An Acad Bras Cienc (2024) 96(3): e20230604 DOI 10.1590/0001-3765202420230604
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

BIOMEDICAL SCIENCES

Phytochemical characterization and antidiabetic analysis of *Bauhinia holophylla* extract on the maternal-fetal outcomes of rats

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Abstract: This study aims to evaluate the phytochemical properties of *Bauhinia holophylla (Bong.) Steud* leaf extract, and their impact on maternal reproductive and fetal development in diabetic rats. For this, adult female Wistar rats (100 days of life) received streptozotocin (40 mg/Kg, intraperitoneal) for induction of diabetes, were mated and distributed into four groups: Nondiabetic; Nondiabetic given *B. holophylla*; Diabetic; and Diabetic given *B. holophylla*. The plant extract was given by gavage at increasing doses: 200, 400, and 800 mg/Kg. At day 21 of pregnancy, liver and blood samples were obtained for oxidative parameters and biochemical analysis, respectively. The uterus was removed for maternal-fetal outcomes. Phytochemical analysis showed a high content of phenolic components and biogenic amines. *B. holophylla* extract did not alter the glycemic levels but improved the lipid profile in diabetic animals. Besides that, the number of live fetuses and maternal weight gain were decreased in Diabetic group, and were not observed in animals treated. The group Diabetic treated presented a higher percentage of fetuses classified as adequate for gestational age compared to the Diabetic group. However, the treatment with plant extract caused embryo losses, fetal growth restriction, and teratogenicity in nondiabetic rats. Thus, the indiscriminate consumption requires carefulness.

Key words: Hyperglycemia, medicinal plants, malformations, pregnancy, serotonin.

INTRODUCTION

Diabetes mellitus (DM) is a syndrome presenting a complex chronic condition characterized by complete or relative insufficiency of insulin secretion and/or action leading to disarrangements in carbohydrate, protein, and fat metabolism (Reece et al. 2004, ADA 2023). The hyperglycemia causes high glycation end products, producing reactive oxygen species, that promote lipid peroxidation, damage cell membranes, and impair several organs, including the liver (Azemi et al. 2012). Moreover, hyperglycemia-mediated oxidative stress negatively influences the pathophysiology of diabetic pregnancy (Zhao & Reece 2005, Sinzato et al. 2022), which contributes to maternal reproductive alterations (increased frequency of spontaneous abortion), neonatal morbidity and mortality or impaired embryofetal development (congenital anomalies) (Eriksson et al. 2003, Bueno et al. 2020).

Several medication interventions are used for diabetes treatment, but the ideal glucose control

is rarely attained (ADA 2023). Therefore, women have utilized plant extracts as an alternative to diabetes therapy and its complications (Azemi et al. 2012). The hypoglycemic effect of many plants has been experimentally demonstrated, both in animals and humans (Damasceno et al. 2017), as species of the genus *Bauhinia* that are typically distributed in tropical regions, including Africa, Asia, and South America (Meng et al. 2014, Saldanha et al. 2021). *Bauhinia sp* is popularly known as "paw-of-cow" due to the bilobate or bifoliolate leaves (Lin et al. 2015, Fortunato et al. 2017). Species including *B. forficata* Link (Pepato et al. 2002), *B. variegate* L. (Kulkarni & Garud 2016), *B. vahlii* Wight & Arn. (Elbanna et al. 2017) and *B. tomentosa* L. (Devaki et al. 2011) have exhibited hypoglycemic effects in rats. Nevertheless, many studies show contradictory results about the antidiabetic potential of certain plants, including *Bauhinia holophylla* (Bong.) Steud (Silva et al. 2010, Pinheiro et al. 2017).

A previous study showed that the treatment with the *B. holophylla* infusion (doses of 400 mg/kg b.w., for 21 days) did not alter the blood glucose levels of female diabetic rats (Pinheiro et al. 2017). However, other studies observed a hypoglycemic action in streptozotocin (STZ)-induced diabetic mice treated with the hydroethanolic extract of *B. holophylla* leaves (400 mg/kg b.w., during 14 days) (Camaforte et al. 2019, Saldanha et al. 2021). Besides that, Rozza et al. (2015) showed antioxidant properties and no toxic effect when using the leaves of *B. holophylla* in rats. This finding indicated that the leaves could be a significant source of several bioactive metabolites.

Nonetheless, there are no studies about the safety of *B. holophylla* use during pregnancy. Diabetic women commonly use the plant during pregnancy without knowing the effects they may have on the maternal and fetal organisms. Experimental studies have proven that medicinal plant components can cause toxic, abortive, and teratogenic effects, leading to embryo-fetal alterations (Leal-Silva et al. 2023, Souza et al. 2023). Therefore, this investigation aimed to evaluate the phytochemical description and antidiabetic effect of *B. holophylla* extract on the maternal reproductive outcomes and fetal development from diabetic rats at the end of pregnancy to advance the knowledge regarding hypoglycemic potential and its safe use.

MATERIALS AND METHODS

Plant material and extraction procedures

B. holophylla specimens were collected from April to May 2015 at Barra do Garças (15°53'26.6" S and 52°19'57" W), Mato Grosso State, Brazil. The plant samples were identified and authenticated by experts from the Universidade Federal de Mato Grosso (UFMT), where a voucher specimen (05718) was deposited at the Herbarium. The nomenclature was verified in the plant list (www.theplantlist.org).

The plant leaves were dried at 50°C for 24 hours in a forced-air oven, ground and powder were prepared for phytochemical analyses and aqueous extract. The preparation of *B. holophylla* aqueous extract was similar to the folk-medicine method by infusion (Pinheiro et al. 2017, Saldanha et al. 2021). The extract was concentrated to 300 mL using a rotary evaporator under reduced pressure for 60 min, with the final concentration of 80 mg/mL on a dry weight basis. The yield of 24 g of crude organic extract was 34.3%. The extract was divided into aliquots and stored at -20°C until use.

