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BIOMEDICAL SCIENCES

Biochemical and histological effects of the subchronic treatment with a beer containing *Baccharis dracunculifolia* in an experimental model of diabetes

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Abstract: This article reports the development of a beer made with *Baccharis dracunculifolia* and its application in an experimental model of diabetes. Initially, the production of the beverage was standardized in order to incorporate the plant extract properly. Next, the beer was analyzed by the UHPLC-MS to identify the substances present. Among others, caffeic acid (5.85 mg / L), m-coumaric acid (5.16 mg / L), pinocembrin (2.99 mg / L), chrysin (10.86 mg / L), myricetin (1.73 mg / L) and spathulenol (9.30 mg / L) were found. Animal tests indicate improvement in biochemical and histological parameters of STZ-induced Wistar rats that ingested the beer made with the plant. The antidiabetic potential of this beverage was observed in the different tests that evaluated insulin resistance and the decrease of the clinical manifestations of diabetes in animals. The use of the drink as an adjunct in clinical treatments for DM2 may be useful, especially in suppressing the oxidative damage caused by the disease.

Key words: bioactive compounds, beer, diabetes, functional beverages.

INTRODUCTION

Characterized by hyperglycemia and the inability of tissues to utilize glucose, diabetes mellitus (DM) is a complex metabolic disease that affects many systems and organs, including the kidneys and liver (Sani et al. 2019). The disease is a significant public health problem, growing to epidemic proportions. It is estimated that currently 9.3% of the world's population has diabetes, and by 2045 the number of cases will reach approximately 700 million (Galaviz et al. 2018, IDF 2019, ADA 2020). Oxidative stress and the mild inflammatory process present in diabetes play a critical role in the pathogenesis of the disease and its comorbidities. The increased production of reactive oxygen species and a substantial reduction in antioxidant defenses promote increased tissue damage (Furuya et al. 2019, Oliveira et al. 2021). This understanding has opened new pathways and targets for the development of improved treatments, as the risk factors associated with diabetes can be reduced through non-pharmacological interventions. In this context, functional foods and beverages emerge as potential allies in the prevention or treatment of these pathological conditions, provided they do not imply additional risks to health (O'Keefe et al. 2014, Nova et al. 2019, Bertuzzi et al. 2020).

Beer, in particular, contains a considerable amount of phenolic compounds with antioxidant activity. However, it is crucial to emphasize that, according to the Gapstur et al. (2023), there is no safe level of alcohol consumption, as it is classified as a toxic and carcinogenic substance. Therefore, any possible benefit associated with beer consumption must be rigorously evaluated against the risks associated with alcohol, especially in patients with chronic conditions such as diabetes mellitus. Our approach in the present study does not aim to suggest beer as a primary or safe therapy but rather as a platform for introducing bioactive compounds, which could, in very specific and controlled contexts, offer antioxidant benefits.

Baccharis dracunculifolia, the plant used in this study, is naturally found in South America and Brazil; it is commonly present in the South, Southeast, and Midwest regions. This plant was previously evaluated in experimental models of gastric ulcer and diabetes; moreover, its anti-inflammatory and antimicrobial activities were analyzed. Different studies reported the presence of bioactive compounds in this plant and their biological activity, demonstrating *B. dracunculifolia* promising therapeutic use (Figueiredo-Rinhel et al. 2019, Costa et al. 2019, Bonin et al. 2020, Brandenburg et al. 2020).

Regarding the choice of beer as a vehicle for the administration of *B. dracunculifolia* extracts, our proposal is based on the search for innovative alternatives that can integrate bioactive components into already popular consumable forms, potentially increasing acceptance among patients. Although the plant extract has already demonstrated antidiabetic effects (Pereira et al. 2022), our approach aimed to investigate the feasibility of a functional beverage that, when consumed moderately and under medical supervision, could offer additional antioxidant benefits. It is worth noting that the formulation was carefully designed to maintain the therapeutic properties of the plant while minimizing the risks associated with alcohol consumption.

Beverages with the addition of plants or fruits are expected to present higher levels of phenolic compounds and, consequently, greater antioxidant activity (Nardini & Garaguso 2020). Given the high prevalence of diabetes and the concomitant consumption of alcoholic beverages (IDF 2019, Marten et al. 2020), developing a beverage with *B. dracunculifolia* that could, within a very specific and controlled context, be used as a complementary approach in managing diabetes is an innovation worth exploring. The present study aimed to characterize clinical and laboratory changes in Wistar rats induced to diabetes by streptozotocin, subjected to subchronic treatment with a craft beer supplemented with *B. dracunculifolia*.

MATERIALS AND METHODS

Plant material and preparation of the ethanol extract

Aerial parts of wild *B. dracunculifolia* were collected at Palmas campus of the Federal Institute of Paraná, located at coordinates 26°30'37.8 "S 51°59'21.0 "W, with an approximate altitude of 1200 meters. Exsiccate of the plant was identified and deposited at the Municipal Botanical Museum of Curitiba (MBM – 398145). In addition, the research was registered at the National System for the Management of Genetic Heritage and Associated Traditional Knowledge – SisGen (A6D3620).

The collected material was dried and stabilized in a circulating hot air oven at 60°C until a constant weight was obtained. The extract was obtained by maceration (cold extraction) as a solvent - absolute ethanol (Synth) at 10%. The contact with the solvent

lasted seven days, with daily homogenizations, at room temperature and protected from light. At the end of this period, the extract was filtered and concentrated in a rotary evaporator using a vacuum percolator with a maximum temperature of 60°C. The resulting extract was placed in an oven with circulating air at 45°C for three days to evaporate the solvent completely. The yield of the alcoholic extract (96%), cold dried (Re), was 14%. The final concentrate was solubilized in an aqueous solution, Tween 80 at 10% (w/v), with a final concentration of 100mg.mL^{-1}.

Brewing process and quality analysis

The single vessel *Microcervejaria Beermax*® equipment was used for the brewing process, and this device automatically controlled the mashing process. The following inputs were used: malt - 62% Pilsen Agrária® (EBC 3.7), 22% Munich Weyermann® (EBC 20) and 16% Carared Weyermann® (EBC 48); Magnum Hallertauer® hops (12.4% Alpha acid); *Saccharomyces cerevisiae* yeast from Lallemand® and; filtered potable water. The production process took place in six main stages: mashing, filtration, boiling, fermentation, carbonation, and filling.

In this experiment, two beers were brewed with varying concentrations of plant extract. The extract, which was pre-solubilized, was introduced at the end of the fermentation process. In Recipe A, 1.0 mg of extract per mL of beer was added; in Recipe B, 0.5 mg of extract per mL of beer was used; for comparison, a third recipe (Recipe C) was also prepared, without any plant extract addition at any stage. The beverages were carbonated in the bottle, after aseptic filling, by adding sucrose. The beverages were carbonated in a bottle, after aseptic filling, by the addition of sucrose. The final product was submitted to the pasteurization technique and microbiological analysis to confirm the absence of pathogenic microorganisms. Additionally, pH, total acidity, and dry extract were analyzed, following the methodology described by the Adolfo Lutz Institute (Zenebon et al. 2005). The tests of density (g/cm^3), alcohol (% v/v), alcohol (% m/m), final sugar concentration (° Plato), original extract (° Plato), real degree of fermentation (RDF %), apparent degree of fermentation (ADF % m/m) and calories (kcal/100mL) were performed using near-infrared measurement in association with a high-precision densimeter in the Antoon Paar Beer Analayser® equipment.

Phenolic compounds and antioxidant activity by DPPH in beverage

The total phenolic compounds were determined by the methodology of Ainsworth & Gillespie (2007), with adaptations. This method uses Folin Ciocalteau reagent and has gallic acid as a reference standard. 500 µL of the sample, 2.5 mL of Folin Ciocalteau reactive (Alphatec®), and 2 mL of sodium carbonate solution - 7.5% Na_2CO_3 (Alphatec®) were added, and the tests were performed in sextuplicate. The test tubes were left to rest protected from light and, subsequently, the absorbance was read in a spectrophotometer at a wavelength of 725nm. A calibration curve with concentrations ranging between 5.0 and 500 µg/mL of gallic acid (Alphatec®) was performed with data expressed in mg of gallic acid equivalents (GAE) per mL of beer.

The methodology used for DPPH was adapted from the work of Brand-Williams et al. (1995), in which 200 µL of the samples were added to 1.8 mL of DPPH reagent [0.13mM] - 2,2-Diphenyl-1-picrylhydrazyl (Alpha Aesar®). The mixture remained in the dark for 30 minutes. After this time, the absorbance reading was performed at 515nm wavelength. Analysis were performed in sextuplicate. The result was compared to a standard curve of Trolox reactive

- 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Sigma-Aldrich) and expressed in mmol of Trolox equivalent per liter of beer (mmol. L^{-1} ET/L).

