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Original Article

Effect of the treatment of *Copaifera duckei* oleoresin (copaiba) in streptozotocin-induced diabetic rats

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ABSTRACT

Diabetes mellitus is a syndrome that reaches more than 382 million people worldwide. It interferes with the metabolism of carbohydrates, causing chronic hyperglycemia. The objective of this study was to evaluate the effect of the *Copaifera duckei*, Dwyer, Fabaceae, oleoresin on streptozotocin-induced (STZ) diabetic rats. This study was based on the induction of diabetes mellitus by streptozotocin (55 mg/kg, *i.p.*) in Wistar rats and treated with doses of *C. duckei* oleoresin (250 and 500 mg/kg, *p.o.*). Subsequently, the clinical, biochemical and histopathological of the pancreas parameters were evaluated. Gas chromatographic analysis indicated that β-bisabolene (22.29%), β-caryophyllene (21.25%) and α-farnesene (15.58%) sesquiterpenes were the major components of the *C. duckei* oleoresin. In streptozotocin-induced diabetes mellitus, it was possible to observe that the *C. duckei* oleoresin treatment had a significant effect ($p < 0.001$) on the clinical parameters, and that there was a positive improvement. This was attenuated by the urea, creatinine, and transaminases alterations ($p < 0.001$) observed in animals with diabetes mellitus, as well as the significantly reduced ($p < 0.001$) values of total cholesterol, triacylglycerides, and glucose. In the histopathological analyses of the pancreas, it was observed that the *C. duckei* oleoresin was able to restore β-cells and to significantly increase the quantity and diameter of the Langerhans islets ($p < 0.05$), when compared to the diabetic group. The treatment with *C. duckei* oleoresin, employed under the conditions of this study, presented antidiabetic activity and can improve the complications found in this syndrome.

Introduction

Diabetes mellitus (DM) is a syndrome that interferes with the metabolism of carbohydrates. The deficiency in the production of insulin by pancreatic β-cells and/or reduced tissue sensitivity to this hormone are the main triggers of chronic hyperglycemia, leading to a variety of complications such as retinopathy, nephropathy, neuropathy, and cardiovascular disease (Sacks et al., 2002; Carvalho et al., 2016b).

Diabetes mellitus is a major public health problem. Recent research claims that more than 382 million people worldwide have DM, many of whom are unaware of their condition as a carrier, making this group more susceptible to unexpected complications. Also, there are prospects that by 2035, DM will reach 592 million carriers (Guarugata et al., 2014). This increase in the prevalence of DM throughout the world makes this pathology a major focus of study with the objective of seeking new sources of treatment and contributing to the reduction of morbidity and mortality rates.

Medicinal plants are a major target for research in the pharmacological field since they have played a significant role in the treatment and prevention of pathologies for thousands of years and continue until today as a source of innovation in the discovery of new drugs (Megraj et al., 2011). There are many plant species

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already described in the literature as adjuvants in the treatment of DM, and it is estimated that there are more than 1200 plants with antidiabetic activity, but less than a third of this total has already been studied and scientifically proven (Chang et al., 2013).

The species *Copaifera duckei* Dwyer belongs to the family Fabaceae (Silva et al., 2008), which produces oleoresin (OR) that comes from the exudation of the trunk of the tree (Veiga-Junior and Pinto, 2002; Oliveira et al., 2006). The OR is used in folk medicine by traditional communities for various medicinal purposes and can be administered topically or orally. Several indications are described, including wound healing, anti-inflammatory action and anti-gastric ulcer and other digestive diseases (Carvalho et al., 2005; Heck et al., 2012).

The literature has described several studies on the pharmacological actions of copaiba OR, such as antimicrobial and antibacterial (Tincusi et al., 2002; Santos et al., 2008), analgesic activity (Carvalho et al., 2005), healing action (Brito et al., 1999), and antitumor (Lima et al., 2003).

Chemically, the OR consists mainly of sesquiterpenes, being prominent in β-caryophyllene; a compound that proves some of the pharmacological properties attributed to the OR due to its high concentration of practically all species of the *Copaifera* genus (Veiga-Junior and Pinto, 2002; Oliveira et al., 2006). Studies have shown that the β-caryophyllene presents antidiabetic action and attenuates oxidative stress in animals with hyperglycemia (Basha and Sankaranarayanan, 2016).

Several studies have scientifically proven the pharmacological activities attributed to OR that are related to the presence of sesquiterpenes. In this sense, the objective of this study was to evaluate the effect of the treatment with *C. duckei* OR in rats with diabetes induced by streptozotocin (STZ).

Materials and methods

Obtaining oleoresin of Copaifera duckei

The OR (Lot 07133310R) was obtained from the Beraca Sabara Chemicals Ingredients Company, located in Ananindeua, State of Pará, Brazil. According to the quality certificate, it was from the species *C. duckei*, which was discussed in previous studies (Carvalho et al., 2015).

