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ETHANOLIC EXTRACT LEAVES OF *EUGENIA dysenterica* DC IMPROVES THE LIPID PROFILE IN MICE WITH DIABETES INDUCED BY STREPTOZOTOCIN

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ABSTRACT

The ethanolic extract of the leaves of *E. dysenterica* (EEC) was evaluated for its antioxidant activity and was then tested for toxicity administered to both the control group and the treated group (2000 mg/kg EEC). For an experimental model of diabetes, animals were divided into experimental groups: a negative control treated with saline, a group treated with streptozotocin (STZ) combined with saline, a group treated with STZ combined with metformin and the others treated with EEC at doses of 25, 50 and 100 mg/kg of body weight (21 days). The antioxidant activity by the β-carotene/linoleic acid method and by the DPPH free radical method was 113 ± 1 and 107 ± 2 μg/ml, respectively. Total flavonoids and total phenols were 147 ± 1 and 204 ± 5 mg/g, respectively. In the acute toxicity test, EEC did not cause alterations. Regarding the DM model and the treatment with the extract, glycemia was not reduced, but there was an increase in HDL cholesterol ($p<0.05$) and a decrease in LDL cholesterol ($p<0.001$) in the group receiving 25 mg/extract/kg. The VLDL and triglycerides levels were significantly reduced at all doses ($p<0.001$). We concluded that EEC did not present hypoglycemic activity, but there was a significant beneficial effect on serum lipids.

INTRODUCTION

Diabetes mellitus (DM) is characterized as a metabolic disorder resulting from defects in the action or secretion of insulin or both, leading to alterations in the metabolism of proteins, carbohydrates and lipids (Oboh, *et al*., 2011).

These alterations cause serious consequences for diabetic patients, such as weight loss, increased lipid levels in the blood, increased incidences of atherosclerosis and peripheral arterial diseases, damage to vision and the kidneys, and death in more severe cases (ADA, 2014). The most current treatments for DM include insulin therapy, oral hypoglycaemics, and changes in lifestyle. However, patients report that their medications cause numerous side effects and discomforts. Therefore, the search for new agents that can control diabetes has become the target of pharmacological studies (Abdel-Hassan, *et al*., 2000).

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As a result, medicinal plants that have hypoglycaemic effects and that have shown significant improvement of diabetic symptoms have aroused the interest of researchers. *Eugenia dysenterica* DC, belonging to the family Myrtaceae and known popularly in Brazil as cagaita or cagaiteira, has leaves with popular indications for the treatment of diabetes, and these leaves have secondary metabolites and antioxidant activity, to which beneficial effects against arterial hypertension, dyslipidaemia and inflammation in general have been attributed (Genovese, *et al.*, 2008; Lima, *et al.*, 2011). Accordingly, the aim of this study was to evaluate the effects of the ethanolic extract of the leaves of *E. dysenterica* (EEC) on food intake, body weight, blood glucose and serum lipids in mice with diabetes induced by streptozotocin (STZ).

MATERIALS AND METHODS

Collection, selection of leaves and preparation of ethanolic extract

The leaves of *Eugenia dysenterica* DC were collected in two residential localities of the municipality of Campo Grande, MS, in May 2014. Species identification was performed by Prof. Arnildo Pott of the Federal University of Mato Grosso do Sul (voucher number: CGMS 52.819). The ethanolic extract was prepared according to the method described by Roesler *et al.* (2007).

Evaluation of antioxidant activity and total phenolic and flavonoid compounds

An antioxidant activity assay using the β-carotene/linoleic acid system was performed as described by Tepe *et al.* (2005) (modified). Antioxidant activity with the free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) was determined according to the method of Kumaran and Karunakaran (2006). Total flavonoids were quantified using the method of Lin and Tang (2007), and total phenols as described by Djeridane *et al.* (2006).

Phytochemical characterization

The phytochemical characterization of the EEC was performed using an HPLC analytical system (Varian 210). The ternary solvent delivery system and autosampler had the same features. The diode array detector (PAD) was monitored at λ = 200-800 nm. The HPLC column was C-18 (25 cm x 4.6 mm; particle size, 5 μm; Luna, Phenomenex, Torrance, CA, USA) with a small pre-column (2.5 cm x 3 mm) containing the same package to protect the analytical column. The flow rate and injection volume were 1.0 ml min^{-1} and 20 ml, respectively. All of the chromatographic analyses were performed at 22°C.
The mobile phase used was composed of 6% acetic acid in water with 2 mM sodium acetate solution (eluent A) and l mM methanol (eluent B). The analyses were performed using the gradient elution system: 0 min 5% B, 30 min, 15% B, 35 min, 30% B, 40 min, 50% B, and 45 min 100%. The extract was injected at a concentration of 100 μg/ml. The pattern in the concentration was 1 μg/ml. To quantify the standards, the method of external standards was used by obtaining a curve with r = 0.9994 for quercetin and r = 0.9992 for gallic acid.

Ethics parameters

All of the experiments followed the experimental protocol approved by the Ethics Committee in the Use of Animals (CEUA) of the Federal University of Mato Grosso do Sul (Protocol no. 663, March 2015) and were conducted in accordance with the National Institutes of Health's Regulations on the Use and Care of Animals for Scientific Purposes.