Chromatographic analysis

Samples of the produced beers and the alcoholic extract of *B. dracunculifolia* were evaluated in a high-resolution Impact II spectrometer (Bruker Daltonics Corporation, Bremen, Germany) with Q-TOF geometry, equipped with an electrospray ionization source - UHPLC/MS-MS. The instrument was calibrated using sodium formate (10 mmol L^{-1}) inserted directly at the beginning of each analysis. The ionization source was operated in positive mode and set to 4500 V with an endplate displacement potential of −500 V. The drying gas parameters were set to a flow rate of 8 L min^{-1} at 200°C and mist at 4 bar pressure. Data were collected from m/z 50 to 1800 with an acquisition rate of 12 spectra per second, and the five most intense ions were selected for automatic fragmentation (auto MS/MS).

The samples were also submitted to high-performance liquid chromatography (HPLC) in the Varian 920-LC equipment, equipped with an automatic sample injector. The chromatographic column used, at a controlled temperature of 30°C, was the Gemini C18 (150 x 4.6 mm d.i, 5 µm particle diameter, Phenomenex, Torrance, CA, USA), using ultrapure water - SA (Milli- Q) and HPLC grade methanol - SB (Sigma) in gradient elution. The gradient started with 30% SB to 64% SB in 15 minutes, 75% SB in 26 minutes, 95% SB in 28 minutes, 30% SB in 32 minutes, and 30% SB in 40 minutes, maintaining this condition for another 10 minutes at a flow rate of 1mL per minute. The compounds were separated and identified in 20 µL sample aliquots by comparing the retention times and absorption spectrum with analytical standards of coumaric acid, ferulic acid, rutin, cinnamic acid, kaempferol, apigenin, galangin, pinocembrin, spathulenol, and quercitin. All samples and standards were prefiltered with 0.45 µm nylon membranes (Millipore). Chromatographic tests for these beers were performed 120 days after filling and pasteurizing the products.

Animals and experimental design

The animal experimentation was performed under the regulations of the Brazilian National Council for the Control of Animal Experimentation and was approved by the Ethics Committee in Animals Use of the Federal Institute of Paraná (registration 009/2018-23411.004490/2018-14). Adult male Wistar rats were kept in rat cages, in a temperature-controlled environment (22°C ± 2°C), with an inverted light-dark cycle (12/12 hours) and acclimated for two weeks before the study (Animal Facility of the Federal Institute of Paraná, Palmas, Paraná, Brazil). Potable water and a standard diet were available *ad libitum* throughout the experiment.

After acclimatization, some animals were induced to an experimental model of diabetes by streptozotocin - STZ (Cayman Chemical Company, Michigan, USA). The STZ was prepared in ice-cold 0.01 M sodium citrate buffer, pH 4.5, and used at a dose of 35 $mg.kg^{-1}$ of body weight – BW (Radenković et al. 2016). The drug was intraperitoneally injected after the animals fasted for 12 hours. After seven days, this procedure was repeated. Fourteen days after the first application, fasting glycemic levels were estimated. Animals were considered diabetic and selected for the study when presented glucose levels above 250 $mg.dL^{-1}$. Animals of non-diabetic groups underwent the same manipulations, being intraperitoneally injected with 0.9% saline solution.

The animals were randomly allocated into 14 groups, consisting of six animals each, totaling

84 rats. The groups were: NORMAL SALINE (NS) – healthy animals, treated with water; NORMAL ETHANOL (NE) – healthy animals, treated with a 5% v/v ethanol solution; NORMAL BASE (NB) – healthy animals, treated with base beer (without addition of plant); NORMAL BACCHARIS HIGH (NBAHIGH) – healthy animals, treated with beer added with *B. dracunculifolia* (1mg of exact/mL of beer); DIABETIC SALINE (DS) – animals induced to diabetes, treated with water; DIABETIC ETHANOL (DE) – animals induced to diabetes, treated with a 5% v/v ethanol solution; DIABETIC BASE (DB) – animals induced to diabetes, treated with base beer (without plant addition); DIABETIC BACCHARIS HIGH (DBAHIGH) – animals induced to diabetes, treated with beer added with *B. dracunculifolia* (1mg of extract/mL of beer); DIABETIC BACCHARIS LOW (DBALOW) – animals induced to diabetes, treated with beer added with *B. dracunculifolia* (0.5mg extract/mL of beer); DIABETIC METFORMIN (DMT) – animals induced to diabetes, treated with water and daily dose of metformin hydrochloride (Medley Farmacêutica LTDA, Brazil); DIABETIC METFORMIN ETHANOL (DME) – animals induced to diabetes, treated with a 5% v/v ethanol solution and daily dose of metformin hydrochloride; DIABETIC METFORMIN BASE (DMB) – animals induced to diabetes, treated with base beer (without addition of plant) and daily dose of metformin hydrochloride; DIABETIC METFORMIN BACCHARIS HIGH (DMBAHIGH) – animals induced to diabetes, treated with beer added with *B. dracunculifolia* (1.0 mg extract mL^{-1} of beer) and daily dose of metformin hydrochloride, and; DIABETIC METFORMIN BACCHARIS LOW (DMBALOW) – animals induced to diabetes, treated with beer added with *B. dracunculifolia* (0.5 mg mL^{-1} extract of beer) and daily dose of metformin hydrochloride. All treatments were daily, uninterrupted, and performed orally by gavage for 32 days. The dose concentrations were calculated according to the animal's body weight (BW), as follows: Water, 0.75 mL/100g BW; 5% v/v ethanol solution, the equivalent to 15g of ethanol for 70kg BW; Beers, the equivalent to 15g of ethanol for 70kg BW; metformin hydrochloride 300 mg kg^{-1} BW.

Dosages of biological parameters and histopathological evaluation

Fasting blood glucose was weekly measured by tail puncture (Accu-Chek Active, Roche, Germany). On the thirtieth day of treatment, a glycemic curve was performed. After fasting for 12 hours, the animals received (via gavage) a single dose of glucose proportional to 1mg. kg^{-1} BW. Glucose quantification was performed at times 15, 30, and 60 minutes after ingestion.

At the end of the treatments, the rats were euthanized, and samples of whole blood, serum, and organs (liver, kidney, and pancreas) were collected for testing. The collection of biological material was carried out according to established protocols (CONCEA 2016). Blood samples were collected by intracardiac puncture, after anesthesia, into EDTA tubes for hematological analyses and into tubes without anticoagulant for serum collection for biochemical analyses. After collection, the samples were centrifuged at 3000 rpm for 10 minutes to separate the serum, which was immediately used for analyses or stored at -80°C until the assays were performed. The organs were weighed and evaluated macroscopically. Samples were stored in Eppendorf tubes and frozen in a freezer at -70°C, until biochemical tests performance. Other organ samples were placed in formaldehyde 10% v/v for the preparation of histological slides. The slides were added to paraffin blocks, cut in a microtome, and stained using the Hematoxylin-Eosin (H.E.) technique. The material was qualitatively evaluated for the presence or absence of histopathological alterations.

Chemicals and reagents

The biochemical analyses conducted in this study utilized specific commercial kits as described below:

Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Creatinine (CREAT), Urea (UR), Total Cholesterol (COL), Triglycerides (TRI), HDL Cholesterol (HDLc), LDL Cholesterol (LDLc), and C-Reactive Protein (CRP): the determinations were performed using specific enzymatic colorimetric and turbidimetric commercial kits from Beckman Coulter® for the AU680 automatic analyzer from Beckman Coulter (Brea, California, USA). Aliquots of 0.5 mL of serum were used for these analyses. The results were expressed in U/L for aminotransferases and in mg/dL for the other assays. The insulin assay was performed using an ELISA commercial kit EZRMI-13K from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany), with results expressed in ng/mL. Serum aliquots were used for quantification. Fructosamine was determined by a fixed-time kinetic method using a commercial kit from Labtest® (Labtest, MG, Brazil). The results were expressed in mmol/L, using serum aliquots. The quantification of glycated hemoglobin was performed using whole blood with EDTA as a sample. The test was carried out with a commercial kit from Labtest® (Labtest, MG, Brazil) by ion-exchange chromatography, with results expressed in %.

Thiobarbituric Acid Reactive Substances (TBARS) in tissues

The TBARS quantification was performed according to Janero (1990), with adaptations, measuring the lipid peroxidation degree by determining the malondialdehyde (MDA) levels. A 10% tissue homogenate in saline at 4°C was prepared in a mixer. First, 125 µL of the homogenate, 500 µL of 10% trichloroacetic acid solution, and 500 µL of 0.67% thiobarbituric acid solution were added. After vortexing, the mixture was placed in a boiling bath for 15 minutes. After cooling to room temperature, 1 mL of n-butanol was added to the samples and again vortexed. After shaking, the material was centrifuged for 5 minutes at 2000 revolutions per minute, and the stained supernatant was read in spectrophotometry at 535 nm (UV Vis Spectrophotometer METROLAB 1700). Then, using the same methodology described above, a concentration curve was performed with MDA as the standard, at concentrations of 1.5, 3.0, 4.5, 6.0, and 10.0 mmol of MDA/mL y=0.0605x+0.0114, R^2 0.9903. The final results were expressed in mmol of MDA/ug of tissue protein, which was measured according to the methodology of Lowry et al. (1951).