Phytochemical investigation of the *B. holophylla* leaves

The total phenolic compounds were determined using the Folin–Ciocalteu's reagent (Singleton

& Rossi 1965). Briefly, 300 mg of the powdered sample was extracted in 80% methanol + 19% distilled water + 1% acetic acid. The samples were centrifuged at 6,000x g, 10 min, 4°C. The supernatant was removed, the residue was remixed with 5 mL of 80% methanol + 19% distilled water + 1% acetic acid, and the same reaction was carried out. The supernatants were pooled, and Folin–Ciocateu reagent and 4% Na_2CO_3 were added. Total phenolic compounds were spectrophotometrically quantified at 725 nm after 60 min of reaction. The calibration curve with gallic acid was used, and the data were expressed in mg gallic acid per g (mg GAE/g).

The total flavonoid content was analyzed according to Awad et al. (2001) with modifications (Souza et al. 2023). 80% methanol + 19% distilled water + 1% acetic acid was added to a 200 mg sample, and after 30 min in an ultrasonic bath, a 5% aluminum chloride solution was added. The tubes were incubated in the dark for 30 min, centrifuged at 6,000 *x g*, and analyzed (425 nm). The results were presented as milligrams of quercetin per gram (mg QE/g).

The identification and quantification of phenolic compounds and amines in leaves of *B. holophylla* were carried out by high-performance liquid chromatography (HPLC) (Ultimate 3000 BioRS, Dionex-Thermo Fisher Scientific Inc®, USA). The profile of phenolic extracts was determined in samples (n=3), quantified at 270, 320, 360, and 520 nm, and the results were expressed as mg/100g (Borges et al. 2020).

To the biogenic amines composition in *B. holophylla* leaves, 300 mg of the powdered sample (n=3) homogenized in 5% cold perchloric acid (1 min) (Merck®, USA) using sonification for 30 min. After centrifugation (6,000 *x g* for 10 min at 4°C), dansyl chloride [(Sigma Chemical Company®, USA), 95%] and saturated Na_2CO_3 were added to the supernatant. After one hour at 60°C, proline (Sigma Chemical Company®, USA) was added. The mixture was maintained in the dark for 60 min at room temperature, with toluene used to extract the dansylated polyamine. Finally, the toluene fraction was collected, dried under gaseous nitrogen, suspended in 1.5 mL of acetonitrile (Tedia®, Brazil), centrifuged (6,000 *x g* for 5 min at 4°C), and the samples (20 µL) were injected into an HPLC. The results were expressed as µg/g (Lima et al. 2008, Cruz et al. 2022).

Antioxidant activity of the *B. holophylla* leaves

The antioxidative activity was evaluated using the stable radical 2,2- diphenyl-1-picrylhydrazyl (DPPH) (Brand-Williams et al. 1995). The antioxidant capacity was determined using a ferric-reducing ability of plasma FRAP assay (Benzie & Strain 1996). The results were expressed as mmol Fe^{2+}/Kg of a sample.

Animals and diabetes induction

This study followed the Guide for Care and Use of Experimental Animals and Animal Research Reporting of In Vivo Experiments (ARRIVE guidelines) (Percie du Sert et al. 2020). The local ethics committee authorized the study protocol (number 23108.001991/13-1 Universidade Federal de Mato Grosso-UFMT). Female Wistar rats (210-240 g, 90 days old) were obtained from the Universidade Estadual de Campinas (UNICAMP) vivarium. They were maintained in the Laboratory of System Physiology and Reproductive Toxicology (UFMT) vivarium under established laboratory conditions (12 h light/dark cycle, temperature 22 ± 3°C). The animals were provided tap water and a standard commercial diet (Purina rat chow, Purina®, Brazil).

In the 100 days of life of the female rats, severe diabetes was induced using a single intraperitoneal (i.p.) injection of streptozotocin (STZ, Sigma Chemical Company®, USA), a

beta-cytotoxic chemical, administered at a dose of 40 mg/Kg diluted in citrate buffer (0.1 mol/L, pH 4.5). The females of the Nondiabetic (Control) group received vehicle (0.01 M citrate buffer, pH 4.5) using the same volume and administration route as in the Diabetic group (Corvino et al. 2015, Cruz et al. 2023). Seven days after diabetes induction, blood samples were collected from the tail vein, and glucose concentrations were measured using a conventional glucometer. As inclusion criteria for the Nondiabetic group, a typical threshold for blood glucose of 6.7 mM (120 mg/dL) was used, and for animals with severe diabetes, a glycemia greater than 16.7 mM (300 mg/dL) was established (Corvino et al. 2015, Neto et al. 2020).

Experimental groups

After inclusion criteria, diabetic and nondiabetic female rats were mated overnight with nondiabetic males. The following morning on which, sperm were seen in the vaginal smear, and this was considered gestational day 0. The pregnant rats were randomized into four experimental groups (n = 12 animals/groups): 1) Nondiabetic: control rats treated with water (vehicle); 2) Nondiabetic Treated: treated with *B. holophylla* aqueous extract; 3) Diabetic: treated with water; 4) Diabetic Treated: treated with *B. holophylla* aqueous extract. The rats were treated with vehicle (water) or plant extract by intragastric route (*gavage*) in the morning during the entire pregnancy. The initial dose of 200 mg/kg of the *B. holophylla* extract was given during the embryonic implantation period (day 0 to day 7 of pregnancy). The dose was increased to 400 mg/kg in the embryonic period (days 8 to 14 of pregnancy) and to 800 mg/kg in the fetal period (days 15 to 21) (Afiune et al. 2017, Pinheiro et al. 2017, Camaforte et al. 2019). The use of increasing doses aiming to reproduce the insulin medication used for diabetic pregnant women based on body weight and advancement of gestational week (Jovanovic 2000, Alfadhli 2015), and doses of 200, 400, and 800 mg/kg of *B. holophylla* extract are equivalent to 32, 64, and 128 mg/kg in humans, based on the body surface area (Reagan-Shaw et al. 2008).

Course of pregnancy

The daily observations for maternal behavioral changes, water and food intake, body weight, and mortality were performed. After 6 h of fasting, the glycemia was measured weekly using a conventional glucometer. On day 21 of pregnancy, the rats were anesthetized with sodium thiopental (Thiopentax®, Cristália Chemical Ltda, Brazil, 120 mg/kg). After confirming the signs of a successful anesthetic procedure, blood samples were collected by decapitation for biochemical parameters analysis. Then, the rats were submitted to laparotomy for exposure to uterine horns. The liver was collected to determine oxidative stress markers.