Acute toxicity test and "Hippocratic screening"

We used female Wistar albino rats of the species *Rattus norvegicus*, distributed into 2 groups (n = 5) according to the OECD (2008) method (Oboh, *et al.*, 2011).

Induction of experimental diabetes

Diabetes was induced in male Swiss mice (*Mus musculus*) 6 weeks of age (25-35 g) by a single injection of streptozotocin (STZ), purchased from Sigma, at a dose of 150 mg/kg (in 20 mM sodium citrate, pH 4.5) after 12 to 14 h of fasting. The animals continued to fast for another 3 h after injection. After 7 days, fasting blood glucose was evaluated, and the animals that showed values greater than 250 mg/dL were considered diabetic and were included in the different groups (Thandavarayan, *et al.*, 2011) [28]: negative control treated with saline (CTL SAL); diabetic control treated with saline (STZ SAL); diabetic control treated with 500 mg/kg metformin hydrochloride (Medley®) (STZ MET); and others treated with EEC at doses of 25, 50 and 100 mg/kg body weight, administered by gavage for 21 days. Blood glucose (tail vein blood) and food and water intake were determined weekly. Euthanasia was performed by exsanguination, and the blood was obtained for biochemical analyses (total cholesterol and fractions, triglycerides and glucose) using commercial kits (LabTest®, Lagoa Santa - GO, Brazil) and a spectrophotometer (PowerWave XS, BioTek).

Histological analysis of the liver and pancreas

After euthanasia, the liver and the pancreas were removed for histological analysis. The material was fixed in 10% formalin, where it was kept until paraffin embedding. Next, sections 7 μm thick were obtained with a microtome and were mounted on glass slides. They were then stained with haematoxylin and eosin and were analysed at 200X magnification with a microscope coupled to a digital camera (Leica Application Suite® – version 4.0.0). Representative fields in the section were selected and examined to determine morphological alterations in the liver and pancreas (Bansal, 2012).

Statistical analysis

The results are expressed as the mean ± standard error of the mean or the mean ± standard deviation of the mean. For multiple comparisons of parametric results, we used ANOVA followed by Tukey's post-test, and for comparison of two groups, Student's t-test was used. The level of significance used was $p < 0.05$. Statistical analysis was performed with Jandel Sigma-Stat Software, version 3.5 (Systal Software, Inc., USA).

RESULTS

Evaluation of antioxidant activity and total phenolic and flavonoid compounds

The results obtained for determination of antioxidant activity with the β-carotene/linoleic acid system and with the free radical DPPH, total flavonoids and total phenols (mg/g) of EEC are shown in Tables 1 and 2.

Table 1. Antioxidant activity with β-carotene/linoleic acid (µg/ml) and antioxidant activity with the free radical DPPH (µg/ml) of the ethanolic extract of the leaves of *E. dysenterica* DC

Ethanolic extract of the leaves of *Eugenia dysenterica* DC.	Values
Antioxidant activity with β-carotene/linoleic acid (µg/ml)	113±0.3
Antioxidant activity with free radical DPPH (µg/ml)	107±0.1

Values expressed as mean ± standard deviation.

Table 2. Total flavonoids (mg/g) and total phenols (mg/g) in the ethanolic extract of the leaves of *E. dysenterica* DC

Ethanolic extract of the leaves of *Eugenia dysenterica* DC	Values
Total flavonoids (mg/g)	147±1.4
Total phenols (mg/g)	204±1.1

Values expressed as mean ± standard deviation.

Phytochemical characterization

In phytochemical analysis of the extract showed quercetin content of 0.9 mg/g and 0.1 mg/g of gallic acid (Figure 1).
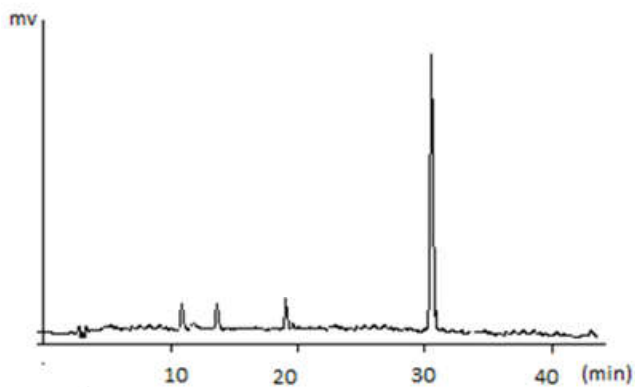

Figure 1. Phytochemical characterization by chromatographic analysis (HPLC). Using the standard Sigma (98% purity) the extract showed to 31.2 min, quercetin and 10.5 min, gallic acid
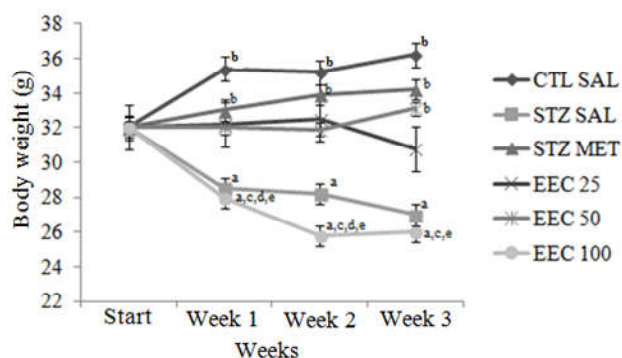