Statistical analysis

Statistical analyses were performed using SPSS Statistics version 23.0 for Windows. Initially, the analysis of normality of the variables was performed using the Shapiro-Wilk test, and then the one-way ANOVA test was applied, considering significance for $p<0.05$. After, to identify significant differences, the Student-Newman-Keuls (SNK) post hoc test was used.

RESULTS AND DISCUSSION

Physicochemical analysis

The results of the physicochemical analysis are presented in Table I below.

A significant statistical difference was found in the pH analysis; the addition of the plant notably increased this parameter compared to beer without the addition of *B. dracunculifolia* ($p < 0.05$). These findings align with Humia et al. (2020), who observed a similar result, where pH levels rose with plant additions. Total acidity, resulting from organic acids in foods, directly influences the product's flavor, odor, color,

Table I. Physicochemical data.

	pH	TA	DE	CLR	Dens	AV	AM	FSC	OE	RDF	ADF	CAL
		#	(%)	(EBC)	(g/cm³)	(% v/v)	(% m/m)	(°Plato)	(°Plato)	(%)	(% m/m)	(kcal/100mL)
Recipe A	4.54***	0.16	2.63	46.7***	1.0049	5.27	4.13	1.73	11.69	70	85	41.93
Recipe B	4.52**	0.17	2.41	36.8**	1.0051	5.25	4.12	1.77	11.68	69.9	84.84	41.92
Recipe C	4.41*	0.15	3.05	31.5*	1.0061	5.17	4.06	2.05	11.8	68.2	82.69	42.39

Source: Author, primary data from the experiment. Data were expressed as mean after sextuplicate. Different amounts of * above the results in the same column indicate statistical difference (p<0.05; SNK after one-way ANOVA). n=24. # expressed in g of acetic acid/dL. pH: ionic hydrogen potential; TA: Total acidity; DE: Dry extract; CLR: color; Dens: Density; AV: Alcohol volume/volume; AM: Alcohol mass/mass; FSC: Final sugar concentration; OE: Original extract; RDF: Real degree of fermentation; ADF: Apparent degree of fermentation; CAL: Calories; Recipe A: beer with higher plant concentration; Recipe B: beer with intermediate plant concentration; Recipe C: beer without plant addition.

stability, and quality. Anderson et al. (2019) determined the desired acidity range for beer, based on the method used in the present study, *to be* 0.09 – 0.18 g of acetic acid/dL of beer. The results in this study fall within these parameters, highlighting their practical relevance for maintaining product quality.

The dry extract represents all the solids present in the beverage, which is associated with the beer's body as it indicates the total amount of dextrins, sugars, and proteins present. Typically, beers contain dry extract between 2 and 10% (Gordon et al. 2018). The dry extract values of all evaluated beers corroborate with the final sugar concentration findings, indicating consistency in the results. A gradual increase in EBC units proportional to the extract added to the drink was observed in the evaluation of color, which is statistically significant ($p < 0.05$). Habschied et al. (2020) found similar results in their studies with beers.

Conversely, no statistical difference ($p > 0.05$) was found between the samples for the final density parameter. Density is closely linked to other quality parameters, such as the apparent extract (% m/m), the real fermentation extract (%), the final sugar concentration (° Plato), and the completion of the fermentation process. The average caloric values found were statistically close to those of beers considered light ($p > 0.05$), up to 35 kcal/dL. It is important to highlight that excessive beer consumption can affect the consumer's diet and health, and the practical implication of this finding is that the maximum amount of drink recommended, considering a diet of 2000 kcal per day, is a 300 mL bottle of beer. This would correspond to 6.1% of the recommended daily calorie intake and 15g of ethanol intake—the maximum recommended dose per day (Salanță et al. 2020). These results highlight the need for moderation in beer consumption, especially considering its caloric and ethanol content.

Phenolic compounds and antioxidant activity

An increase in phenolic compounds was observed as plant concentrations increased in the beverage. *B. dracunculifolia* contains various phenolic compounds, including coumaric acid, ferulic acid, rutin, cinnamic acid, camperol, apigenin, galangin, pinocembrin, quercitin, and artepilin C (Salazar et al. 2018, Bonin et al. 2020).

Phenolic compounds, which naturally originate through the secondary metabolism of plants, may act on the activity of free radicals, thereby influencing the reduction of tissue lipid oxidation when incorporated into food (Samborska et al. 2019). Figure 1, below, shows the results of the phenolic compounds dosages and the antioxidant activity by DPPH.

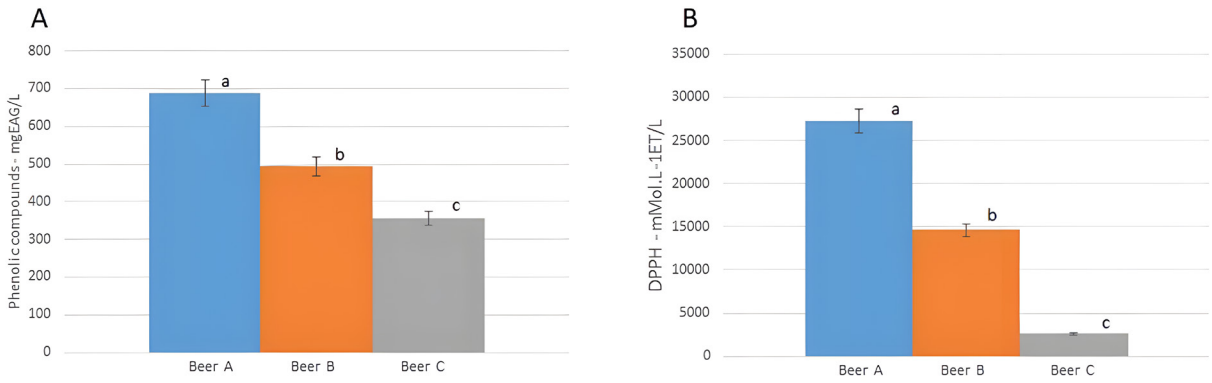


Figure 1. a) Dosage of phenolic compounds; b) Dosage of DPPH. Data were expressed as mean ± SEM (standard error of the mean). Different letters [a, b, c] indicate statistical difference ($p<0.05$; SNK after one-way ANOVA). n=24. Beer A: beer with the highest concentration of plant; Beer B: beer with intermediate plant concentration; Beer C: beer without plant addition. Source: Author, primary data from the experiment.

B. dracunculifolia is rich in a variety of phenolic compounds, including coumaric acid, ferulic acid, rutin, cinnamic acid, kaempferol, apigenin, galangin, pinocembrin, quercetin, and artepilin C (Salazar et al. 2018, Bonin et al. 2020). These bioactive compounds have been identified using high-resolution spectrometry. However, it is important to note that the concentration and activity of these compounds can vary significantly depending on several environmental factors, such as the geographical location, climate, and soil conditions where *B. dracunculifolia* is grown (Park et al. 2004). This variation is crucial when comparing results across different studies, as these factors may influence both the quantity and effectiveness of the phenolic compounds extracted from the plant. For instance, Park et al. (2004) observed that *B. dracunculifolia* cultivated in regions with higher sunlight exposure and lower rainfall exhibited higher concentrations of artepilin C, a compound with potent antioxidant properties. In contrast, Bonin et al. (2020) reported lower levels of artepilin C in plants harvested from more humid regions, suggesting that environmental stress may enhance the synthesis of certain phenolics. Therefore, the bioactive profile of *B. dracunculifolia* in this study reflects not only the plant's intrinsic properties but also the environmental conditions under which it was cultivated.

In addition, the *in vitro* antioxidant activity of the beverage, based on the capture of organic radicals, registered an increasing behavior, in line with the added plant concentrations and with the increase in the levels of phenolic compounds in the beverage. Other studies have also reported this correlation between increased phenolic compounds and radical orgone capture (Do Nascimento et al. 2018, Guiné et al. 2020).

Chromatographic analysis

High-resolution spectrometer analysis identified 34 compounds (Table II and Figure 2a). The present results showed a different chemical composition from those previously reported by (Bonin et al. 2020, Iurckevicz et al. 2021). This variation may be related to the local environments and conditions in which the plants were exposed, seasonality, temperature, altitude, sun exposure, nutritional factors, and plant development stage (Lemos et al. 2015). The plant was collected in August 2019, and in the

previous 12 months, the average temperatures were between 10 and 25°C, with regular rainfall. Under spontaneous vegetation, the plant was cultivated in acidic, deep, and low natural fertility soil.

Beer samples (A and C) were chemically characterized by the same methodology, as shown in Figures 2a, b, c, d, e. Afterward, samples were submitted to an HPLC in the presence of reference standards with some of the substances found in the previous test, as shown in Table III.

The *B. dracunculifolia* ethanolic extract demonstrated a complex sample matrix rich in large mass components. The drinks with the addition of extract showed chromatographic correlation with beer without addition of plant, with peaks in retention times equivalent to substances present in the extract.