Analysis of maternal biochemical parameters and redox status

The maternal blood samples were collected in dry tubes and centrifuged at 1,575 × *g* at 4°C for 10 min. The serum was collected to determine biochemical parameters using commercial kits (Winner®, Argentina). An Optimized UV test estimated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) determinations. Concentrations of total protein, total cholesterol, triglycerides, and high-density lipoprotein (HDL-c) were measured using the colorimetric method. Very-low-density lipoprotein (VLDL-c) serum concentrations were calculated from the triglyceride concentrations (Knopfholz et al. 2014).

Collected liver samples were rapidly washed with phosphate buffer saline (0.01 M, NaCl 0.138 M, KCl 0.0027 M, pH 7.4). Hepatic

malondialdehyde (MDA) and total glutathione (GSH-t) level, and superoxide dismutase (SOD) activity were determined using commercial kits (Cayman® Chemical Co., USA). Catalase (CAT) activity was determined following decreases in the initial hydrogen peroxide (H_2O_2) level (20 nM as the initial substrate) at 240 nm and 25°C, over a time frame of two min, according to Sinzato et al. (2023). Reduced thiol group (-SH) levels in liver homogenates were based on the development of a yellow color when 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB) was added to compounds containing sulfhydryl groups. The absorbance was read at 412 nm (Sinzato et al. 2023).

Maternal reproductive outcomes and fetal analysis

The uterus was weighed and dissected to observe and count the number of corpora lutea, dead and live fetuses, points of implantation, and reabsorption (embryonic death). The number of undetectable implantation sites was determined by Salewski's method (Salewski 1964). The rates of losses before (pre-implantation) and after (postimplantation) embryo implantation were calculated (Leal-Silva et al. 2023). The live fetuses were weighed and classified as small (SGA), adequate (AGA), or large (LGA) for gestational age (Sinzato et al. 2022). The ratio between fetal and placental weights was calculated to determine the placental efficiency (Macedo et al. 2021).

The fetuses were also evaluated in a microscope to determine the incidence of external anomalies. After external analysis, half the fetuses were fixed in Bodian's solution, and serial sections were prepared for the visceral examination, as described by Wilson (1965). The remaining fetuses were ready for the assessment of the skeletons by the staining procedure of Staples & Schnell (1964). Additionally, the skeletal analyses and the counting of the ossification sites were performed according to the methodology proposed by Aliverti et al. (1979), which determines the degree of fetal development.

Statistical evaluation

Based on previous experiments conducted in our laboratory to calculate the sample size for full-term pregnant severe diabetic rats (Bueno et al. 2020), and using 90% power and an error type I of 5%, approximately 12 animals/groups have been established.

Data are presented as mean ± standard deviation (SD). After normalizing the results, to compare the mean values among experimental groups, one-way analysis of variance (ANOVA) succeeded by Tukey's Multiple Comparison Test was used. Fisher's Exact test calculated the proportions values. All data were statistically significant when $p<0.05$.

RESULTS

Phytochemical investigation and antioxidant activity of the *B. holophylla* leaves

The *B. holophylla* leaves contained high levels of phenolic compounds. We detected 18.1 mg/g of total phenol and 11.0 mg/g of total flavonoid (methanolic extract). Four phenolic compounds were identified, i.e., rutin, catechin, 3-hydroxytyrosol, and gallic acid (Table I). Additionally, the leaf extracts of *B. holophylla* showed considerable antioxidant activity based on the level of DPPH scavenging and FRAP assay (Table I).

The phytochemical analysis showed that this species is abundant in biogenic amines (Figure 1). Biogenic amines and 11 amino acids (cadaverine, dopamine, histamine, putrescine, serotonin, spermidine, spermine, tryptophan, tryptamine, tyramine, and 5-hydroxytryptophan) were identified in the extract from *B.*

Table I. Total phenolic compounds, flavonoids, biogenic amines, antioxidant capacity measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing ability of plasma (FRAP) of aqueous extract of *Bauhinia holophylla* leaves.

Compounds	Concentration
Total phenol (mg GAE/g)	18.1 ± 0.6
Total flavonoids (mg QE/g)	11.0 ± 0.4
DPPH (*µg/g Trolox)*	15.4. ± 15.6
FRAP (mmol Fe^{2+}/Kg)	85.3 ± 0.2
Quantifications of total phenolic compounds (mg/100g)	
Rutin	4.3 ± 0.1
Catechin	3.7 ± 0.0
3-hydroxytyrosol	1.5 ± 0.1
Gallic acid	1.5 ± 0.0
Quantifications of biogenic amines (*µg/g*)	
Tryptophan	106.2 ± 4.2
Tyramine	95.3 ± 1.7
5-hydroxytryptophanhttps://www.sigmaaldrich.com/BR/pt/substance/5hydroxyltryptophan220224350098?context=product	44.8 ± 1.2
Serotonin	12.5 ± 0.3
Dopamine	6.7 ± 0.5
Putrescine	3.0 ± 0. 1
Spermine	2.6 ± 0.3
Tryptamine	1.7 ± 0.1
Spermidine	1.3 ± 0.1
Cadaverine	0.8 ± 0.0
Histamine	0.5 ± 0.0

Values are represented as means of three repetitions ± standard deviation (SD).

holophylla leaves, and tryptophan, tyramine, 5-hydroxytryptophan, and serotonin were in higher concentration (Figure 1 and Table I).

Maternal glycemia of rats

As shown in Figure 2, the glycemic levels of all rats in the Nondiabetic group remained around 100 mg/dL during pregnancy. However, the rats with STZ-induced diabetes presented higher blood glucose levels than 300 mg/dL than the Nondiabetic groups. The treatment with *B. holophylla* aqueous extract caused no influence on the glycemia of Nondiabetic or Diabetic groups compared to the corresponding untreated groups.