Figure 2 – Body weight (g) of control animals (CTL – non-diabetic treated with saline/STZ SAL – diabetic treated with saline/STZ MET – diabetic treated with metformin) and diabetic animals treated with ethanolic extract of the leaves of *E. dysenterica* (EEC) at doses of 25, 50 and 100 mg/kg body weight for 21 days. Values expressed as mean ± standard error of the mean. The letters indicate statistical difference as follows: [a]compared to CTL SAL; [b]compared to STZ SAL; [c]compared to STZ MET; [d]compared to EEC 25, and [e]compared to EEC 50. p<0,05. n = 6-10. ANOVA followed by the Tukey post-test.

Acute toxicity and "Hippocratic screening"

EEC administered at a dose of 2000 mg/kg in normal animals (CTL EXT) did not cause any alteration in the behavior parameters analyzed compared to the animals treated with only saline (CTL SAL), where there was no difference in food or water intake between the groups. No deaths occurred during the observation period of 14 days. The body weight of the animals, as well as the weight of the liver, kidneys, lungs and heart did not show a significant difference between the groups, and there were no macroscopic changes (data not shown). This test demonstrated that the extract of this plant belonged to class 5 (substance with a lethal oral dose [LD50] over 2000 mg/kg), thereby considered having a low toxicity (OECD, 2008).

Induction of experimental diabetes

Evaluation of food and water intake

The animals in the EEC 25 and 50 groups showed a food intake similar to that of the group treated with metformin, but 100 mg/kg extract caused a higher food intake, where there was a significant difference in the first and last week of the study (Table 3). Diabetic animals treated with EEC showed greater water intake than the STZ MET group (p<0.05) (Table 4).

Evaluation of body weight

The weight of the control animals treated with saline (CTL SAL) or EEC at doses of 25 and 50 mg/kg showed no change in weight or weight gain, as seen with diabetic mice treated with metformin (Figure 2).

Evaluation of fasting blood glucose

The animals of the STZ SAL group showed initial levels of fasting blood glucose that were statistically higher in relation to the non-diabetic animals in the control group (p<0.001). The groups that received the treatment with EEC at doses of 25, 50 or 100 mg/kg did not show significantly different values in relation to the STZ SAL and STZ MET groups, with the exception of EEC 50 at week 1 and EEC 100 at week 2. The values described in parentheses represent the percentual difference in weekly blood glucose in relation to initial glycemia in the respective study groups (Table 5).

Evaluation of serum lipid profile

The values for total cholesterol did not differ statistically between groups, although the group that received EEC 25 mg/kg showed lower values when compared to the other groups. With regard to HDL-cholesterol, only the STZ MET and EEC 25 groups displayed significantly higher values in relation to the others. The opposite was observed for LDL-cholesterol, where the highest values were seen in the CTL SAL and STZ SAL groups, differing statistically from the STZ MET and EEC 25 groups. VLDL-cholesterol and triglycerides were elevated in the diabetic animals of the STZ SAL group in comparison to CTL SAL, where a significant decrease was seen in the other groups, in comparison with either STZ SAL or CTL SAL (p<0.001). The figure 3 displays the serum lipids values of the groups studied and the differences between them.

Histological analysis of the liver and pancreas

In the histological analysis of the liver, the groups that received EEC 25 and 50 mg/kg body weight exhibited hepatic tissue integrity, that is, normal hepatic parenchyma, as well as the CTL SAL and STZ MET groups (Figure 4).

18184

Lidiani Figueiredo Santana et al. Ethanolic extract leaves of eugenia dysenterica dc improves the lipid profile in mice with diabetes induced by Streptozotocin

Table 3. Food intake of control animals and diabetic animals treated with e thanolic extract of the leaves or *Eugenia dysenterica* DC

Food intake (g/animal ± S.E.M.)			
Group	Week 1	Week 2	Week 3
CTL SAL	6.23±0.61	8.66±1.41	5.94±0.46
STZ SAL	8.03±0.61	9.00±0.83	9.14±0.41[a]
STZ MET	10.53±0.27[a]	8.12±0.69	8.78±0.37[a]
EEC 25	12.86±0.67[a,b]	10.33±0.89	8.91±0.47[a]
EEC 50	7.88±0.46[d]	9.05±0.58	9.74±0.46[a]
EEC 100	13.60±0.97[a,b,c,e]	10.57±0.91	11.40±0.76[a,b,c,d]

Food intake (g/animal) of control animals (CTL – non-diabetic treated with saline/STZ SAL – diabetic treated with saline/STZ MET – diabetic treated with metformin) and diabetic animals treated with ethanolic extract of the leaves of *E. dysenterica* (EEC) at doses of 25, 50 and 100 mg/kg body weight, for 21 days. Values expressed as mean ± standard error of the mean (S.E.M.). In the same column, the letters indicate statistical difference as follows: [a] compared to CTL SAL; [b] compared to STZ SAL; [c] compared to STZ MET; [d] compared to EEC 25, and [e] compared to EEC 50. p<0.05. n = 6-10. ANOVA, followed by the Tukey post-test.