Studies demonstrated the correlation of the compounds quantified in this work with beneficial biological activities. Cafeic acid (PubChemCID 689043), a derivative of m-Coumaric acid (PubChemCID 637541), also identified in this work, possess antioxidant, anti-inflammatory, anticancer, and antidiabetic activities (Pittalà et al. 2018). Pinocembrim (PubChemCID 68071) is found in different products, especially in bee propolis and plants of the Piperaceae family. Anti-inflammatory, antioxidant, anticancer, antidiabetic activities, in addition to vasoprotective and neuroprotective actions, were reported for pinocembrin (Shen et al. 2019). Chrysin (PubChemCID 5281607) is a natural compound found in flowers and foods, such as mushrooms, honey, and propolis. It has potent anti-inflammatory, antioxidant, anti-allergic, antidiabetic, anti-hypertensive activities, in addition to hepatoprotective and neuroprotective actions (Naz et al. 2019). Myricetin (PubChemCID 5281672) showed biological action against inflammatory diseases, atherosclerosis, thrombosis, cerebral ischemia, diabetes, Alzheimer's disease, and pathogenic microbial infections (Song et al. 2021). Spathulenol (PubChemCID 92231) showed antioxidant, anti-inflammatory, antidiabetic, anti-Alzheimer's disease, antiproliferative and antimycobacterial actions (Bahadori et al. 2017).

Biological parameters and histopathological evaluation

The injection of STZ triggers a selective cytotoxicity process on pancreatic beta cells, generating an experimental model of DM. The STZ provokes a series of morphophysiological changes in the animals, such as the increase in the levels of oxidation markers in the studied organs, such as TBARS, and macroscopic and microscopic tissue changes (Radenković et al. 2016). Metformin was used as the reference antidiabetic drug. The animals showed biochemical and morphological alteration in serum and tissue, respectively, compatible with the experimental model used. No clinical manifestation that could characterize the presence of another underlying disease in the animals was observed.

Table IV shows the weekly dosage of fasting capillary blood glucose in the treated groups. At the beginning of treatment, the groups showed statistical similarity according to their experimental condition STZ-induced and non-induced. The STZ-induced animals treated with metformin showed a drop in glycemic levels in the first days of treatment. In the tests of the following two weeks (14 and 21 days), another important result trend was noticed, the drop in the glycemic levels of STZ -induced animals treated only with beer added with *B. dracunculifolia* (DBAHIGH and DBALOW). At the end of the treatment, the groups DBAHIGH and DBALOW were statistically different from the control groups (DS, DE, and DB). This difference in glucose levels showed the positive and isolated effect of treatment with the beverage containing

Table II. Chemical constitution of *Baccharis dracunculifolia* extract.

N	Compounds	RT (min)	Molecular formula	Mass (Da)	Class
1	Cafeic acid	5.09	$C_9H_8O_4$	180.1574	Cinnamic acids
2	m-Coumaric acid	9.96	$C_9H_8O_3$	164.1580	Cinnamic acids
3	1,3-Dicaffeoylquinic acid	13.03	$C_{25}H_{24}O_{12}$	516.4509	Organooxygen compounds
4	Dihydroactinidiolide	13.03	$C_{11}H_{16}O_2$	180.2435	Benzofurans
5	Naringenin	13.35	$C_{15}H_{12}O_5$	272.2528	Flavonoids
6	Kaempferol	14.54	$C_{15}H_{10}O_6$	286.2363	Flavonoids
7	Spathulenol	14.75	$C_{15}H_{24}O$	220.3560	Sesquiterpenoids
8	Alpha-curcumene	15.34	$C_{15}H_{22}$	202.3410	Sesquiterpenoids
9	2-Phenylethanol	15.49	$C_8H_{10}O$	122.1644	Benzene and subst derivatives
10	Sphinganine	16.02	$C_{18}H_{39}NO_2$	301.5078	Organonitrogen compounds
11	Naproxen	16.08	$C_{14}H_{14}O_3$	230.2592	Naphthalenes
12	Biocytin	16.5	$C_{16}H_{28}N_4O_4S$	372.4830	Carboxylic acids and derivatives
13	Pinocembrin	17.28	$C_{15}H_{12}O_4$	256.2534	Flavonoids
14	(9Z,11E,13S,15Z)-13-hydroxyoctadeca-9,11,15-trienoic acid	18.55	$C_{18}H_{30}O_3$	294.4290	Organooxygen compounds
15	Chrysin dimethylether	18.77	$C_{17}H_{14}O_4$	282.2907	Flavonoids
16	α-Dimorphecolic acid	19.1	$C_{18}H_{32}O_3$	296.4449	Fatty Acyls
17	Caryophyllene epoxide	19.21	$C_{15}H_{24}O$	220.3505	Triterpenoids
18	2-Isopropyl-5-methyl-9-methylene-bicyclo-1-decene(4.4.0)	19.37	$C_{15}H_{24}$	204.1878	Triterpenoids
19	(S)-2,3-Epoxysqualene	19.46	$C_{30}H_{50}O$	426.7174	Triterpenoids
20	Myricetin	19.68	$C_{15}H_{10}O_8$	318.2351	Flavonoids
21	Cyclosativene	19.78	$C_{15}H_{24}$	204.3411	Sesquiterpenoids
22	Omega-3 Arachidonic acid	19.79	$C_{20}H_{32}O_2$	304.2402	Fatty Acyls
23	Maslinic acid	20.08	$C_{30}H_{48}O_4$	472.7100	Triterpenoids
24	DEhydrO (11,12)URSolic acid lactone	20.11	$C_{30}H_{46}O_3$	454.3447	Organooxygen compounds
25	9(Z),11(E),13(E)-Octadecatrienoic acid ethyl ester	20.22	$C_{20}H_{34}O_2$	306.2558	Fatty Acyls
26	R-Mellein	20.48	$C_{10}H_{10}O_3$	178.1846	Benzopyrans
27	2-Linoleoyl glycerol	20.55	$C_{21}H_{38}O_4$	354.5240	Fatty Acyls
28	Betulin	21.14	$C_{30}H_{50}O_2$	442.7280	Triterpenoids
29	Oleanolic acid	21.22	$C_{30}H_{48}O_3$	456.7110	Triterpenoids
30	Uvaol	21.26	$C_{30}H_{50}O_2$	442.7280	Triterpenoids
31	Linoleic acid	21.28	$C_{18}H_{32}O_2$	280.4455	Fatty Acyls
32	Alpha-Cubebene	21.57	$C_{15}H_{24}$	204.3570	Sesquiterpenoids
33	Bis(2-ethylhexyl) phthalate	21.79	$C_{24}H_{38}O_4$	390.5561	Benzoic acids and derivatives
34	Ethyl Oleate	22.84	$C_{20}H_{38}O_2$	310.5145	Fatty Acyls

N: Number of compounds; RT: Retention time. Source: Author, primary data from the experiment.

