table 3). However the higher concentration of serum urea in the HFD + STZ (35 mg/kg bw) group found in our experiments (Table 3) could be partially explained based on the increase of muscle protein catabolism in the T2DM rats due to the loss of body weight (Table 1 and Fig. 1 A, B). This observation was also reported in experiments for T2DM animal model induced with HFD and a low-dose STZ (Sundaram *et al*., 2013; Subramanian *et al*., 2015). Diabetes could lead to cell oxidative stress, causing decreased serum albumin level due to the increased urinary excretion of protein as a result of diabetic nephropathy (Stehouwer *et al*., 2002). Histological data indicates hyaline degeneration in the proximal contorted tubules, shown in Figure 2, consequentelly promoting albumin leakage trough the urinary track. The relative low serum level of albumin in the HFD + STZ (35 mg/kg bw) experimental group (Table 3) could also be explained because of the lower biosynthesis of the protein in the liver related to the deleterious effect of STZ on the hepatocytes. Albumin is a major protein component of the human serum, representing approximately 60% of total plasma protein and is accounting for 25% of total hepatic protein synthesis (Park *et al*., 2014).

Histological examination of the liver, kidney and pancreas

Histological changes in the liver, kidney and pancreas for the control and the experimental groups are shown in Figure 2. In the liver of HFD control group showed mild steatosis (Fig. 2 B) and in the HFD + STZ (15, 25 and 35 mg/kg bw) groups moderate steatosis (Fig. 2 C, D, E), however a mild necrosis was observed in all groups except in the STD group (Fig. 2 A). Kidney steatose was absent in STD and HFD control groups (Fig. 2 F, G), in the HFD + STZ (15 and 25 mg/kg bw) groups presented mild steatosis in the proximal contorted tubules (Fig. 2 H, I) and moderate in the HFD + STZ 35 mg/kg bw group (Fig. 2 J). Hyaline degeneration was absent in the STD group (Fig. 2 F) and moderate in all other groups (Fig. 2 G-J).