Table 4. Water intake of control animals and diabetic animals treated with ethanolic extract of the leaves of *E. dysenterica*

Water intake (mL/animal± S.E.M.)			
Group	Week 1	Week 2	Week 3
CTL SAL	9.64±0.35	8.93±0.74	9.64±0.35
STZ SAL	38.57±2.61[a]	36.43±3.57[a]	42.14±2.14[a]
STZ MET	28.57±3.81[a,b]	21.43±0.00[a,b]	17.35±1.44[b]
EEC 25	48.81±1.19[a,c]	45.24±2.47[a,c]	47.62±1.53[a,c]
EEC 50	30.00±1.54[a,d]	35.71±3.35[a,c,d]	40.71±2.76[a,c]
EEC 100	42.85±4.73[a,c,e]	40.00±3.45[a,c]	40.71±3.68[a,c]

Water intake (mL/animal) of control animals (CTL – non-diabetic treated with saline/STZ SAL – diabetic treated with saline/STZ MET – diabetic treated with metformin) and diabetic animals treated with ethanolic extract of the leaves of *E. dysenterica* (EEC) at doses of 25, 50 and 100 mg/kg body weight, for 21 days. Values expressed as mean ± standard error of the mean (S.E.M.). In the same column, the letters indicate statistical difference as follows: [a] compared to CT SAL; [b] compared to STZ SAL; [c] compared to STZ MET; [d] compared to EEC 25, and [e] compared to EEC 50. p<0.05. n = 6-10. ANOVA followed by the Tukey post-test.
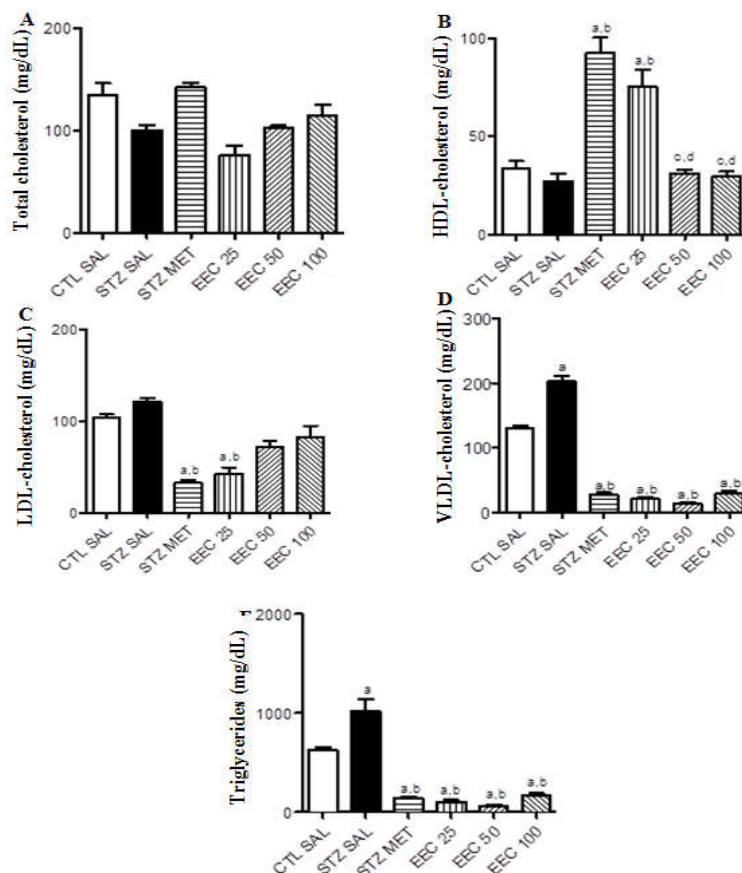

Figure 3 – Serum of control animals (CTL – non-diabetic treated with saline/STZ DM – diabetic treated with saline/STZ MET – diabetic treated with metformin) and diabetic animals treated with ethanolic extract of the leaves of *E. dysenterica* (EEC) at doses of 25, 50 and 100 mg/kg body weight Figure 3 – Serum of control animals (CTL – non-diabetic treated with saline/STZ DM – diabetic treated with saline/STZ MET – diabetic treated with metformin) and diabetic animals treated with ethanolic extract of the leaves of *E. dysenterica* (EEC) at doses of 25, 50 and 100 mg/kg body weight for 21 days. A) Total cholesterol (mg/dL); B) HDL-cholesterol (mg/dL); C) LDL-cholesterol (mg/dL); D) VLDL-cholesterol (mg/dL); and E) triglycerides (mg/dL). Values expressed as mean ± standard error of the mean. The letters indicate

Table 5. Fasting blood glucose of control animals and diabetic animals treated with ethanolic extract of the leaves of *E. dysenterica*