Maternal body weight, water, and food intake during pregnancy

The treated animals showed no clinical signs of toxicity (piloerection, irritability, diarrhea, or death). Still, the body weight in the Nondiabetic Treated group was lower on day 20 of pregnancy compared to the Nondiabetic group. In both diabetic groups, the body weight was decreased, and the water and food intake increased on all days in relation to nondiabetic rats. The Diabetic Treated group presented increased food intake at day 0 of pregnancy compared to nondiabetic rats. The aqueous extract of *B. holophylla* leaves decreased the water intake at days 7 to 20 and the food intake at day 20 of pregnancy in relation to diabetic rats without plant treatment (Table II).

Maternal biochemical parameters and oxidative stress markers

Figure 3 shows the serum biochemical parameters (A-G) and oxidative stress markers in the liver (H-L). The Diabetic group presented increased levels of serum triglyceride, total cholesterol, VLDL-c, ALT, and AST compared to the Nondiabetic group. In the Diabetic Treated group, the same parameters were decreased compared to the Nondiabetic group, except for ALT and AST levels. The treatment with *B. holophylla* aqueous extract decreased HDL-c concentration in nondiabetic and diabetic rats more than in the untreated Nondiabetic group. In addition, the serum concentrations of triglyceride, cholesterol, and VLDL-c levels were decreased. HDL-c concentration and AST

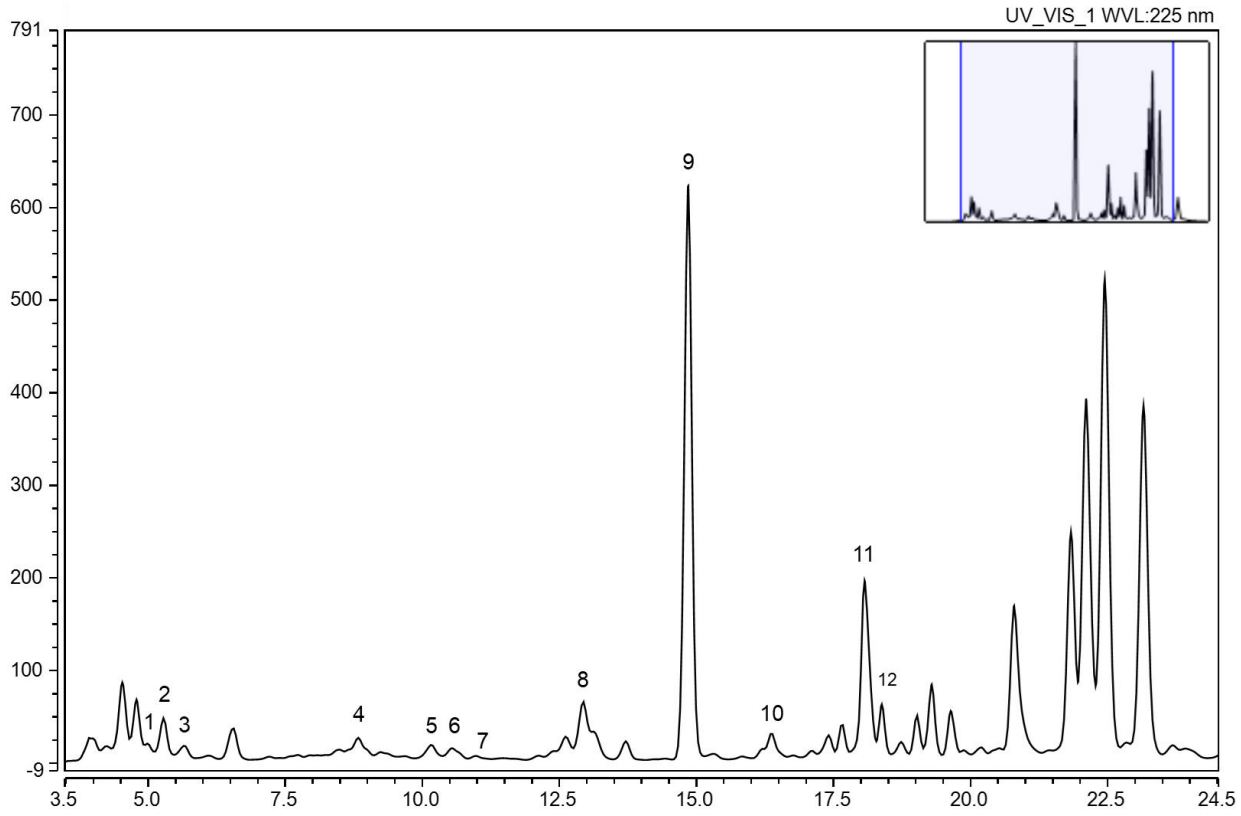

Figure 1. Composition of biogenic amines by HPLC analytical chromatography of leaf extract of *Bauhinia holophylla*. Experimental conditions: eluents A (100% acetonitrile) and B (50% acetonitrile). Gradient system: 0–2 min, 40% A + 60% B; 2–4 min, 60% A + 40% B; 4–8 min, 65% A + 35% B; 8–12 min, 85% A + 15% B; 12–15 min, 95% A + 5% B; 15–21 min, 85% A + 15% B; 21–22 min, 75% A + 25% B; 22–25 min, 40% A + 60% B. Column: Ace 5C18 column (250 × 4.6 mm, 5 µm). Flow rate: 0.7 mL· mL/min and λ = 225 nm. Injected volume: 20 µL. Identified compounds: 1) 5-hydroxytryptophan; 2) tryptophan; 3) serotonin (mono-hydrate); 4) tryptamine; 5) putrescine; 6) cadaverine; 7) histamine; 8) serotonin (hydrochloride); 9) tyramine; 10) spermidine; 11) dopamine; and 12) spermine.

activity increased in the Diabetic Treated group compared to the diabetic rats.

The analyses of liver homogenate samples showed that regardless of plant treatment, the MDA levels were increased in diabetic groups compared to the nondiabetic rats at the end of pregnancy. The CAT activity was lower in both groups of diabetic rats in relation to the Nondiabetic groups. There was no difference in total protein, SOD activity, GSH-t, and -SH concentrations among experimental groups.