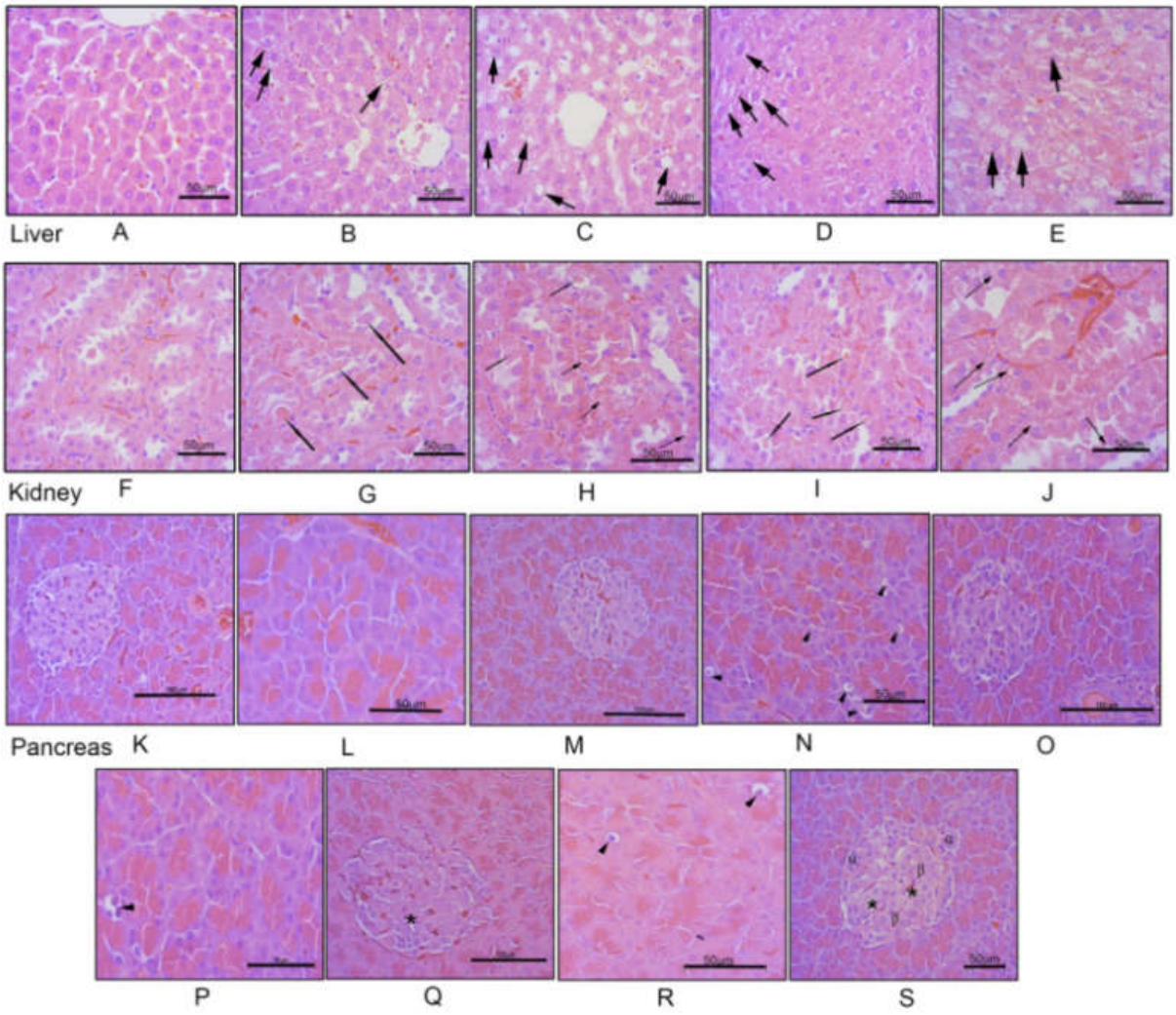

Figure 2. Effects of HFD and low doses of STZ on histology of liver, kidney and pancreas (H&E 50X). (A) STD control group showing normal hepatocytes. (B) HFD control group showing mild steatosis micro droplets (arrows). (C) HFD + STZ (15 mg/kg bw), (D) HFD + STZ (25mg/kg bw) and (E) HFD + STZ (35 mg/kg bw) groups showing moderate steatosis micro droplets (arrows). (F) STD control group showing normal morphology, without renal tubules alterations. (G) HFD control group showing hyaline cylinders present in the inner part of the renal tubules (thin arrows). (H) HFD + STZ (15 mg/kg bw) and (I) HFD + STZ (25 mg/kg bw) groups showing hyaline cylinders present (thin arrows) and steatosis micro droplets (arrows) in the renal tubules. (J) HFD + STZ (35 mg/kg bw) group showing moderate steatosis (arrows) in the renal tubules. (K & L) STD control group showing normal pancreas morphology, waxy acini and pancreatic islets (PI) without morphological alterations. (M & N) HFD control group showing pancreatic islets (PI) without morphological alterations and moderate apoptotic waxy cells (arrows head). (O & P) HFD + STZ (15 mg/kg/bw) group showing pancreatic islets (PI) normal morphology and mild apoptotic waxy cells (arrows head). (Q & R) HFD + STZ (25 mg/kg/bw) group showing pancreatic islets mild vacuolization (*) and apoptotic waxy cells (arrows head). (S) HFD + STZ (35 mg/kg/bw) group showing waxy acini with normal morphology, pancreatic islets hypotrophy (PI), pancreatic islets moderate vacuolization (*), see β and α cells in the pancreatic islets (PI)

Normal pancreatic architecture was noted in STD, HFD and HFD + STZ (15 mg/kg bw) (Fig. 2 K-P), moderate atrophy islets were found in HFD + STZ (25 mg/kg bw) (Fig. 2 Q, R) and intense in HFD + STZ (35 mg/kg bw) presenting moderate vacuolization, but partially maintaing pancreatic islets anatomy (Fig. 2 S). Pancreas exocrine portion showed moderate apoptotic bodies in the HFD group and mild in the other groups (Fig. 2 M-S).

Conclusion

The results presented indicates the establishment of a rat T2DM model in the HFD and low dose of STZ (35 mg/kg bw) group, confirmed by hyperglycemia, insulin level and the HOMA-IR value and aditional biochemical data and histological analyses. This model is adequate for testing folk's medicine with hypoglycemia activity and alternative energy sources for individuals carrying T2DM, obtained from the local biodiversity of the Mato Grosso do Sul State, mainly from the both Pantanal region and the Cerrados.

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