Analyze of oleoresin by gas chromatography

The OR was filtered on a 0.45 mm × 13 mm nylon filter membrane, Millex. Subsequently, aliquots of 100 μl were diluted in 2 ml hexane and analyzed in triplicate. The quantitative analyses of the β-caryophyllene content were carried out in a gas chromatograph with a flame ionization detector (CG-FID, Shimadzu corporation-CG-plus 2010), the conditions of the study were: Rtx-5 capillary column (30 m × 0.25 mm ID × 0.25 μm) and helium as entraining gas, with a flow rate of 1.5 ml/min. The temperature of the oven was programmed to remain 120 °C for 2 min and increase by 3 °C/min to 160 °C, which was to be maintained for 2 min and followed by an increase of 8 °C/min to a final temperature of 290 °C, maintained for 2 min; the injector temperature was 270 °C, and the detector temperature 290 °C, and we injected a volume of 1.0 μl in a split ratio of 20:1 (Carvalho et al., 2015). The β-caryophyllene standard retention time (purity of 98.5%, BCBJ3860V batch, Sigma-Aldrich Co., St. Louis, USA) was utilized for standard curve construction at concentrations of 0.25, 0.5, 1, 2, 4, 6 and 8 mg/ml.

The qualitative analyses were carried out in gas chromatography, coupled with mass spectrometry (CG-MS, Shimadzu Corporation – QP2010SE). They were performed under conditions similar to that of the CG-FID, with the mass detector operating in scan mode 40–400 (*m/z*), and with an electronic impact of 70 eV. The identification of the compounds was performed by comparing the mass spectra of the chromatogram with the equipment library (Nist 5.0).

Ethical consideration and animals used

The study was submitted to the Animal Use Ethics Committee of the Federal University of Amapá, CEUA-UNIFAP, and it was approved in May 2017, under Protocol No. 01/2017. Twenty-five male Wistar rats were used, each with a weight of around 210 ± 40 g. During the study, the animals were housed in individual stainless steel metabolic cages, measuring 60 cm × 50 cm × 22 cm. They were kept in an air-conditioned environment, with a temperature of 25 ± 3 °C, and a humidity of 50 ± 10%, a photoperiod of 12 h of light and dark, and they were fed with standard balanced rations for rodents and water ad libitum.

Induction of diabetes mellitus

The induction of DM was performed in the fasting animals every 16 h, by intraperitoneal injection of streptozotocin (STZ) (Sigma-Aldrich Inc., St. Louis, MO, USA) dissolved in 0.01 M sodium citrate buffer (pH 4.5), with a dose of 55 mg/kg in a volume of 1 ml/kg of body weight. Four days after the STZ injection, animals with glycemia greater than 300 mg/dl were considered diabetic.

Experimental design

The animals were randomly divided into five groups ($n = 5$) and treated orally for 30 days in the following groups:

Normoglycemic group treated with 1 ml/animal 1% tween solution (normal);
Diabetic group treated with 1 ml/animal 1% tween solution (DTC);
Diabetic group treated with glibenclamide 5 mg/kg (GBC);
Diabetic group treated with OR 250 mg/kg (1 ml/animal) delivered in 1% tween solution (OR250);
Diabetic group treated with OR 500 mg/kg (1 ml/animal) delivered in 1% tween solution (OR500).

The doses used were based on the toxicity study of Sachetti et al. (2011), and the lowest dose that did not present toxic effect (500 mg/kg) and 50% of that dose (250 mg/kg) was used.

The diabetic animals were kept in metabolic cages, and body mass, water intake, food intake, and diuresis were evaluated daily. For glucose dosing, blood collection of the retro-orbital plexus and urine were performed every five days. The glucose dosage was performed using the photocolorimetry glucose-oxidase method (Glucox 500, Doles Reagents and Equipment for Laboratory Co., Goiânia, GO, Brasil).

Biochemical analyses

On the 30th day of treatment, the blood was collected (1.5 ml) from the retro-orbital plexus for biochemical analysis of total triacylglycerides, total cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine. The collected blood was centrifuged for 10 min at 7000×*g*, and then the analyses were performed using Doles® (Goiânia-GO, Brasil) reagent kits, in UV–Vis model UVmini-1240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) (Faria e Souza et al., 2017).

Histopathological analysis

At the end of the treatments, the euthanasia of the animals was performed, and, then, the mass of each of the organs was measured: liver, kidneys, pancreas, heart, and lungs. The histopathological analysis of the pancreas were prepared, following the technique described by Vasconcelos et al. (2011), with cuts of 5 μm and the sheets stained by hematoxylin–eosin (H/E). The microscopic analyses were performed, using an Olympus microscope coupled to an image software (Bel View 3.0). The characteristics of the islets of Langerhans were evaluated for damage that were caused by STZ, following the method described by Rouhi et al. (2017), were modified and analyzed 100 islets per microscopic field ($n = 5$ cuts per pancreas) and the average diameter of the islets.