Maternal reproductive outcomes

The number of implantation sites, live fetuses, maternal weight gain, gravid uterus weight, and maternal weight gain less gravid uterus were decreased by diabetic status compared to the nondiabetic condition. In addition, the Diabetic group showed higher loss before and after embryo implantation than the nondiabetic rats. The use of *B. holophylla* aqueous also caused an increased percentage of pre-implantation embryonic loss compared to nondiabetic rats (Table III).

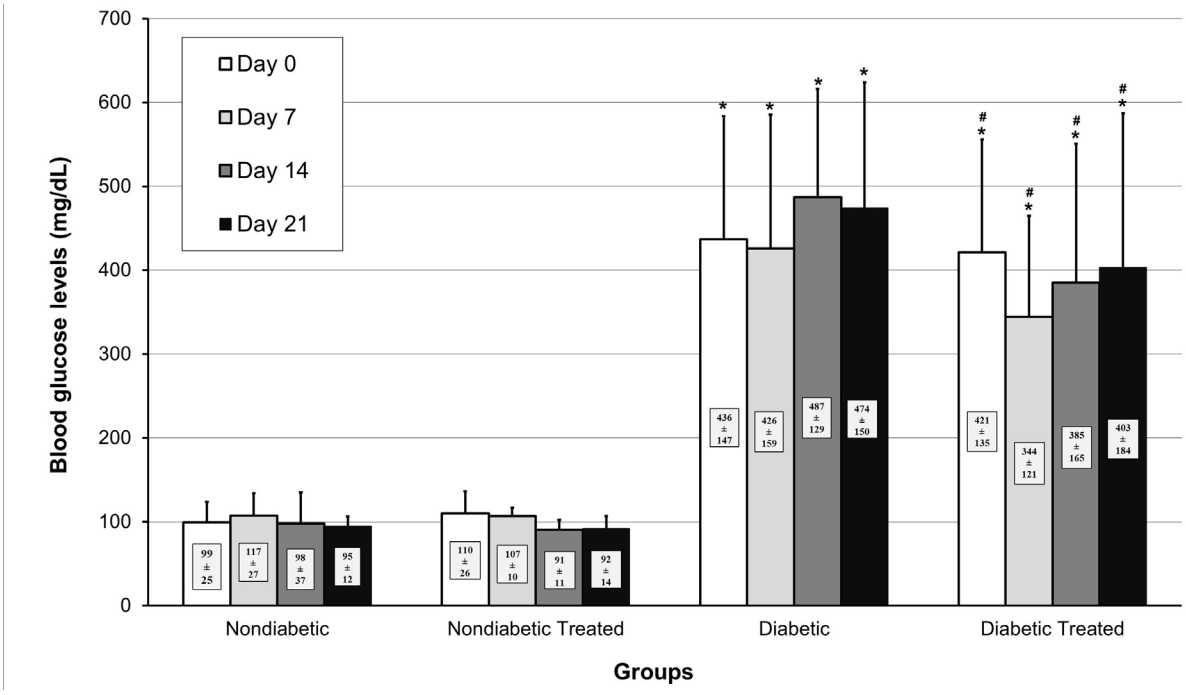

Figure 2. **Fasting blood glucose levels on gestational days 0, 7, 14, and 21 of nondiabetic and diabetic rats treated with water or *Bauhinia holophylla* aqueous extract.**
Data are shown as mean ± standard deviation (SD), n = 12 rats/group.
***p<0.05 - compared to the Nondiabetic group, #p<0.05 compared to the Nondiabetic Treated group (One-way ANOVA followed by Tukey's Multiple Comparison test).**

Table II. **Body weight, water intake, and food consumption on gestational days 0, 7, 14, and 20 of nondiabetic and diabetic rats treated with water or *Bauhinia holophylla* aqueous extract.**

	Groups			
	Nondiabetic (n=12)	**Nondiabetic Treated (n=13)**	**Diabetic (n=12)**	**Diabetic Treated (n=14)**
Body weight (g)				
Day 0	271.2 ± 16.8	254.8 ± 26.6	240.7 ± 19.5*	247.5 ± 22.5*
Day 7	287.7 ± 26.2	265.8 ± 28.0	251.9 ± 16.6*	259.9 ± 17.8*
Day 14	307.0 ± 18.4	289.6 ± 22.4	268.3 ± 17.5*	280.4 ± 22.3*
Day 20	386.8 ± 21.0	349.5 ± 32.2*	310.9 ± 29.9*	335.7 ± 34.1*
Water intake (mL)				
Day 0	37.8 ± 11.5	36.8 ± 6.5	99.3 ± 42.7*	75.6 ± 36.6*#
Day 7	41.1 ± 15.3	42.1 ± 7.7	126.8 ± 46.6*	90.6 ± 41.8*#$
Day 14	45.5 ± 12.6	46.9 ± 7.7	174.6 ± 66.5*	113.2 ± 52.3*#$
Day 20	54.2 ± 10.8	51.3 ± 9.6	172.5 ± 64.6*	99.6 ± 50.2*#$
Food intake (g)				
Day 0	16.5 ± 5.5	16.7 ± 1.3	25.3 ± 7.8*	24.0 ± 4.4*#
Day 7	20.0 ± 5.8	20.1 ± 4.7	31.3 ± 8.5*	26.4 ± 6.4
Day 14	23.5 ± 2.9	21.8 ± 2.7	39.0 ± 9.0*	31.0 ± 12.0
Day 20	25.5 ± 5.3	22.2 ± 2.3	40.6 ± 10.1*	31.8 ± 9.7$

Data are expressed as mean ± standard deviation (SD).
***p<0.05 - compared to the Nondiabetic group, #p<0.05 compared to the Nondiabetic Treated group, $p<0.05 compared to the Diabetic group (One-way ANOVA followed by Tukey's Multiple Comparison test).**