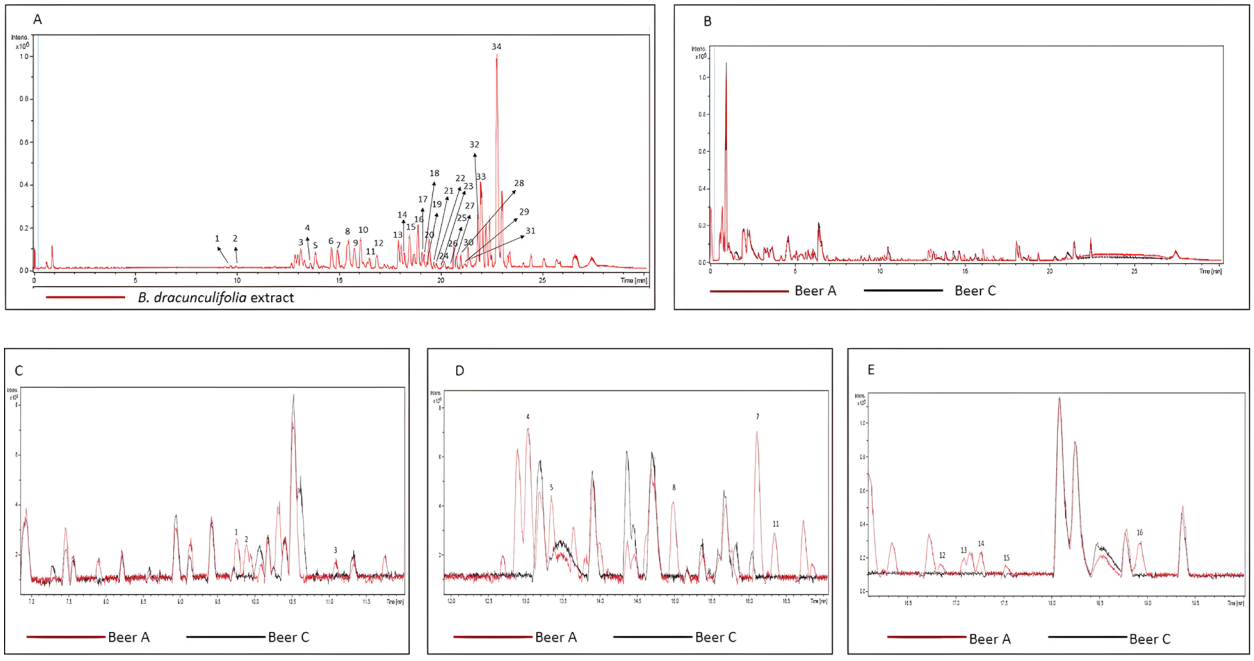


Figure 2. Chromatogram. Graphics UHPLC/MS-MS – Signal intensity / retention time. a) *Baccharis dracunculifolia* extract analysis; b) Overlap Chromatogram Beers A e C; c) Overlap Chomatogram Beers A e C expansion RT 7 – 12; d) Overlap Chromatogram Beers A e C expansion RT 12 – 16,5; e) Overlap Chromatogram Beers A e C RT 16,5 – 20. 1: Cafeic acid; 2: m-Coumaric acid; 3: 1,3-Dicaffeoylquinic acid; 4: Dihydroactinidiolide; 5: Naringenin; 6: Kaempferol; 7: Spathulenol; 8: Alpha-curcumene; 9: 2-Phenylethanol; 10: Sphinganine; 11: Naproxen; 12: Biocytin; 13: Pinocembrin; 14: (9Z,11E,13S,15Z)-13-hydroxyoctadeca-9,11,15-trienoic acid; 15: Chysin dimethylether; 16: α-Dimorphecolic acid; 17: Caryophyllene epoxide; 18: bicyclo-1-decene(4.4.0); 19: (S)-2,3-Epoxysqualene; 20: Myricetin; 21: Cyclosativene; 22: Omega-3 Arachidonic acid; 23: Maslinic acid; 24: DEhydrO (11,12) URSolic acid lactone; 25: 9(Z),11(E),13(E)-Octadecatrienoic acid ethyl ester; 26: R-Mellein; 27: 2-Linoleoyl glycerol; 28: Betulin; 29: Oleanolic acid; 30: Uvaol; 31: Linoleic acid; 32: Alpha-Cubebene; 33: Bis(2-ethylhexyl) phthalate; 34: Ethyl Oleate. RT: Retention Time (min). Source: Author, primary data from the experiment.

the plant in the experimental model applied since the STZ-induced group that received the base beer (DB) is statistically similar to the DS group. Mateo-Gallego et al. (2020) demonstrated clinical improvement in diabetic patients who consumed beer with reduced carbohydrates. In the present experiment, beers with carbohydrate amounts similar to commercial beers (approximately 5g per 100mL) were used, which indicates greater activity and therapeutic potential for the proposed product when compared to this experiment. These results suggest the beneficial action of the compounds from *B. dracunculifolia* present in beer.

An improvement in glucose absorption was observed, after overload, in STZ-induced animals that received the beers added with *B. dracunculifolia*, as observed in the glycemic curve data presented in Figure 3. The DBAHIGH and DBALOW groups, as in the glucose evolution fasting, showed better glycemic indexes when compared to other STZ-induced groups, especially in the 120-minute dose. DMBAHIGH and DMBALOW showed statistical similarity with the normal groups in the first 30 minutes of the experiment. The DMT animals only showed this pattern in the last sample quantification. These results reinforce the biological activity of the

Table III. Compound quantification.

Sample	Cafeic acid	Coumaric acid	Pinocembrin	Chrysin	Myricetin	Spathulenol
	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Extract	9.24	34.07	93.45	220.75	8.27	83.1
Beer A	5.85	5.16	2.99	10.86	1.73	9.30
Beer B	2.22	-	-	2.33	0.55	3.25
Beer C	-	-	-	-	-	-

Extract: *B. dracunculifolia* ethanolic extract; Beer A: Beer with a higher concentration of extract; Beer B: Beer with a lower concentration of extract; Beer C: Beer without extract addition.

Table IV. Analyses of fasting blood glucose during treatment.

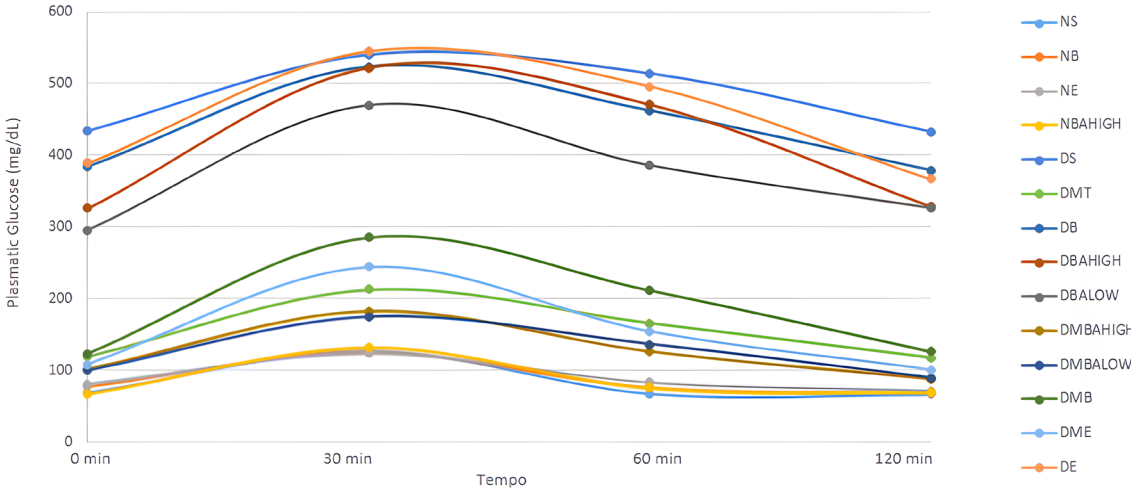
	Beginning				7 days				14 days				21 days				30 days			
NS	82.0	±	4.0	*	90.2	±	2.2	*,#	83.8	±	3.4	*	78.0	±	2.4	*	68.8	±	1.6	*
NE	74.8	±	2.2	*	78.3	±	6.4	*,#	100.2	±	3.7	*	85.0	±	4.0	*	80.7	±	2.5	*
NB	70.7	±	4.6	*	95.7	±	6.4	*,#	89.0	±	2.1	*	76.8	±	2.4	*	77.3	±	4.2	*
NBAHIGH	73.8	±	3.4	*	62.7	±	2.8	*	97.2	±	2.6	*	75.7	±	1.3	*	66.8	±	2.5	*
DS	391.3	±	38.5	#	434.0	±	49.8	@	404.8	±	35.2	$	377.2	±	23.8	@	433.3	±	18.1	&
DE	403.8	±	31.4	#	419.0	±	42.2	@	341.3	±	16.0	@	307.5	±	15.8	#,&,@	389.2	±	41.6	&
DB	404.3	±	30.4	#	404.5	±	34.1	@	278.7	±	33.6	&,@	339.0	±	55.3	&,@	383.5	±	29.9	&
DBAHIGH	405.8	±	30.5	#	338.5	±	37.3	$,@	310.5	±	31.8	@	285.8	±	16.6	#,&	326.0	±	25.3	#
DBALOW	398.8	±	22.6	#	283.8	±	33.3	&,@	223.0	±	28.2	#,&	253.3	±	14.6	#	295.3	±	24.0	#
DMT	389.3	±	24.6	#	201.3	±	20.6	#,&	171.8	±	25.3	*,#	131.0	±	15.9	*	118.2	±	4.2	*
DME	388.5	±	24.6	#	152.8	±	19.8	*,#	115.0	±	10.6	*	135.2	±	22.1	*	108.3	±	3.2	*
DMB	396.0	±	24.6	#	186.8	±	24.8	*,#,&	144.5	±	26.3	*,#	160.7	±	17.3	*	123.0	±	0.9	*
DMBAHIGH	394.0	±	16.7	#	189.2	±	39.1	*,#,&	144.7	±	28.2	*,#	148.3	±	21.1	*	101.0	±	7.6	*
DMBALOW	395.8	±	26.6	#	144.7	±	25.5	*	122.3	±	15.3	*	121.5	±	12.1	*	99.3	±	4.9	*

Source: Author, primary data from the experiment. Data were expressed as mean ± standard error of the mean (SEM). Different symbols *, #, &, @, $, in the same column indicate statistical difference ($p<0.05$; SNK after one-way ANOVA). n= 84. NS – Normal saline; NE – Normal ethanol; NB – Normal base; NBAHIGH – Normal *Baccaharis* high; DS – Diabetic Saline; DE – Diabetic ethanol; DB – Diabetic Basic; DBAHIGH – Diabetic *Baccharis* high; DBALOW – Diabetic *Baccharis* low; DMT – Diabetic Metformin; DME – Diabetic Metformin Ethanol; DMB – Diabetic Metformin Base; DMBAHIGH – Diabetic Metformin *Baccharis* high; DMBALOW – Diabetic Metformin *Baccharis* low.

drink used, suggesting that the observed effects result from chemical constituents present in the plant extract added to the drink.