Statistical analysis

Linear regression analysis was used for the quantification of β-caryophyllene. For the other tests, the analysis of variance (one-way ANOVA) was used, followed by the Turkey's test for multiple comparisons. Results with significance levels of $p < 0.05$ were considered statistically significant. All results were expressed as the mean ± standard deviation. The statistical programs used were GraphPad Instat and Prism (Version 5.03).

Results and discussion

The OR consists mainly of a volatile fraction formed by sesquiterpenes; its chemical evaluation encompasses several chromatographic and spectroscopic methodologies, which allow the identification and quantification of most sesquiterpene components (Carvalho et al., 2015).

From the chromatographic profile of the OR, it was possible to identify 84.99% of the sesquiterpene composition present in the OR, allowing the identification of fifteen compounds; among the majorities are β-bisabolene (22.29%), β-caryophyllene (21.25%), and α-farnesene with a percentage of 15.58% (Fig. 1 and Table 1).

These results are in agreement with those reported by other authors since a large variety of sesquiterpenes has been found ranging from 70% to 90% of the OR constitution, being the main sesquiterpenes described: β-caryophyllene, β-bisabolene, α-copaene, α-farnesene, α e β-selinene, α-humulene, and γ-cadinene (Cascon and Gilbert, 2000; Leandro et al., 2012).

The quantitative analysis using β-caryophyllene as the standard compound for the construction of the analytical curve and obtaining the equation of the line ($y = 1635.106 + 952,542$ and $R^2 = 0.9980$) (Fig. 2) demonstrated that the β-caryophyllene content in the ORC was 3.342 ± 0.026 mg/ml ($n = 3$).

The β-caryophyllene content found in the OR was higher than described by other authors, who described a content ranging from 1640 mg/ml to 2996 mg/ml (Tappin et al., 2004; Veiga-Junior et al., 2005; Gomes et al., 2007; Sant'anna et al., 2007).

Several investigations have shown that the terpenes contained in volatile oils have a high antidiabetic potential; in principle, this pharmacological action is attributed to the antioxidant

Table 1
Sesquiterpene components identified in *Copaifera duckei* OR by CG-MS.

Sesquiterpenes	RT (min)	Percentual (%)
α-Copaene	4.589	1.41
α-Gurjunene	5.252	0.68
α-Cubebene	5.584	0.43
α-Farnesene	5.810	15.58
α-Selinene	6.012	7.02
β-Caryophyllene	6.273	21.25
Germacrene D	6.745	1.59
β-Farnesene	7.194	1.52
α-Humulene	7.481	1.81
δ-Bisabolene	7.658	1.65
γ-Muurolene	7.845	1.68
γ-Cadinene	8.015	0.78
γ-Elemene	8.316	1.77
β-Bisabolene	8.513	22.29
Oxide of caryophyllene	9.521	5.53
Total identified		84.99

RT (min), retention time (minutes).

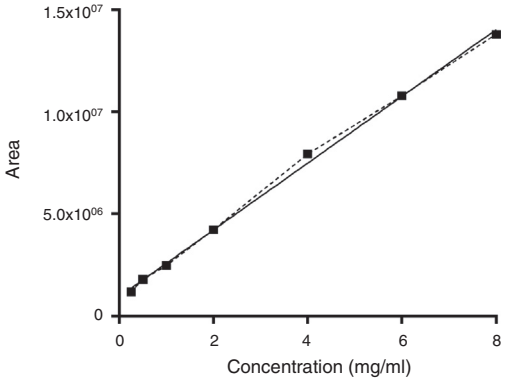

Fig. 2. Standard curve of β-caryophyllene obtained by CG-FID at concentrations of 0.25–8 mg/ml, with equation $y = 1.635 \times 10^6 + 952,542$. Coefficient of correlation $R^2 = 0.9980$.

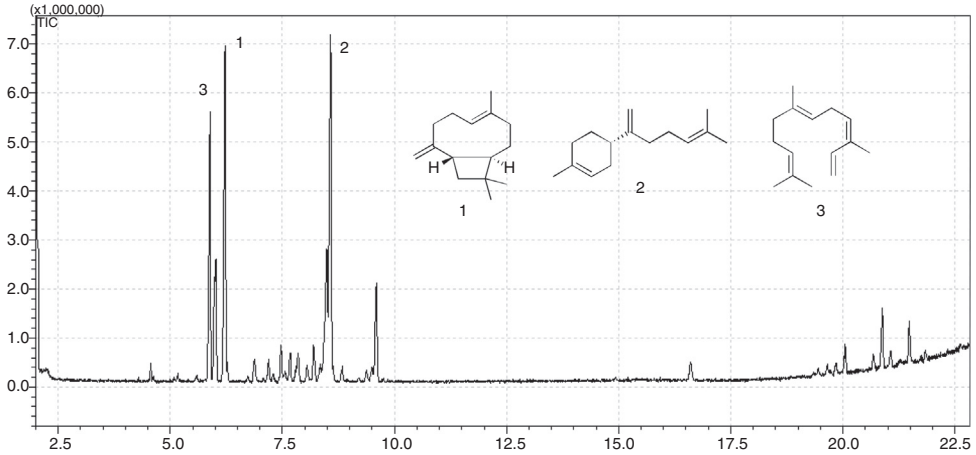

Fig. 1. Chromatographic profile of *Copaifera duckei* OR obtained by CG-MS, presenting as majority constituents: β-caryophyllene (**1**), β-bisabolene (**2**) and α-farnesene (**3**).