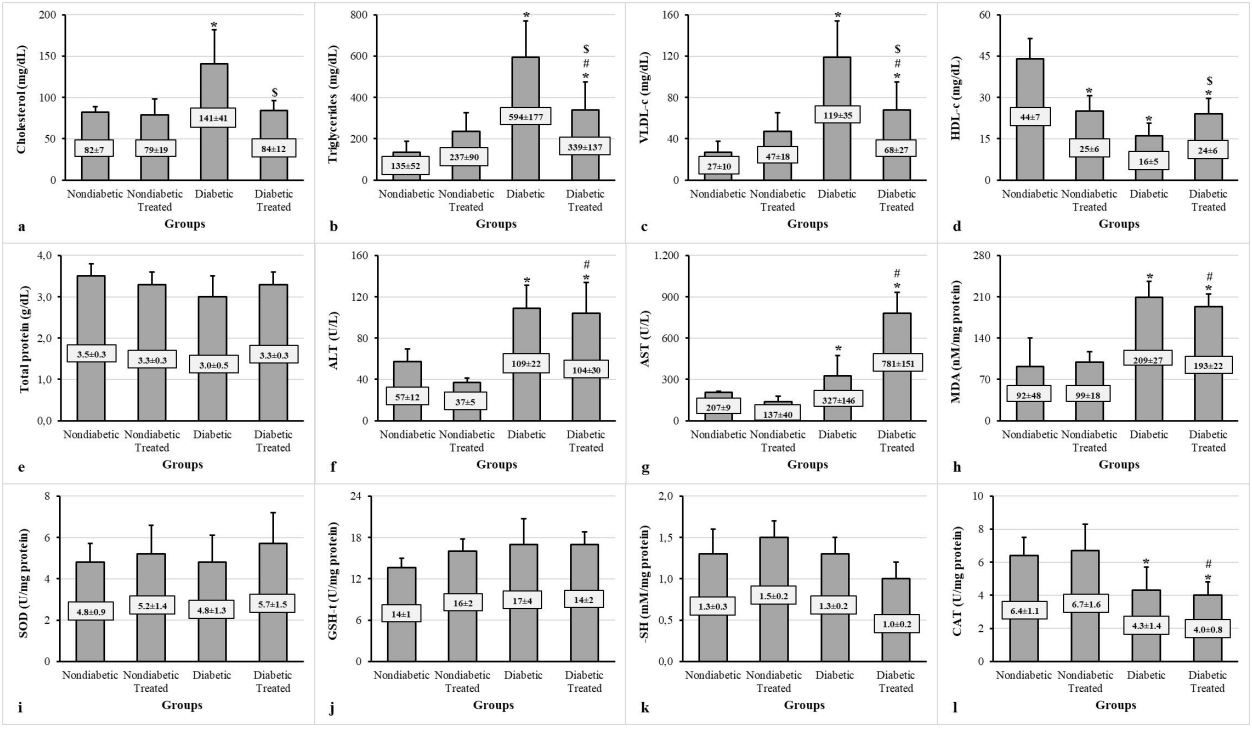

Figure 3. Maternal blood biochemical profile (a-g) and liver oxidative stress markers (h-l) on gestational day 21 of nondiabetic and diabetic rats treated with water or *Bauhinia holophylla* aqueous extract.
Data are shown as mean ± standard deviation (SD), n = 12 rats/group.
(a) cholesterol; (b) triglycerides; (c) very-low-density lipoprotein, VLDL-c; (d) high-density lipoprotein, HDL-c, (e) total protein; (f) alanine aminotransferase, ALT; (g) aspartate aminotransferase, AST; (h), malondialdehyde, MDA; (i) superoxide dismutase, SOD; (j) total glutathione, GSH-t; (k) total reduced thiol groups, -SH; and (l) catalase, CAT. *p<0.05 - compared to the Nondiabetic group, #p<0.05 compared to the Nondiabetic Treated group, $p<0.05 compared to the Diabetic group (One-way ANOVA followed by Tukey's Multiple Comparison test).

Fetal and placental analyses

Table IV shows fetal and placental data. The Nondiabetic Treated group presented lower fetal and placental weights, ossification sites, AGA fetuses rate, and increased SGA fetuses rates compared to the Nondiabetic group. The Diabetic group also showed decreased fetal and placental weight, ossification sites, rate of AGA fetuses, and placental efficiency. It exhibited a high percentage of fetuses classified as SGA in relation to the Nondiabetic group. The treatment with *B. holophylla* caused lower fetal weight, ossification sites, and placental efficiency, and increased placental weight in diabetic rats compared to the Nondiabetic group. In addition, this group presented a higher fetal weight, rate of AGA fetuses, and placental efficiency, and a decreased percentage of fetuses classified as SGA compared to the Diabetic group.

There were no significant differences in the external anomalies among experimental groups. The total number of skeletal abnormalities significantly increased in all experimental groups compared to the Nondiabetic group. The percentage of fetuses presenting incomplete ossification of sternebra was more common in all experimental groups than those from the Nondiabetic group. The anomalies in the vertebral centers, supernumerary ribs, and sternebra agenesis were more frequent in the fetuses from both diabetic rats compared to the Nondiabetic group. Regarding the visceral abnormalities, there was a higher percentual mean of fetuses

Table III. Maternal reproductive outcomes on gestational day 21 of nondiabetic and diabetic rats treated with water or *Bauhinia holophylla* aqueous extract.

	Groups			
	Nondiabetic	***Nondiabetic treated***	***Diabetic***	***Diabetic treated***
Pregnant at term (N)	12	13	12	14
Corpora lutea Total (N) Mean ± SD[a]	154 12.8 ± 1.5	180 13.8 ± 2.1	141 11.7 ± 1.4	175 12.5 ± 1.8
Implantation Total (N) Mean ± SD[a]	147 12.2 ± 1.7	146 11.2 ± 2.5	115 9.6 ± 3.0*	151 10.8 ± 2.0
Live fetuses Total (N) Mean ± SD[a]	136 11.3 ± 1.7	139 10.7 ± 2.7	97 8.1 ± 3.4*	137 9.8 ± 2.4
Dead fetuses Total (N) Mean ± SD[a]	2 0.2 ± 0.6	0 0.0 ± 0.0	0 0.0 ± 0.0	0 0.0 ± 0.0
Resorptions Total (N) Mean ± SD[a]	9 0.7 ± 1.6	7 0.5 ± 0.9	18 1.5 ± 1.8	14 1.0 ± 1.4
Pre-implantation loss (%)[b]	4.5	18.9*	18.4*	13.7*
Postimplantation loss (%)[b]	7.5	4.8	15.6*	9.3
Maternal weight gain (g)[a]	115.7 ± 12.8	94.6 ± 33.9	70.2 ± 22.7*	88.2 ± 33.6
Gravid uterus weight (g)[a]	76.8 ± 9.9	67.4 ± 18.6	54.5± 26.4*	65.1 ± 15.9
Maternal weight gain minus gravid uterus weight (g)[a]	38.8 ± 14.9	27.2 ± 20.3	15.6 ± 11.5*	23.1 ± 21.1