In the assessment of insulin resistance (IR) and glucose monitoring parameters (Table V), tests for insulin, HbA1c, and fructosamine were conducted, along with the use of mathematical formulas to estimate IR, specifically HOMA-IR and TyG.

The results of HbA1c and fructosamine in animal experiments represent a good methodological alternative in carrying out intergroup comparisons. The statistical difference of the changes in glycated hemoglobin levels during the test was discrete. Locally, an increase in the mean values for the STZ- induced animals without metformin treatment may be observed; however, this group showed no statistical difference compared to the animals treated with metformin. This result can be explained by the total treatment time of the experiment, 32 days. The HbA1c is a measure



	Jejum	30 min	60 min	120 min
NS	68,8 ± 1,6*	127,2 ± 7,2*	67,3 ± 3,1*	66,3 ± 3,1*
NE	80,7 ± 2,5*	123,5 ± 3,5*	82,7 ± 6,7*	71,3 ± 4,1*
NB	99,0 ± 3,8*	124,7 ± 6,7*	75,5 ± 3,1*	68,0 ± 2,4*
NBAHIGH	66,8 ± 2,5*	131,2 ± 7,0*	75,2 ± 1,8*	68,5 ± 4,0*
DS	433,3 ± 18,1$^{\&}$	539,8 ± 16,0$^{\%}$	513,8 ± 19,8$^{\$}$	432,5 ± 9,4$^{\&}$
DE	389,2 ± 41,6$^{\&}$	544,8 ± 13,7$^{\%}$	496,0 ± 22,0$^{\$}$	367,0 ± 6,1$^{\&}$
DB	383,5 ± 29,9$^{\&}$	523,2 ± 15,2$^{\$,\%}$	461,7 ± 29,8$^{\$}$	378,5 ± 38,5$^{\&}$
DBAHIGH	326,0 ± 25,3$^{\#}$	521,8 ± 8,2$^{\$}$	470,5 ± 5,8$^{\$}$	328,7 ± 14,5$^{\#}$
DBALOW	295,3 ± 24,0$^{\#}$	470,2 ± 41,4$^{\$,\%}$	385,8 ± 24,6$^{@}$	326,2 ± 36,0$^{\#}$
DMT	118,2 ± 4,2*	212,7 ± 22,0$^{\#,\&}$	165,7 ± 18,4$^{\#,\&}$	117,0 ± 8,0*
DME	108,3 ± 3,2*	244,3 ± 28,5$^{\&,@}$	154,2 ± 13,2$^{\#}$	100,5 ± 5,8*
DMB	123,0 ± 0,9*	285,2 ± 13,3$^{@}$	211,2 ± 13,1$^{\&}$	125,5 ± 4,2*
DMBAHIGH	101,0 ± 7,6*	182,3 ± 13,8$^{*,\#,\&,@}$	125,7 ± 10,4$^{*,\#}$	87,5 ± 6,7*
DMBALOW	99,3 ± 4,9*	174,0 ± 22,2$^{*,\#,\&,@}$	136,0 ± 26,3$^{*,\#}$	89,2 ± 9,8*

Figure 2. Chromatogram. Graphics UHPLC/MS-MS – Signal intensity / retention time. a) *Baccharis dracunculifolia* extract analysis; b) Overlap Chromatogram Beers A e C; c) Overlap Chomatogram Beers A e C expansion RT 7 – 12; d) Overlap Chromatogram Beers A e C expansion RT 12 – 16,5; e) Overlap Chromatogram Beers A e C RT 16,5 – 20. 1: Cafeic acid; 2: m-Coumaric acid; 3: 1,3-Dicaffeoylquinic acid; 4: Dihydroactinidiolide; 5: Naringenin; 6: Kaempferol; 7: Spathulenol; 8: Alpha-curcumene; 9: 2-Phenylethanol; 10: Sphinganine; 11: Naproxen; 12: Biocytin; 13: Pinocembrin; 14: (9Z,11E,13S,15Z)-13-hydroxyoctadeca-9,11,15-trienoic acid; 15: Chysin dimethylether; 16: α-Dimorphecolic acid; 17: Caryophyllene epoxide; 18: bicyclo-1-decene(4.4.0); 19: (S)-2,3-Epoxysqualene; 20: Myricetin; 21: Cyclosativene; 22: Omega-3 Arachidonic acid; 23: Maslinic acid; 24: DEhydrO (11,12) URSolic acid lactone; 25: 9(Z),11(E),13(E)-Octadecatrienoic acid ethyl ester; 26: R-Mellein; 27: 2-Linoleoyl glycerol; 28: Betulin; 29: Oleanolic acid; 30: Uvaol; 31: Linoleic acid; 32: Alpha-Cubebene; 33: Bis(2-ethylhexyl) phthalate; 34: Ethyl Oleate. RT: Retention Time (min). Source: Author, primary data from the experiment.

that represents the glycemic status in the last 120 days, the average lifespan of a red blood cell (Ng et al. 2019).

In contrast, when evaluating fructosamine, which represents the presence of glycated proteins (mainly albumin) in the samples, a more uniform statistical division between the experimental groups was observed. This marker shows the glycemic status of the last two weeks (Bergman et al. 2020). The statistical difference between the non-induced control groups (NS, NB, NE, and NBAHIGH) and the STZ- induced control groups without metformin treatment (DS, DE, and DB) is evident. The STZ-induced groups treated with metformin, as expected, presented mean values of fructosamine intermediate to

Table V. Insulin resistance and glucose monitoring parameters.

	Plasma Insulin				HOMA-IR				TyG				HbA1c				Frutosamine			
	ng/mL												%				mmol/L			
NS	3.41	±	0.17	*	16.70	±	0.91	*	7.02	±	0.13	*	9.00	±	0.43	*	0.18	±	0.02	*
NE	4.40	±	0.34	#	25.36	±	2.36	*,#	7.60	±	0.06	#	10.64	±	0.68	*,#	0.18	±	0.01	*
NB	4.22	±	0.45	#	22.59	±	1.56	*,#	7.62	±	0.04	#	9.72	±	0.61	*,#	0.17	±	0.00	*
NBAHIGH	4.80	±	0.38	#	23.04	±	2.54	*,#	7.52	±	0.08	#	9.73	±	0.42	*	0.18	±	0.02	*
DS	1.50	±	0.17	&,@	47.09	±	7.00	&	9.89	±	0.06	%	23.16	±	1.03	%	0.74	±	0.05	@
DE	1.21	±	0.08	@	34.00	±	5.09	#,&	9.51	±	0.20	$	18.55	±	0.60	$,@	0.72	±	0.04	@
DB	2.14	±	0.24	&,@	48.29	±	7.78	&	9.29	±	0.13	$	19.13	±	0.47	$	0.77	±	0.06	@
DBAHIGH	1.85	±	0.21	&,@	42.41	±	5.09	&	9.19	±	0.11	$	16.47	±	0.84	@	0.55	±	0.06	#,&
DBALOW	1.91	±	0.23	&,@	38.82	±	3.41	&	8.69	±	0.05	@	17.15	±	0.59	$,@	0.57	±	0.04	&
DMT	2.49	±	0.21	&	20.81	±	1.76	*,#	8.17	±	0.12	&	14.35	±	0.42	&	0.42	±	0.02	#
DME	2.49	±	0.21	&	19.18	±	1.74	*,#	8.03	±	0.09	&	13.27	±	0.54	#,&	0.50	±	0.03	#,&
DMB	2.60	±	0.20	&	22.76	±	1.73	*,#	8.57	±	0.12	@	12.81	±	0.73	#,&	0.48	±	0.03	#,&
DMBAHIGH	2.09	±	0.11	&,@	12.84	±	1.19	*	8.08	±	0.13	&	11.77	±	1.10	*,#,&	0.42	±	0.02	#
DMBALOW	1.97	±	0.17	&,@	14.15	±	1.88	*	8.22	±	0.17	&	11.15	±	0.94	*,#	0.42	±	0.02	#

Source: Author, primary data from the experiment. Data were expressed as mean ± standard error of the mean (SEM). Different symbols *, #, &, @, $, % in the same column indicate statistical difference (p<0.05; SNK after one-way ANOVA). n= 84. NS – Normal saline; NE – Normal ethanol; NB – Normal base; NBAHIGH – Normal *Baccaharis* high; DS – Diabetic Saline; DE – Diabetic ethanol; DB – Diabetic Basic; DBAHIGH – Diabetic *Baccharis* high; DBALOW – Diabetic *Baccharis* low; DMT – Diabetic Metformin; DME – Diabetic Metformin Ethanol; DMB – Diabetic Metformin Base; DMBAHIGH – Diabetic Metformin *Baccharis* high; DMBALOW – Diabetic Metformin *Baccharis* low.

the groups described above, with statistical differentiation. Two groups stand out in this parameter, DBAHIGH, and DBALOW, that, although not treated with metformin, presented statistical differences with such groups. This result corroborates with data described and discussed previously in this section regarding the improvement of the biochemical profile of STZ-induced animals treated with beer added with *B. dracunculifolia*.