Table 2
Effect of treatment for 30 days with *Copaifera duckei* OR (250 and 500 mg/kg, *p.o.*) and GBC (5 mg/kg, *p.o.*) on clinical parameters of diabetic rats.

Parameters	Normal	DTC	OR250	OR500	GBC
Weight (g)	280 ± 5.3[b]	231 ± 6.6	260 ± 5.6[b]	271 ± 7.3[b]	259 ± 5.5[b]
Food (g)	28 ± 2.8[b]	44 ± 4.6	34 ± 3.8[b]	31 ± 3.8[b]	33.6 ± 4.7[a]
Water (ml)	51 ± 3.3[b]	139 ± 8.1	79 ± 6.1[b]	70 ± 6.9[b]	67 ± 9.3[b]
Urine (ml)	13.5 ± 3.4[b]	109 ± 7.7	61 ± 6.5[b]	53 ± 5.7[b]	42 ± 5.2[b]

The values represent the mean ± standard deviation ($n = 5$/group).
[a] $p < 0.05$ expresses statistically significant results compared to the DTC group.
[b] $p < 0.001$ expresses statistically significant results compared to the DTC group.

effect of these compounds (Boukhris et al., 2012; Basha and Sankaranarayanan, 2015).

Experimental DM induced with streptozotocin exhibits selective β-cytotoxic action for pancreatic cells and can provide pathophysiological conditions for the study of therapeutic and preventive agents of the disease, making possible the evaluation of relevant parameters of the diabetic syndrome, mainly biochemical and clinical (Carvalho et al., 2016b).

In the assessment of the clinical parameters of diabetic animals (Table 2), when compared to the DTC group with the normal group of animals, there was a significant increase in food consumption, water, and diuresis ($p < 0.001$). However, there was a significant reduction in body mass ($p < 0.001$).

DM is a factor that causes the imbalance in homeostasis of energy expenditure because this condition favors the catabolism of structural proteins, hydrolysis of lipids, and β-oxidation of fatty acids, forming by-products for gluconeogenesis and energy expenditure, thus contributing to the reduction of body mass. In contrast, the lack of insulin also conditions the decrease of leptin and consequently increases food intake (Damiani et al., 2010; Mahendran et al., 2014).

The increase in water consumption and diuresis, observed in the DTC group, is due to the hyperglycemia that causes an imbalance of the blood osmolality, causing the water to pass from the intracellular to the extracellular environment, causing dehydration. In this way, the brain osmoreceptors generate a response to cellular dehydration, triggering an increase in water consumption and increased diuresis in order to maintain osmotic balance (Lerco et al., 2003; Mahendran et al., 2014).

The groups of diabetic animals treated with OR250 and OR500 were able to reduce ration consumption significantly, as well as water and diuresis ($p < 0.001$, Table 2). They were able to significantly increase body weight gain when compared to the DTC group ($p < 0.001$). The GBC-treated group also presented a statistical difference of $p < 0.001$ in most of the parameters. However, for feed intake, there was only a difference of $p < 0.05$. Therefore, the groups treated with OR and GBC demonstrated that these treatments were effective concerning the improvement of the clinical condition caused by DM.

In the evaluation of the relative mass of the organs (Table 3), it was possible to observe that the DTC group presented a statistically significant difference in the increase of the relative mass of the liver and the decrease of the mass of the pancreas. However, there was no difference in the relative mass of kidneys, heart, and lungs between the groups. The treatments with OR250 and OR500 evidenced a significant increase in the relative mass of the pancreas ($p < 0.01$), as observed in the group treated with GBC in relation to the DTC group. The OR250 and OR500 groups, as well as the GBC, presented reduced and significant values of liver mass when compared to the DTC group ($p < 0.001$).

Diabetes mellitus induced with cytotoxic drugs into the β-pancreatic cells present a reduction of the relative mass of the pancreatic tissue due to the high cellular destruction (Carvalho et al., 2016a). The increase in pancreatic mass seen in the OR250 and OR500 groups, as compared to the DTC group, is a strong indication that OR treatment may have attenuated the destruction of pancreatic tissue, since it was observed by El-Soud et al. (2011) that the destruction of pancreatic tissue caused by STZ is also capable of promoting dysfunction of exocrine pancreatic tissue (acini).