Fasting blood glucose (mg/dL± S.E.M.)(%)			
Group	Initial	Week 1	Week 2	Week 3
CTL SAL	127.44±7.57	127.44±6.51 (+0%)	104.75±4.62 (-17,80%)	101.25±4.96 (-20,55%)
STZ SAL	458.77±41.97[a]	482.66±39.00[a] (+5,21%)	479.20±32.82[a] (+4,45%)	270.00±28.61 (-41,15%)
STZ MET	306.16±15.11[a]	289.83±12.22[b] (-5,33%)	196.33±16.47[b] (-35,87%)	203.66±11.72 (-33,48%)
EEC 25	338.50±30.62[a]	306.16±26.43[a] (-9,55%)	306.16±20.87 (-9,55%)	351.80±27.34[a] (+3,93%)
EEC 50	292.50±20.44[a]	224.66±19.01[b] (-23,19%)	373.40±45.28[a] (+27,66%)	348.33±62.89[a] (+19,09%)
EEC 100	438.90±27.92[a]	385.00±30.27[a] (-12,28%)	455.44±48.22[a,c] (+3,77%)	357.00±20.24[a] (-18,66%)

Fasting blood glucose (mg/dL) of control animals (CTL – non-diabetic treated with saline/STZ DM – diabetic treated with saline/STZ MET – diabetic treated with metformin) and diabetic animals treated with ethanolic extract of the leaves of *Eugenia dysenterica* DC (EEC) at doses of 25, 50, 100 mg/kg body weight for 21 days. Values expressed as mean ± standard error of the mean (S.E.M.). In the same column, the letters indicate statistical difference as follows: [a]compared to CTL SAL; [b]compared to STZ SAL; [c]compared to STZ MET. $p<0.05$. n = 6-10. ANOVA followed by the Tukey post-test.
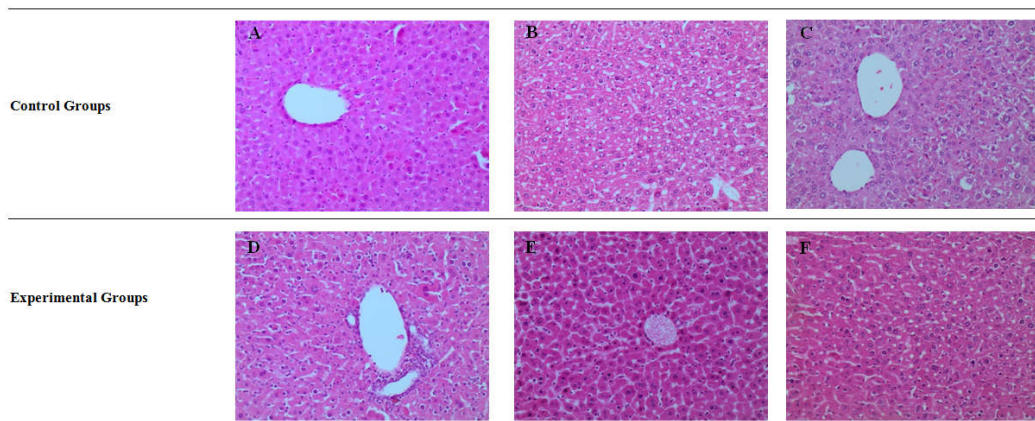

Figure 4 - Histopathological analysis of the liver stained with hematoxylin and eosin (H&E – 200X) in control animals (CTL SAL – non-diabetic treated with saline/STZ DM – diabetic treated with saline/STZ MET – diabetic treated with metformin) and diabetic animals treated with ethanolic extract of *Eugenia dysenterica* DC leaves (EEC) at doses of 25, 50, 100 mg/kg body weight for 21 days. A) CTL SAL – compatible with normal hepatic parenchyma; B) STZ DM – mild hepatic steatosis; C) STZ MET – compatible with normal hepatic parenchyma; D) EEC 25 - compatible with normal hepatic parenchyma; E) EEC 50 - compatible with normal hepatic parenchyma; F) EEC 100 - mild hepatic steatosis
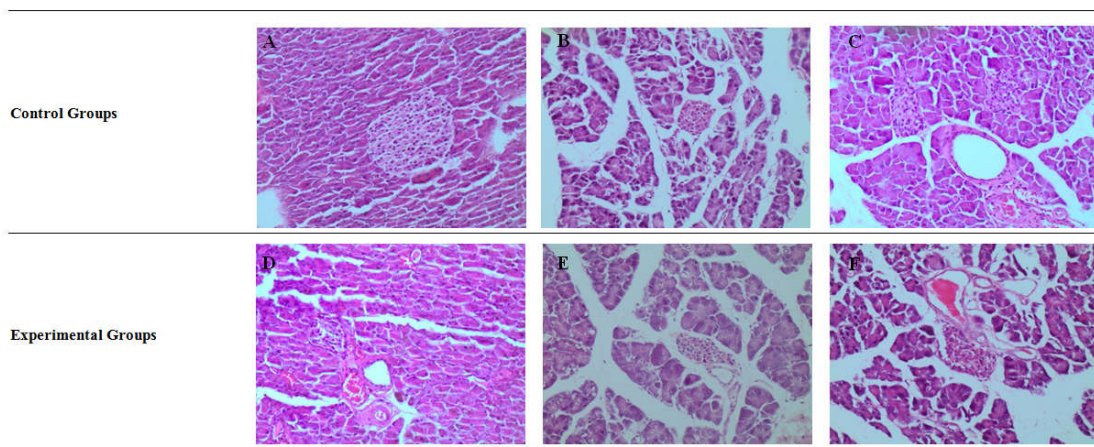