Statistical differences were found in serum insulin levels between STZ-induced and non-induced groups. No significant differences were observed in this parameter between the STZ-induced groups. It is important to highlight that, in general, when evaluating the therapeutics of medicinal plants in DM, insulin alone may not present the real therapeutic potential of these products added to natural drugs (Unuofin & Lebelo 2020). Therefore, mathematical estimates are suggested in interpreting experimental results such as those demonstrated in the present study. The HOMA-IR index was statistically relevant between the STZ-induced groups treated or not treated with metformin. For this analysis, the results of DMBAHIGH and DMBALOW were considered statistically similar to the NS group. Bao et al. (2020) reported a similar result, showing the improvement of this parameter after treating experimental diabetes using a plant. The relationship between dyslipidemia and IR, assessed by TyG, showed correlation and consistency with the results presented by HOMA-IR. IR leads to the release of accumulated lipids in adipocytes, in addition to the fact that insulin influences, via hormonal signaling, the hepatic metabolism of triglycerides – TG (Sánchez-García et al. 2020). The STZ-induced groups treated with beer added with the extract showed an improvement in the

IR parameters compared to their respective STZ-induced controls. The IR is linked to a condition of subclinical inflammation associated with adaptive and innate immunity events. Thus, inflammatory pathways are one of the main forms of DM2 pathogenesis and its complications, including the vascular ones, an increase in the expression of pro-inflammatory substances and their respective molecular actions is observed. This data suggests that anti-inflammatory agents from plants and products derived from them, such as the one presented in this study, may improve plasma glucose levels and the functionality of pancreatic beta cells (Granato et al. 2017, Luc et al. 2019).

The STZ induction promotes changes in hepatic and renal metabolism; therefore, the STZ-induced groups tend to express higher serum levels of liver enzymes, renal markers, inflammatory markers, and lipid dysregulation when compared to animals without STZ induction. The results expressed in Table VI present these data.

DM is often associated with lipid metabolic changes and is a significant risk factor for the development of atherosclerosis and cardiovascular damage (Sani et al. 2019). Increased CT and TG are markers of lipid alteration in STZ-induced albino rats. The evaluation of the results showed a trend, with mathematical differentiation, of improvement in the biochemical parameters of ALT, AST, CREAT, and TRI in the groups treated with the beverage added with *B. dracunculifolia* when compared to their control groups. These observations corroborate the results of the morphological improvement of the evaluated organs, suggesting a decrease in tissue and cell structural damage associated with treatment with beer with plant addition. Pereira et al. (2022) found similar results of morphological and biochemical parameters improvement in STZ-induced animals treated with the methanol extract of *B. dracunculifolia*.

Figure 4 shows the results of the morphological evaluation, the relative weight, and the evaluation of the lipid peroxidation profile of the kidney (4.1.), liver (4.2), and pancreas (4.3).

Histopathological evaluation showed typical tissue alterations of the induced pathological process, especially in animals from the DS group (Figure 4.1.a, 4.2.a, 4.3.a). In addition to changes in volume and weight, tissue tone was paler than un-induced controls. A decrease in the number of normal cells, tissue destruction, lymphocyte infiltration, and slight loss of cell architecture was found. The liver showed changes in the parenchyma, accumulation of fat, and small vacuoles inside the cells. In the pancreas, the inflammatory process is well evidenced, with fibrosis in the islets and leukocyte infiltration in the acinar region. Boland et al. (2017) indicated that insulin depletion might result in degenerative structural tissue changes. With alterations compatible with cloudy swelling, the animals' kidneys were accumulating water inside, in addition to nuclear displacement to the cell periphery, increase in the nucleus/cytoplasm ratio and eosinophilic granules, results that are compatible with the literature (Ebrahimi et al. 2019). The non-diabetic groups (NS, NE, NB, NBAHIGH) showed histological structure characteristics of normal organs. Cellular morphological and tissue architecture improvement was observed in STZ-induced diabetic groups, with or without metformin, treated with the beers (DBAHIGH, DBALOW, DMBAHIGH, DMBALOW) when compared to control groups. These results corroborate other observations of this study regarding the improvement of pathophysiological parameters in STZ diabetic animals treated with beer containing *B. dracunculifolia*.

Table VI. Biochemical dosages.

	ALT				AST				CREAT				UREIA				PCR				COL				TRI				HDL				LDL			
NS	44,0	±	4,9	*	226,4	±	38,7	*	0,29	±	0,02	*	40,3	±	1,3	*	0,14	±	0,02	*	93,5	±	6,7	*	33,5	±	3,8	*	29,9	±	0,8	*	57,0	±	5,6	*
NE	42,4	±	1,9	*	218,8	±	28,3	*,#	0,31	±	0,01	*,#	37,5	±	1,0	*	0,13	±	0,02	*	97,7	±	4,6	*	50,9	±	3,0	*,#,&,@	32,2	±	0,7	*	55,3	±	4,0	*,#
NB	48,8	±	5,3	*	267,0	±	55,5	*,#	0,31	±	0,02	*,#	39,1	±	1,5	*	0,13	±	0,02	*	99,0	±	3,8	*	49,0	±	3,0	*,#,&	30,9	±	0,5	*	58,3	±	3,4	*
NBAHIGH	38,4	±	2,0	*	157,4	±	14,4	*	0,26	±	0,04	*	35,5	±	0,8	*	0,14	±	0,01	*	96,4	±	1,9	*	57,7	±	3,3	*,#,&,@	33,1	±	0,5	*	51,8	±	2,3	*,#,&
DS	124,9	±	37,8	#	258,9	±	58,1	&	0,40	±	0,02	&	51,1	±	9,1	&	0,16	±	0,01	&	86,1	±	3,7	*	95,9	±	6,5	%	32,8	±	1,5	*	34,2	±	3,7	&
DE	157,5	±	82,3	#	376,4	±	141,2	&	0,37	±	0,01	#,&	45,0	±	4,8	#,&	0,16	±	0,01	&	79,5	±	3,9	*	75,6	±	7,6	&,@,$,%	32,0	±	0,7	*	32,4	±	3,8	&
DB	115,0	±	34,2	#	241,5	±	57,2	&	0,36	±	0,01	#,&	50,7	±	7,7	&	0,14	±	0,01	*,#,&	78,9	±	6,6	*	69,4	±	6,0	&,@,$	31,9	±	1,5	*	33,1	±	5,7	&
DBAHIGH	147,0	±	53,0	#	261,0	±	72,6	#,&	0,26	±	0,01	*	53,6	±	8,5	&	0,15	±	0,01	*,#,&	81,7	±	7,7	*	61,1	±	5,2	#,&,@	33,8	±	1,6	*	35,7	±	6,8	#,&
DBALOW	87,4	±	49,8	#	222,8	±	76,8	*,#,&	0,26	±	0,01	*	40,3	±	5,7	#,&	0,15	±	0,01	*,#,&	80,0	±	5,4	*	61,3	±	7,0	#,&,@	31,0	±	2,2	*	36,7	±	2,6	#,&
DMT	84,3	±	25,0	*	217,4	±	25,0	*,#	0,27	±	0,02	*	44,1	±	4,3	*	0,14	±	0,01	*	86,0	±	4,9	*	41,1	±	3,5	*,#	30,6	±	1,6	*	47,2	±	5,3	*,#,&
DME	50,0	±	4,4	*	188,1	±	26,4	*,#	0,39	±	0,01	&	44,3	±	4,2	*,#	0,15	±	0,01	*,#	98,9	±	2,6	*	76,9	±	9,8	$,@,%	35,6	±	0,9	*	47,9	±	3,0	*,#,&
DMB	48,1	±	5,1	*	159,8	±	13,3	*,#	0,39	±	0,02	&	47,0	±	6,4	*,#	0,13	±	0,02	*,#	87,3	±	6,7	*	89,0	±	9,1	$,%	33,9	±	1,3	*	35,6	±	5,1	#,&
DMBAHIGH	51,0	±	3,8	*	187,4	±	15,3	*,#	0,38	±	0,02	&	41,6	±	2,4	*	0,17	±	0,01	*,#,&	96,9	±	6,8	*	56,7	±	3,6	*,#,&,@	35,0	±	2,0	*	50,6	±	5,8	*,#,&
DMBALOW	40,2	±	2,5	*	171,2	±	8,0	*,#	0,38	±	0,01	&	39,0	±	1,8	*	0,16	±	0,01	*,#,&	90,7	±	3,3	*	56,9	±	4,2	*,#,&,@	32,5	±	0,9	*	46,8	±	3,5	*,#,&

Source: Author, primary data from the experiment. Serum levels of biochemical markers. Data were expressed as mean ± standard error of the mean (SEM). Different symbols *,#,&,@,$,% in the same column indicate statistical difference (p<0.05; SNK after one-way ANOVA). n= 84. ALT – Alanine transaminase; AST – Aspartate transaminase; CREAT – Creatinine; UREIA – Ureia; PCR - C reactive protein; COL – Cholesterol; TRI – Triglycerides; HDL – HDL cholesterol; LDL – LDL cholesterol; NS – Normal saline; NE – Normal ethanol; NB – Normal base; NBAHIGH – Normal *Baccaharis* high; DS –Diabetic Saline; DE – Diabetic ethanol; DB – Diabetic Basic; DBAHIGH – Diabetic *Baccharis* high; DBALOW – Diabetic *Baccharis* low; DMT – Diabetic Metformin; DME – Diabetic Metformin Ethanol; DMB – Diabetic Metformin Base; DMBAHIGH – Diabetic Metformin *Baccharis* high; DMBALOW – Diabetic Metformin *Baccharis* low.