Chronic hyperglycemia due to DM contributes to the hyperactivity of hepatocytes in the liver, since the lack of insulin or its peripheral resistance causes the tissues to use the lipids as an energy source, as well as storing them in the hepatic tissues; in this way, the increased synthesis of triacylglycerides and fatty acids contribute to the increase of liver mass (Okamoto et al., 2002; Jun et al., 2007), which was observed in the DTC group. On the other hand, the reduction observed in the groups treated with OR and GBC indicates that the treatment with OR has the ability to avoid the increase of liver mass due to the accumulation of lipids evident in DM.

Biochemical results (Table 4) demonstrate that the DTC group in relation to the normal group showed a significant increase in most of the evaluated parameters, except for total proteins ($p < 0.001$). Changes in urea, creatinine, and transaminases (AST and ALT) observed in the DTC group are indications of renal and hepatic dysfunction (Sá et al., 2015). However, treatments with OR250, OR500, and GBC were able to significantly reduce these levels ($p < 0.001$), demonstrating that such treatments attenuate renal and hepatic dysfunction by reducing levels of transaminases, urea, and creatinine.

A study describes that streptozotocin, besides promoting cytotoxicity due to oxidative damage in β-cells of the pancreas, can cause damage to renal and liver cells in the same way as observed in pancreatic cells. This is because these organs express in their cells the GLUT 2 glucose transporter which presents as the STZ pathway to the intracellular environment (Eleazu et al., 2013). In part, this may explain the high concentration of renal and hepatic markers in the DTC group in relation to the other groups. Regarding the reduction of these parameters, it was observed that the groups treated with OR250 and OR500 reduced the concentrations of hepatic (AST and ALT) and renal (urea and creatinine) toxicity markers. In this way, the treatment with OR can contribute to the protection of these organs against the damage caused by the DM. In the study conducted by Calleja et al. (2013), the potential of β-caryophyllene as a potent natural antioxidant and its ability to attenuate oxidative stress and injury caused by free radical-mediated diseases has

Table 3
Effect of treatment for 30 days with *Copaifera duckei* OR (250 and 500 mg/kg, *p.o.*) and GBC (5 mg/kg, *p.o.*) on the variation of the relative mass of the organs of diabetic rats.

Organs	Normal	DTC	OR250	OR500	GBC
Liver	7.02 ± 0.26[b]	11.01 ± 0.36	7.79 ± 0.29[b]	7.68 ± 0.21[b]	8.21 ± 0.33[b]
Kidneys	1.24 ± 0.14	1.31 ± 0.17	1.25 ± 0.12	1.24 ± 0.15	1.26 ± 0.18
Pancreas	0.69 ± 0.08[b]	0.44 ± 0.09	0.54 ± 0.06[a]	0.59 ± 0.09[a]	0.54 ± 0.08[a]
Heart	0.54 ± 0.09	0.57 ± 0.13	0.55 ± 0.08	0.54 ± 0.07	0.57 ± 0.07
Lungs	1.33 ± 0.14	1.37 ± 0.16	1.35 ± 0.21	1.32 ± 0.17	1.35 ± 0.15

The values represent the mean ± standard deviation ($n = 5$/group).
[a] $p < 0.05$ expresses statistically significant results compared to the DTC group.
[b] $p < 0.001$ expresses statistically significant results compared to the DTC group.

Table 4
Effect of treatment for 30 days with *Copaifera duckei* OR (250 and 500 mg/kg, *p.o.*) and GBC (5 mg/kg, *p.o.*) on biochemical parameters of diabetic rats.

Parameters	Normal	DTC	OR250	OR500	GBC
Total proteins (g/dl)	6.53 ± 0.12	6.22 ± 0.18	6.02 ± 0.11	6.23 ± 0.15	6.41 ± 0.13
Urea (mg/dl)	52.2 ± 3.7[b]	89.4 ± 5.8	61.9 ± 3.3[b]	58.3 ± 3.1[b]	56.7 ± 4.1[b]
Creatinine (mg/dl)	0.52 ± 0.06[b]	0.83 ± 0.08	0.67 ± 0.05[b]	0.65 ± 0.08[b]	0.63 ± 0.06[b]
AST (U/dl)	35.5 ± 4.2[b]	70.2 ± 3.6	55.2 ± 3.4[b]	40.9 ± 4.4[b]	43.1 ± 3.1[b]
ALT (U/dl)	44.7 ± 4.6[b]	79.5 ± 4.9	60.7 ± 3.8[b]	51.4 ± 4.4[b]	53.3 ± 3.9[b]
Triglycerides (mg/dl)	75.3 ± 12.1[b]	221.9 ± 17.3	169.1 ± 15.5[b]	132.7 ± 12.9[b]	141.4 ± 16.3[b]
Total cholesterol (mg/dl)	69.5 ± 6.7[b]	170,11,3	137.1 ± 14.6[b]	115.1 ± 12.4[b]	99.1 ± 14.9[b]

The values represent the mean ± standard deviation ($n = 5$/group).
[a] $p < 0.05$ expresses statistically significant results compared to the DTC group.
[b] $p < 0.001$ expresses statistically significant results compared to the DTC group.

been demonstrated. The improvement of the biochemical profile observed in the GBC group, in part can be explained by the reduction of glycemic levels, thus contributing to the reduction of oxidative damage mediated by hyperglycemia (Erejuwa et al., 2011).