Figure 5 - Histopathological analysis of the pancreas stained with hematoxylin and eosin (H&E – 200X) in control animals (CTL – non- diabetic treated with saline/STZ DM – diabetic treated with saline/STZ MET – diabetic treated with metformin) and diabetic animals treated with ethanolic extract of *Eugenia dysenterica* DC leaves (EEC) at doses of 25, 50, 100 mg/kg body weight for 21 days. A) CTL SAL – normal pancreatic parenchyma; B) STZ DM - dilated pancreatic acini, an atrophic islet of Langerhans, alterations compatible with the alterations of diabetes; C) STZ MET - dilated pancreatic acini, an atrophic islet of Langerhans, alterations compatible with the alterations of diabetes; D) EEC 25 - normal pancreatic parenchyma; E) EEC 50 - dilated pancreatic acini, an atrophic islet of Langerhans, alterations compatible with the alterations of diabetes; F) EEC 100 - dilated pancreatic acini, an atrophic islet of Langerhans, alterations compatible with the alterations of diabetes

However, the STZ SAL and EEC 100 groups showed hepatocytes with normal aspects and some with lipid inclusion bodies, compatible with mild hepatic steatosis. With regard to histology of the pancreas, it was seen that the STZ SAL, EEC 50 and EEC 100 groups showed dilated pancreatic acini and atrophic islets of Langerhans, thereby exhibiting characteristics compatible with alterations of DM (Figure 5) and demonstrating that the extract at these doses did not impede the atrophy of pancreatic cells. The other groups showed typical pancreatic acini with normal islets of Langerhans, compatible with normal pancreatic parenchyma.

DISCUSSION

The literature has reported that the leaves of *Eugenia dysenterica* DC, in the form of tinctures using alcohol in the preparation, are empirically used for the treatment of diabetes (Souza *et al*., 2012). In addition, Couto *et al*. (2009) identified high quantities of phenolic compounds (flavonoid, tannin and quercetin), and Pellegrina *et al*. (2005) first identified powerful antioxidants in gallic acid species that are directly related to the reversal of biochemical parameters altered in chronic non-communicable diseases, including dyslipidaemia, hypertension and inflammatory processes in general (Dufrens and Farnworth, 2001). The evaluation of antioxidant activity and the total phenolic and flavonoid compounds of the extract used here corresponded to that described in the literature (Genovese, *et al*., 2008; Couto *et al*., 2009). To our knowledge, this study was the first to evaluate the effects of an ethanolic extract of *E. dysenterica* leaves on lipid profiles, blood glucose and body composition in an experimental model of STZ-induced diabetes.

In toxicity studies, clinical observations, organ weights and changes in body weight could serve as useful indicators of the state of general health of animals (Jahn and Gunzel, 1997). The animals treated with the extract did not show atypical behavioural manifestations according to "Hippocratic screening," and there were no deaths during the observation period. The data obtained in this study were relevant, demonstrating the beneficial use of this species of great economic, food and medical importance. However, other studies based on protocols established by regulatory agencies should be conducted (such as studies of subacute toxicity, chronic toxicity, and reproductive toxicity) to evaluate the overall safety of the use of this plant in humans. In light of EEC's lack of acute toxicity and the popular indication of the leaves of *E. dysenterica* for the treatment of diabetes, this study was undertaken to investigate the hypoglycaemic activity of EEC and its effects on the lipid profiles, food intake and body weight of mice with STZ-induced diabetes.

DM is characterized by the presence of hyperglycaemia due to the lack of insulin action (ADA, 2014), and to reproduce the symptomology of DM, studies have used an experimental model of DM in rodents because of the clinical, laboratory and histological similarities with human DM. The disease is induced in animals by the administration of drugs such as STZ, which causes hyperglycaemia and hyperinsulinaemia, followed by transitory hypoglycaemia and finally, chronic hyperglycaemia, as demonstrated in this study (Carvalho, *et al*., 2003). Once DM is established, animals begin to show some characteristic symptoms, such as polydipsia, which arises as a consequence of blood hyperosmolarity due to high levels of circulating glucose, which is recognized by osmoreceptors in the brain and causes intense thirst as a response (Graber, 2013). Treatment with EEC showed no benefits with regard to polydipsia, probably due to the osmotic imbalance initiating a process of dehydration.

Another characteristic symptom of DM, as seen in the induction model chosen here, is polyphagia, followed by significant weight loss (ADA, 2014). This increase in food intake can be associated with the regulatory process of hunger in the satiety centre located in the ventromedial hypothalamic nucleus, which requires insulin for glucose uptake. Once taken up, the hunger centre is inhibited. Therefore, in DM, there is a lack of insulin, the ventromedial hypothalamic nucleus does not provide for glucose uptake, and the hunger centre is not inhibited, thus stimulating greater food intake (Jacobson, 1996). Weight loss is due to the loss or degradation of structural proteins and an increase in lipolysis, which occurs due to dysregulation of carbohydrate metabolism and the formation and storage of lipids by insulin (Guilherme, *et al*., 2008). Studies conducted with plants of the family Myrtaceae and genus *Eugenia*, such as that of Pepato *et al*. (2001), who used an extract of *Eugenia jambolana* leaves, have demonstrated that this plant does not have hypoglycaemic effects in animals with STZ-induced DM.