Relative organ weights showed minor significant differences between groups (Figure 4.1.b, 4.2.b, 4.3.b). Statistical significance was found for the relative weight of liver and kidney for the DS group, demonstrating an increase in the size of these organs. During the natural course of diabetes, systemic metabolic and inflammatory changes may be caused, leading the body to carry out physiological and morphological adaptations to maintain homeostasis. These adaptations are important in an attempt to circumvent the disease and could be the factor that influenced this result (Cole & Florez 2020).

DM is related to several oxidative stress alterations that lead to tissue damage, particularly in the liver, kidney, and pancreatic tissues (Figure 4.1.c, 4.2.c, 4.3.c). This damage is likely a result of lipid peroxidation after the production of free radicals (Ighodaro 2018). The bioactive compounds present in the drink containing the plant helped reduce and control oxidative stress. When evaluating liver peroxidation data, a classic distribution pattern of the extent of this type of damage may be observed. When analyzing the kidney and pancreas TBARS plots, mathematical differences and trends are greater. In both evaluations, the levels of lipooxidation for DBAHIGH and DBALOW groups are reduced compared to the DS group. Specifically for pancreas data, these animals showed statistical comparison with groups treated with metformin (DME, DMB, DMBAHIGH, and DMBALOW).

CONCLUSIONS

The study findings indicate an improvement in biochemical and histological parameters of STZ-induced Wistar rats treated with beer containing *B. dracunculifolia* extract. This drink's antidiabetic potential is observed in different tests that evaluated insulin resistance and the reduction of clinical manifestations of diabetes in animals. The presence of various phenolic compounds in the beer may be directly or indirectly responsible for its antidiabetic and hypolipidemic effects, as well as the improvement of liver and kidney parameters, especially for the DBAHIGH and DBALOW groups. Additionally, a protective effect on liver, kidney, and pancreas tissues was observed.

Although the exact hypoglycemic mechanism involved is not clearly understood, it is possible that the anti-inflammatory properties of the plant's chemical compounds contribute to this effect. Phenolic compounds such as artepillin C and quercetin may play a role in reducing oxidative stress and inflammation, which are commonly associated with diabetes progression (Salazar et al. 2018, Bonin et al. 2020). The study's findings suggest that the drink was safe for healthy Wistar rats based on biochemical and histopathology analyses. However, while the results are promising, there are several limitations to consider. The sample size was relatively small, and the study duration was limited, which may affect the generalizability of the results. Moreover, the costs associated with producing the plant extract-enriched beer could be a limiting factor for large-scale applications. Additionally, while the study indicates potential antidiabetic benefits, more extensive research is required to fully understand the mechanisms at play and to confirm the clinical relevance of these findings. Such studies should include longer treatment periods, a larger cohort of animals, and an exploration of the dose-response relationship.

Future research should also focus on the identification and isolation of specific bioactive compounds responsible for the observed effects. Furthermore, clinical trials are essential to evaluate the efficacy and safety

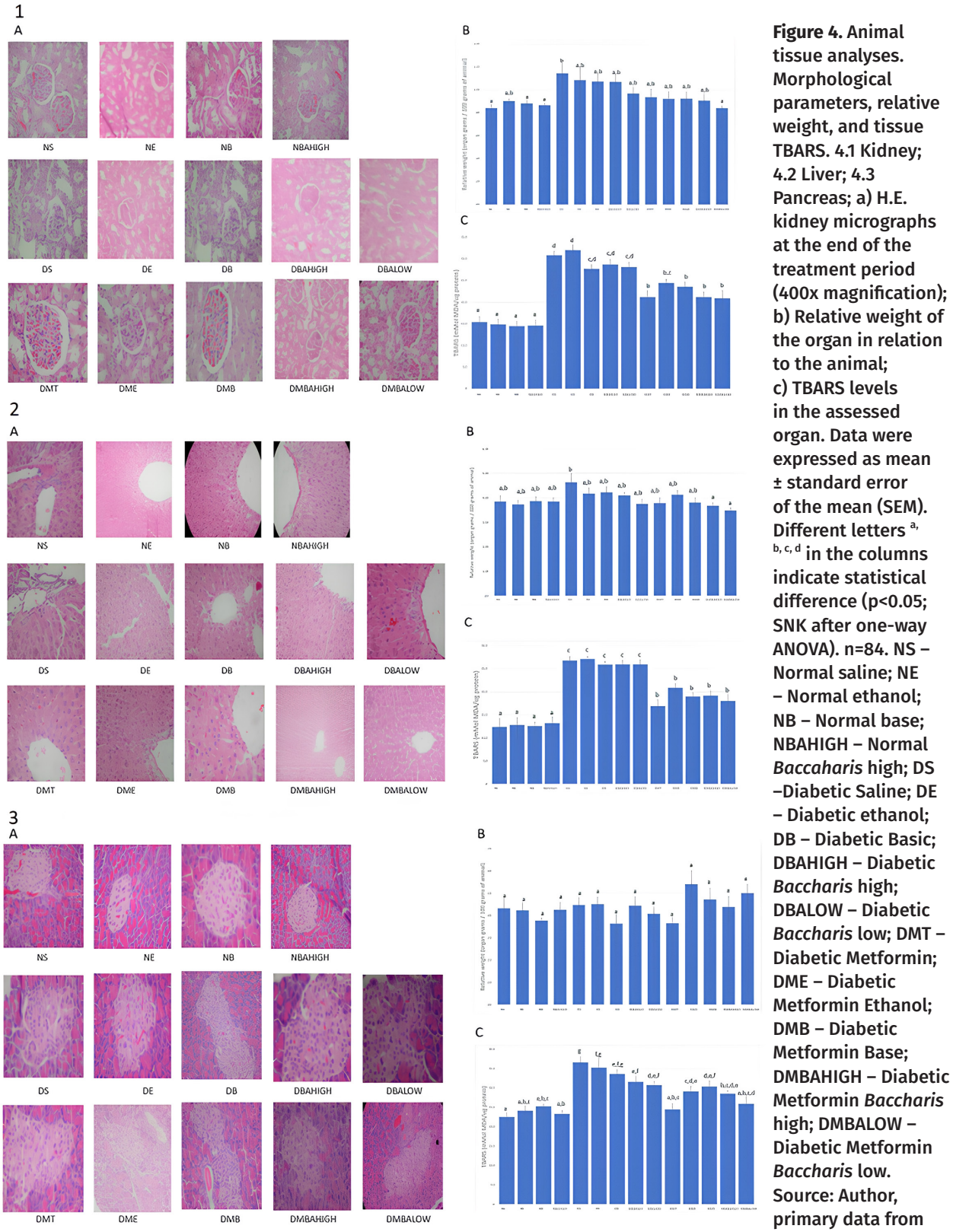


Figure 4. Animal tissue analyses. Morphological parameters, relative weight, and tissue TBARS. 4.1 Kidney; 4.2 Liver; 4.3 Pancreas; a) H.E. kidney micrographs at the end of the treatment period (400x magnification); b) Relative weight of the organ in relation to the animal; c) TBARS levels in the assessed organ. Data were expressed as mean ± standard error of the mean (SEM). Different letters [a, b, c, d] in the columns indicate statistical difference ($p<0.05$; SNK after one-way ANOVA). n=84. NS – Normal saline; NE – Normal ethanol; NB – Normal base; NBAHIGH – Normal *Baccaharis* high; DS –Diabetic Saline; DE – Diabetic ethanol; DB – Diabetic Basic; DBAHIGH – Diabetic *Baccharis* high; DBALOW – Diabetic *Baccharis* low; DMT – Diabetic Metformin; DME – Diabetic Metformin Ethanol; DMB – Diabetic Metformin Base; DMBAHIGH – Diabetic Metformin *Baccharis* high; DMBALOW – Diabetic Metformin *Baccharis* low. Source: Author, primary data from the experiment.

of *B. dracunculifolia*-enriched beverages in human subjects. Understanding the precise molecular pathways involved in its antidiabetic action could lead to the development of novel therapeutic strategies for managing Type 2 Diabetes Mellitus (DM2).

In conclusion, while the current study highlights the potential of *B. dracunculifolia*-enriched beer as an adjunct in DM2 treatment, it is imperative to approach the application with caution. More robust evidence is needed before considering clinical applications, and future studies should address the gaps in knowledge regarding the long-term safety and efficacy of this intervention. The potential advantages, such as the natural origin and the dual benefit of dietary and therapeutic use, are promising but must be weighed against the limitations discussed.

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