Diabetes mellitus is a pathology that is directly related to the elevation of serum levels of triacylglycerides and cholesterol. The reduction of insulin production is the main factor that contributes to the increase of lipid levels; this insulin deficiency results in the non-activation of the enzyme LPL (lipoprotein lipase) that is responsible for the hydrolysis of the triacylglycerides contained in the lipoproteins; thus, favoring hypertriacylglyceridemia. Insulin deficiency may also result in the activation of HMG-CoA reductase, favoring the production of cholesterol-rich lipoproteins, since under normal conditions, insulin exerts an inhibitory action on HMG-CoA reductase (Jarald et al., 2013; Sebai et al., 2013).

Regarding the levels of triacylglycerides and total cholesterol (Table 4), the results demonstrate that these levels in the DTC group significantly increased when compared to the normal group ($p < 0.001$), presenting values of 221.9 ± 17.3 mg/dl and 170.11 mg/dl, respectively. The groups treated with OR250 and OR500 showed lower and extremely significant values when compared to the DTC group ($p < 0.001$) indicating that these treatments were able to avoid the increase of triacylglycerides and total cholesterol in the animals with DM, as well as that observed in the group treated with GBC.

These results are in agreement with those obtained by other authors, that observed a significant reduction of total cholesterol and triacylglyceride levels in diabetic rats treated with volatile oil of the *Lavandula stoechas* and *Cinnamomum tamala* species (Kumar et al., 2012; Sebai et al., 2013)

In the evaluation of glycosuria (Fig. 3), it was possible to observe that the normal group had a mean value of 9.30 ± 3.20 mg/dl, and when it was compared with the DTC group, a significant difference with an extremely high mean value was observed (340.40 ± 32.20 mg/dl, $p < 0.001$). It was observed that diabetic groups treated with OR250, OR500, and GBC presented a significant reduction of glycosuria, compared to the DTC group, with values of 222.70 ± 29.80, 182.90 ± 31.40, and 102.60 ± 31.60 mg/dl, respectively ($p < 0.001$). This action on glycosuria is possibly due to the reduction of the glycemic levels observed in these groups. A similar result was described in the study carried out by Carvalho et al. (2016a).

In the glycemic evaluation (Fig. 4), the normal group presented a result of 121.01 ± 15.62 mg/dl, whereas the DTC group showed high levels during the 30 days of study, with a mean of 425.58 ± 21.64 mg/dl, and when comparing this result with those of the OR250 and OR500 groups, we can observe that there was a significant reduction ($p < 0.001$), with mean values of 255.76 ± 26.53 and 212.10 ± 21.21 mg/dl, respectively. The group treated with GBC also presented a significant reduction with a mean of 162.55 ± 21.61 mg/dl ($p < 0.001$).

This result demonstrates the hypoglycemic potential of OR. Possibly, this hypoglycemic effect observed in the OR-treated groups is due to the high content of sesquiterpene compounds, since the high content of β-caryophyllene and β-bisabolene, in addition to other sesquiterpenes, has already been demonstrated. Several studies have described the antidiabetic effect of volatile oils, and in part, this pharmacological effect is attributed to terpenic compounds (Boukhris et al., 2012; Kumar et al., 2012; Yen et al., 2015).

In a study using β-caryophyllene sesquiterpene, it has been shown that it is able to reduce the glycemia of diabetic mice induced by STZ by acting on increased insulin production (Basha and Sankaranarayanan, 2015). A study described by Suijun et al. (2014) demonstrated that β-caryophyllene acts on the signaling pathway of insulin release mediated by activation of the G protein Rac1. The Rac1 protein participates in the regulation of the translocation of type 4 glucose transporter vesicles (GLUT4) from the intracellular compartment to the plasma membrane,

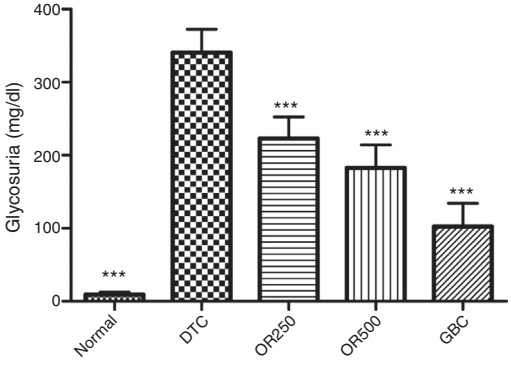

Fig. 3. Effect of treatment for 30 days with *Copaifera duckei* oleoresin (250 and 500 mg/kg, *p.o.*) and GBC (5 mg/kg, *p.o.*) on glycosuria of diabetic rats. The bars represent the mean ± standard deviation ($n = 5$/group). ***$p < 0.001$ represents a statistically significant result compared to the DTC group.