In this experimental model, the ethanolic extract from the leaves of *Eugenia dysenterica* did not present as satisfactory a hypoglycaemic effect over the weeks of treatment as the group treated with metformin. However, the EEC 25 and EEC 100 groups presented a percentage reduction in or maintenance of the glycaemia values throughout the evaluation period. It was observed that the induction of diabetes in the EEC 100 group generated hyperglycaemia with fasting glycaemia values more severe than the other groups. Therefore, despite the reduction in fasting blood glucose values, the levels were high, which might justify the maintenance of the low body weights and the high water intake presented by this group. In the present study, this finding was corroborated by histopathology of the pancreas, which showed, except in the CTL SAL and EEC 25 groups, dilated pancreatic acini and atrophy of the islets of Langerhans, which are characteristics consistent with the alterations in DM. As described above, insulin deficiency in DM leads to an alteration in lipid metabolism, resulting in an increase in plasma triglycerides, probably as a consequence of the high synthesis of triglycerides from fatty acids transported to the liver due to greater lipolysis in adipose tissue (Guilherme, *et al*., 2008).

Therefore, high hepatic VLDL synthesis occurs, accompanied by lesser catabolism of VLDL by the activity of lipoprotein lipase (Jain, *et al*., 2007). Consequently, among of the most frequent complications found in patients with DM are dyslipidaemias, which lead to serious risk for the onset of cardiovascular diseases, which are primarily responsible for the decrease in survival of patients with DM and which are considered the most common cause of mortality, among which various vascular disorders are included (Yadav, *et al*., 2005). In the study in question, all of the treated groups, regardless of the dose, experienced a significant reduction in triglycerides and VLDL cholesterol compared with the CTL SAL and STZ SAL groups, showing the same effectiveness as metformin. Jelastin *et al*. (2011) found that the treatment of diabetic rats with an ethanolic extract of the leaves of *Eugenia floccosa* Bedd (Myrtaceae) caused decreases in triglycerides ($p<0.05$) and VLDL ($p<0.01$) compared with the DM group treated with saline. Our results also corroborated the histological findings of the liver, demonstrating the presence of steatosis only in histological sections of the STZ SAL and EEC 100 groups, which maintained higher fasting blood glucose levels throughout the study. These results indicated that the extract had protective activity on the serum lipid profile since it increased HDL cholesterol, which protects against cardiovascular diseases, and decreased LDL cholesterol, VLDL cholesterol and triglycerides, which in excess can cause or aggravate such diseases. Kahraman *et al*. (2003) demonstrated in their study that the intake of flavonoids and other phenolic compounds was directly associated with a reduced risk of cardiovascular diseases.

This finding stems from these compounds being considered effective antioxidants with the capacity to scavenge free radicals and to chelate metal ions, protecting cells from free radicals and lipid peroxidation; they also act as membrane stabilizers (Galati, *et al*., 2002). Studies aimed at understanding lipid metabolism and the use of flavonoids have demonstrated that serum and liver concentrations of total cholesterol and triglycerides are reduced, while the serum HDL cholesterol level is increased after the administration of flavonoids (Bao *et al*., 2016). Considering that the analysis of EEC showed the presence of flavonoids, these compounds might have an effect on lipid metabolism through their antioxidant activity, thereby decreasing the levels of triglycerides, total cholesterol and LDL cholesterol, followed by an increase in HDL cholesterol levels, especially at lower extract doses. The lowest dose of extract also promoted the regeneration of pancreatic tissue, which in histological analysis showed integrity.

Acknowledgements

Conflict Of Interest

The authors declare no conflicts of interest.

REFERENCES

Abdel-Hassan IA, Abdel-Barry JA, Mohammeda ST 2000. The hypoglycaemic and antihyperglycaemic effect of Citrullus colocynthis fruit aqueous extract in normal and alloxan diabetic rabbits. *Journal of ethnopharmacolog*y. 71:325-330.

American Diabetes Association 2014. Diagnosis and classification of diabetes mellitus. Diabetes care, *37* (Supplement 1), S81-S90.

Bansal P, Paul P, Mudgal J, Nayak PG, Pannakal ST, Priyadarsini KI, Unnikrishnan MK 2012. Antidiabetic, antihyperlipidemic and antioxidant effects of the flavonoid rich fraction of *Pilea microphylla* (L.) in high fat diet/streptozotocin-induced diabetes in mice. *Experimental and Toxicologic Pathology*. 64:651-658.

Bao L, Hu l, Zhang Y, Wang Y 2016. Hypolipidemic effects of flavonoids extracted from Lomatogonium rotatum. *Exp Ther Med*. 11:1417-1424.

Carvalho EN, Carvalho NAS, Ferreira LM. 2003. Experimental model of induction of diabetes mellitus in rats. *Acta Cir. Bras*. 8: 60-64.

Couto ROD, Valgas AB, Bara MTF, Paula JRD 2009. Caracterização físico química do pó das folhas de Eugenia *dysenterica* DC. (Myrtaceae). Rev Elet Farm. 7:59-69.

Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal, N. 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food chemistry*. 97:654-660.