N= number. Data are expressed as mean ± standard deviation (SD) and proportions (%).
***p<0.05 - compared to the Nondiabetic group ([a]One-way ANOVA followed by Tukey's Multiple Comparison test; [b]Fisher's Exact test).**

with dilated trachea in all experimental groups compared to the Nondiabetic group. There was an increased frequency of hydroureter in both Diabetic groups and a higher frequency of hydronephrosis in the diabetic rats treated with plant extract compared to the Nondiabetic group (Table V).

DISCUSSION

Bauhinia holophylla leaves are traditionally used for diabetes treatment. For the first time, the biological effects were evaluated of the traditional infusion in nondiabetic and diabetic rats administered during the pregnancy period. The use of *B. holophylla* aqueous extract in nondiabetic rats showed teratogenicity and lower body weight at the end of treatment, probably due to this group's pre-embryo losses and lower fetal weight. However, the female diabetic animals that received leaf extract demonstrated amelioration of some symptoms associated with diabetes, verified in this study by reducing the excessive water intake and better lipid profile, although the plant treatment did not change the glycemia and liver oxidative stress. The treatment also contributed to a few changes in intrauterine conditions for better

Table IV. Fetal body weight, placental weight, and efficiency on gestational day 21 of nondiabetic and diabetic rats treated with water or *Bauhinia holophylla* aqueous extract.

	Groups			
	Nondiabetic (n=136)	***Nondiabetic treated (n=139)***	**Diabetic (n=97)**	**Diabetic treated (n=137)**
Fetal body weight (g) [a] Mean ± SD	5.1 ± 0.5	4.7 ± 0.6*	4.3 ± 0.6*	4.8 ± 0.5*$
SGA Fetuses (%)[b]	3 (2.2)	20 (14.4)*	43 (44.3)*	14 (10.2)$
AGA Fetuses (%)[b]	128 (94.1)	118 (84.9)*	53 (54.6)*	121 (88.3)$
LGA Fetuses (%)[b]	5 (3.7)	1 (0.7)	1 (1.0)	2 (1.5)
Ossification sites [a]	23.7 ± 1.7	21.4 ± 2.4*	21.3 ± 2.3*	21.6 ± 2.2*
Placental weight (g) [a] Mean ± SD	0.5 ± 0.0	0.4 ± 0.1*	0.6 ± 0.1*	0.6 ± 0.1*#
Placental efficiency [a] Mean ± SD	12.2 ± 2.2	12.2 ± 2.3	7.8 ± 1.8*	8.9 ± 1.9*#$

Legend: SGA – small for gestational age; AGA – adequate for gestational age; LGA – large for gestational age.
Data are expressed as mean ± standard deviation (SD) and proportions (%).
***p<0.05 - compared to the Nondiabetic group, #p<0.05 compared to the Nondiabetic Treated group, $p<0.05 compared to the Diabetic group ([a]One-way ANOVA followed by Tukey's Multiple Comparison test; [b]Fisher's Exact test).**

fetal development, confirmed by decreasing adverse maternal repercussions followed by a lower number of SGA and LGA fetuses and a higher number of AGA ones. These effects may be related to the components in the plant extract, which were also examined, such as phenolic compounds and biogenic amines.

The aqueous extract of *B. holophylla* leaves presented a significant phenolic compounds concentration and markers of antioxidant activity, including 3-hydroxytyrosol, rutin, gallic acid, and catechin, which corroborated other phytochemical analyses already published (Cechinel Filho 2009, Rozza et al. 2015, Da Fonseca et al. 2022). Additionally, this study identified several biogenic amines and amino acids, such as 5-hydroxytryptophan, tryptophan, and serotonin, that were not previously described in this species, showing the originality of this study.

This study showed the glycemia of the female rats similar to uncontrolled human type 1 diabetes due to chemical induction by a high dose of STZ in adult rats (Kleinert et al. 2018). Nonetheless, *B. holophylla* treatment did not play with the glycemic control of the diabetic dams and caused no changes in the glycemia of the nondiabetic pregnant rats. Also, Pinheiro et al. (2017) showed no hypoglycemic effect in non-pregnant female rats treated with an aqueous extract of *B. holophylla* at 400 mg/kg. However, the hypoglycemic effect was demonstrated using hydroethanolic extract of *B. holophylla* leaves in STZ-induced diabetic male mice (Camaforte et al. 2019). These different data might be due to the type of plant material, extract preparation, sex, animal species, and treatment period. Besides, this study evaluated biological data from the gestational period of rats treated with plant extract.

Under normal conditions, pregnancy is characterized by increased food consumption and progressive weight gain. Nonetheless, the loss or maintenance of body weight associated with polydipsia and hyperphagia are interpreted as hyperglycemia-induced symptoms (ADA

Table V. Frequency of anomalies of fetuses from nondiabetic and diabetic rats treated with water or *Bauhinia holophylla* aqueous extract.