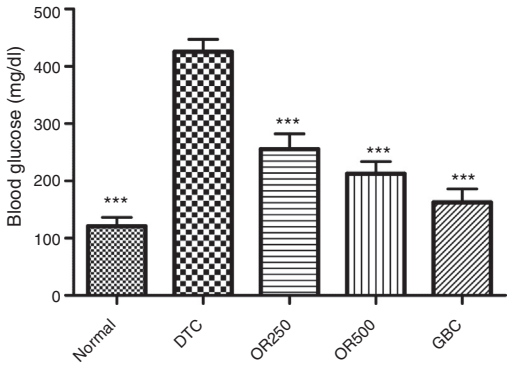

Fig. 4. Effect of treatment for 30 days with *Copaifera duckei* oleoresin (250 and 500 mg/kg, *p.o.*) and GBC (5 mg/kg, *p.o.*) on blood glucose of diabetic rats. The bars represent the mean ± standard deviation ($n = 5$/group). ***$p < 0.001$ represents a statistically significant result compared to the DTC group.

favoring the uptake of glucose in insulin-sensitive tissues (Ueda et al., 2010). In part, this mechanism may explain the antidiabetic effect observed in OR treatment.

A study showed the antidiabetic potential of 29 essential oils; the data suggested that the essential oil of *Melissa officinalis* activates the adenosine monophosphate-activated protein kinase pathway (AMPK) in a dose-dependent manner and that terpenic compounds with limonene, β-citronellal, caryophyllene, and α- and β-citral may be involved in this mechanism of activation (Yen et al., 2015). AMPK plays a significant role in insulin signaling and glucose metabolism. Its activation favors the uptake of glucose (Russo et al., 2013).

The histopathological results (Fig. 5) showed that the induction of DM with STZ was able to promote the necrosis of β-pancreatic cells. It can be observed in the histological photomicrographs that there is a reduction in the number of cell nuclei in the islet of Langerhans of the DTC group. On the other hand, it is possible to notice the largest number of cells in the islets of the other groups treated. Other results show that there was a significant reduction in the number and diameter of the islets of Langerhans when compared to the DTC group in the Normal group (Table 5). It is observed that the groups treated with OR500 and GBC present a significant difference, as compared to the DTC ($p < 0.05$). Although there was an increase in the amount and diameter of the islets of Langerhans in the group treated with OR250, there was no significant difference compared to the DTC group. These results suggest that the effect of OR in the pancreas is dose-dependent since the highest dose had a significant effect on the number and diameter of the islets of Langerhans.