Dufrense CJ, Farnworth ER 2001. A review of latest research findings on health promotion properties of tea. *J Nutr Biochem*. 12: 404-421.

Galati G, Sabzevari O, Wilson JX, O'brien PJ. 2002. Prooxidant activity and cellular effects of the phenoxyl

radicals of dietary flavonoids and other polyphenolics. Toxicolog. 1: 91-104.

Genovese MI, Da Silva Pinto M, De Souza Schmidt Gonçalves AE, Lajolo FM 2008. Bioactive compounds and antioxidant capacity of exotic fruits and commercial frozen pulps from Brazil. *Revista de Agroquimica y Tecnologia de Alimentos*. 14: 207-214.

Graber MN. 2013. Diabetes and hyperglycemia. In: Adams JG, ed. *Emergency Medicine: Clinical Essentials*. 2nd *e*d. Philadelphia, PA: Elsevier Saunders, 162.

Guilherme A, Virbasius JV, Puri V, Czech MP 2008. *Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nat Rev Mol Cell Biol. 9:367-377.*

Jacobson AM 1996. Current concepts: the psychological care of patients with insulindependent diabetes mellitus. *N Engl J Med*. 334:1249-1253.

Jahn AL, Gunzel PKH 1997. The value of spermatology in male reproductive toxicology: do spermatologic examinations in fertility studies provide new and additional information relevant for safety assessment? Reprod Toxicol. 11:171-178.

Jain SK, Rains JL, Croad JL 2007. Effect of chromium niacinate and chromium picolinate supplementation on lipid peroxidation, TNF-α, IL-6, CRP, glycated hemoglobin, triglycerides and cholesterol levels in blood of streptozotocin-treated diabetic rats. *Rad Biolog Med*., 43:1124-1131.

Jelastin KS, Tresina PS, Mohan, VR 2011. Antioxidant, antihyperlipidaemic and antidiabetic activity of Eugenia floccose bedd leaves in alloxan induced diabetic rats. *J Basic Clin Pharm*. 3:235-240.

Kahraman A, Erkasap N, Koken T, Serterser M, Aktepe F, Erkasap S. 2003. *The antioxidative and antihistaminic properties of quercetin in ethanol induced gastric lesion.* Toxicol. 183:133-142.

Kumaran A, Karunakaran RJ. 2006. Antioxidant and free radical scavenging activity of an aqueous extract of Coleus aromaticus. Food chemistry. 97:109-114.

Lima TB, Silva ON, Silva LP, Rocha TL, Grossi-de-Sá MF, Franco OL, Leonardecz E. 2011. In vivo effects of cagaita (Eugenia dysenterica, DC.) leaf extracts on diarrhea treatment. *Evidence-Based Complementary and Alternative Medicine*. 2:185-189.

Lin JY, Tang CY. 2007. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food chemistry*. 101:140-147.

Oboh G, Ademiluyi AO, Faloye YM 2011. Effect of combination on the antioxidant and inhibitory properties of tropical pepper varieties against α-amylase and α-glucosidase activities in vitro. *Journal of medicinal food*. 14:1152-1158.

OECD 2008. Organization for Economic Co-Operation and Development. Guidelines. 425: Acute Oral Toxicity: Up-and-Down Procedure, OECD, Paris, 2008. 27p.

Pellegrina CD, Padovani G, Mainente F, Zoccatelli G, Bissoli G, Mosconi S, Veneri G, Peruffo A, Andrighetto G, Rizzi C, Chignola R. 2005. Anti-tumour potential of a gallic acid-containing phenolic fraction from Oenothera biennis. Cancer Lett. 226:17-25.

Pepato MT, Folgado VBB, Kettelhut C, Brunetti L (2001). Lack of antidiabetic effect of a Eugenia jambolana leaf decoction on rat streptozotocin diabetes. *Braz J Med Biol Res. 34:389-395.*

Roesler R, Malta LG, Carrasco LC, Holanda RB, Sousa CAS, Pastore GM 2007. Antioxidant activity of cerrado fruits. *Food Science and Technology* (Campinas). 27:53-60.

Souza PM, Elias ST, Simeoni LA, de Paula JE, Gomes SM, Guerra ENS, Magalhaes PO 2012. Plants from Brazilian Cerrado with potent tyrosinase inhibitory activity. PLoS One, 7:9-11.

Tepe B, Sokmen M, Sokmen A, Daferera D, Polissiou M. 2005. Antimicrobial and antioxidative activity of the essential oil and various extracts of Cyclotrichium origanifolium (Labill.) Manden. & Scheng. *Journal of Food Engineering*. 69:335-342.

Thandavarayan RA, Giridharan VV, Sari FR, Arumugam S, Veeraveedu PT, Pandian GN, Watanabe K. (2011). Depletion of 14-3-3 protein exacerbates cardiac oxidative stress, inflammation and remodeling process via modulation of MAPK/NF-κB signaling pathways after streptozotocin-induced diabetes mellitus. Cellular Physiology and Biochemistry. 28:911-922.

Yadav UC, Moorthy K, Baquer NC. 2005. Combined treatment of sodium orthovanadate and Mormodica charantia fruit extract prevents alterations in lipid profile and lipogenic enzymes in alloxan diabetic rats. *Mol Cell Biochem*. 268:111–120.

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