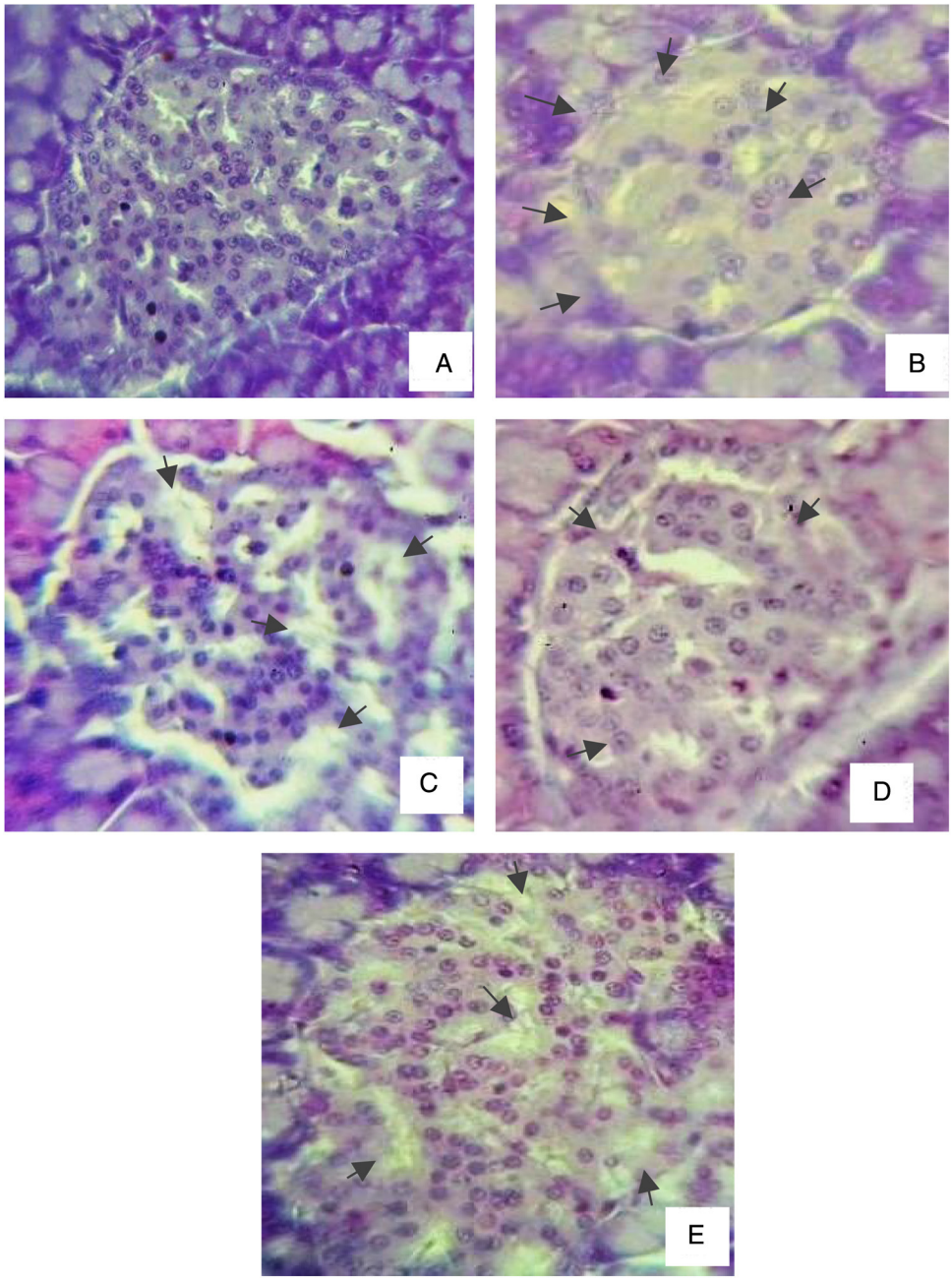

Fig. 5. Photomicrographs of the histopathology of pancreas presenting islets of Langerhans stained by H&E, increase of 400×. (A) Normal group, showing normal number of cells; (B) group DTC, showing reduced numbers of cells (black arrows indicate regions with necrosis caused by STZ); (C) group treated with OR250; (D) group treated with OR500, both present an increase in the number of cell nuclei, indicating possible cellular restoration, with few necrotic regions; (E) GBC group, showing an increase in islet cells and few focus of necrosis.

Table 5
Effect of *Copaifera duckei* Dwyer OR on the number and diameter of islets of Langerhans, assessed on histopathological slides of pancreatic tissue of diabetic rats.

Groups	Number of islets of Langerhans[c]	Diameter of the islets of Langerhans (μm)
Normal	130.9 ± 15.60[b]	83.35 ± 14.66[a]
DTC	69.75 ± 13.53	49.21 ± 11.43
OR250	81.50 ± 16.72	66.72 ± 16.64
OR500	103.40 ± 21.05[a]	78.54 ± 13.89[a]
GBC	108.66 ± 15.03[a]	72.08 ± 16.90

The values represent the mean ± standard deviation ($n = 5$/group).
[a] $p < 0.05$ expresses statistically significant results compared to the DTC group.
[b] $p < 0.001$ expresses statistically significant results compared to the DTC group.
[c] Number of islets of Langerhans quantified in 100 microscopic fields analyzed by histological slide ($n = 5$/pancreas, 400× magnification).

A study by El-Soud et al. (2011) used the essential oil of *Foeniculum vulgare* Mill. In the treatment of diabetic rats, they demonstrated that the treatment was able to regenerate the islet cells of Langerhans and that the antidiabetic effect observed for this volatile oil is due to the restoration of β-pancreatic cells. This finding corroborates our results as it may be suggested that OR also contributed to the reinstatement of these cells since treatment with OR500 demonstrated an increase in the number of cells in the islets in relation to the DTC group. Moreover, there are reports that essential oils rich in terpenes have anti-diabetic properties due to the increase of insulin production due to the restoration of β-pancreatic cells. This fact is fundamental to attenuate the adverse metabolic state observed in DM (Abdelmeguid et al., 2010; Sebai et al., 2013; Basha and Sankaranarayanan, 2016).

Conclusions

In view of the results obtained in this study, the oleoresin of *C. duckei* (copaíba) was able to act on the clinical and biochemical parameters with a reduction of total cholesterol, triacylglyceride, and glucose levels as well as contributing to the restoration of pancreatic tissue. Such effects may be due to their chemical composition with sesquiterpenes as majority compounds. Therefore, we can conclude that the OR treatment used in the conditions of this study has antidiabetic activity and is capable of improving the complications resulting from the DM.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contributions

HOC carried-out laboratory work as part of her final master dissertation. IVFS, CFR, ASAB, BSFS, IMF, RMB, CSL helped to carry out the various experiments. ANC and JCTC supervised this work and prepared the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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