	Groups			
	Nondiabetic	**Nondiabetic Treated**	**Diabetic**	**Diabetic Treated**
External anomalies				
Number of fetuses examined (litter)	136 (12)	139 (13)	97 (12)	137 (14)
Mean % fetuses with alteration per litter (mean ± SD) [a]	0.0 ± 0.0	0.0 ± 0.0	4.2 ± 14.4	0.0 ± 0.0
Total number of fetuses (%) with alteration [b]	0 (0.0%)	0 (0.0%)	2 (2.1%)	0 (0.0%)
Body edema [b]	0 *(0.0 %)*	0 *(0.0 %)*	2 *(2.1 %)*	0 *(0.0 %)*
Skeletal anomalies				
Number of fetuses examined (litter)	73 (12)	73 (13)	50 (12)	73 (14)
Mean % fetuses with alteration per litter (mean ± SD) [a]	52.6 ± 28.0	93.3 ± 18.8*	90.9 ± 17.6*	92.1 ± 13.9*
Total number of fetuses (%) with alteration [b]	39 (53.4%)	68 (93.1%)*	47 (93.9%)*	68 (93.1%)*
Incomplete ossification of cranium [b]	1 *(1.3%)*	3 *(4.1%)*	2 *(4.0%)*	1 *(1.3%)*
Abnormally shaped vert. centrum [b]	1 *(1.3%)*	0 *(0.0%)*	4 *(8.0%)*	12 *(16.4%)**
Bipartite ossif. of vertebral centrum [b]	0 *(0.0%)*	1 *(1.3%)*	4 *(8.0%)**	14 *(19.1%)**
Supernumerary rib [b]	4 *(5.5%)*	5 *(6.8%)*	25 *(50.0%)**	18 *(24.6%)*#*
Wavy rib [b]	3 *(4.1%)*	7 *(9.6%)*	2 *(4.0%)*	13 *(17.8%)*#*
Sternebra agenesis [b]	3 *(4.1%)*	2 *(2.7%)*	1 *(2.0%)*	0 *(0.0%)*
Unossified sternebrae [b]	7 *(9.6%)*	9 *(12.3%)*	5 *(10.0%)*	7 *(9.6%)*
Incomplete ossification of sternebrae [b]	12 *(16.4%)*	40 *(54.8%)**	29 *(58.0%)**	30 *(41.1%)**
Abnormally shaped sternebrae [b]	9 *(12.3%)*	16 *(21.9%)*	39 *(78.0%)**	45 *(61.6%)**
Visceral anomalies				
Number of fetuses examined (litter)	63 (12)	66 (13)	47 (12)	64 (14)
Mean % fetuses with alteration per litter (mean ± SD) [a]	30.5 ± 21.0	30.1 ± 15.7	63.9 ± 36.7*	64.1 ± 31.2*#
Total number of fetuses (%) with alteration [b]	19 (31.7%)	19 (28.8%)	33 (70.2%)*	40 (62.5%)*
Microphthalmia [b]	0 *(0.0%)*	0 *(0.0%)*	1 *(2.1%)*	0 *(0.0%)*
Expanded spinal canal [b]	0 *(0.0%)*	1 *(1.5%)*	0 *(0.0%)*	0 *(0.0%)*
Dilated trachea [b]	0 *(0.0%)*	7 *(10.6%)**	4 *(8.5%)**	6 *(9.4%)**
Bladder hyperplasia [b]	0 *(0.0%)*	0 *(0.0%)*	0 *(0.0%)*	1 *(1.6%)*
Dilated renal pelvis [b]	1 *(1.6%)*	0 *(0.0%)*	2 *(4.2%)*	3 *(4.7%)*
Hydroureter [b]	13 *(20.6%)*	12 *(18.2%)*	21 *(44.7%)**	24 *(37.5%)**
Sinuosis Ureter [b]	1 *(1.6%)*	0 *(0.0%)*	0 *(0.0%)*	1 *(1.6%)*
Hydronephrosis [b]	1 *(1.6%)*	1 *(1.5%)*	5 *(10.6%)*	10 *(15.6%)**
Kidney agenesis [b]	1 *(1.6%)*	0 *(0.0%)*	1 *(2.1%)*	0 *(0.0%)*
Ectopic testis [b]	0 *(0.0%)*	0 *(0.0%)*	1 *(2.1%)*	0 *(0.0%)*

Data are expressed as mean ± standard deviation (SD) and proportions (%).
***p<0.05 - compared to the Nondiabetic group, #p<0.05 compared to the Nondiabetic Treated group, ([a]One-way ANOVA followed by Tukey's Multiple Comparison test; [b]Fisher's Exact test).**

2023). Our data showed a significant beneficial effect of *B. holophylla* extract that contributed to reducing the secondary complications of diabetes. In this investigation, both Diabetic groups showed lower body weight and higher water and food intake during pregnancy. However, the treatment with the plant in Diabetic rats reduced water during all pregnancy and food intake at the end of pregnancy, which might be related to phenolic compounds in *B. holophylla* (Coskun et al. 2005, Kobori et al. 2011, Rozza et al. 2015).

The presence of phenolic compounds in *B. holophylla* also be related to improving the lipid profile in these rats (Rozza et al. 2015, Ribeiro et al. 2018). The diabetic rats exhibited an inadequate lipid profile, with increased serum cholesterol, triglycerides, and VLDL-c levels, similar to diabetic patients. The insulin activates the lipoprotein lipase, which hydrolyzes triglycerides, and its deficiency results in a lack of this enzyme activation, causing hypertriglyceridemia (Georg & Ludvik 2000, Rahman 2007). Our data showed reduced levels of triglycerides, total cholesterol, and VLDL-c. They increased HDL-c concentration in diabetic animals after 21 days of medication, confirming a substantial beneficial effect of *B. holophylla* extract.

However, the *B. holophylla* treatment increased AST activity in diabetic rats, suggesting this plant impaired hepatic metabolism. These findings corroborate Pinheiro et al. (2017), who verified higher ALT and AST activities in non-pregnant diabetic rats treated with this plant. Furthermore, using *B. holophylla* during pregnancy did not prevent oxidative damage in the liver. In the present study, higher lipoperoxidation was observed by MDA higher levels in liver samples of diabetic rats at the end of the gestational period. Hyperglycemia leads an increased reactive oxygen species (ROS) production due to increased input of reducing equivalents in the classical metabolic pathway of the mitochondrial electron transport chain (Giacco & Brownlee 2010). The liver plays an essential role in the redox status and glucose tolerance; and it is a significant organ damaged by ROS hyperglycemia exposure (McGuinness & Cherrington 2003), which limits the redox balance, decreasing the enzymatic antioxidant defense, like catalase (Matough et al. 2012), corroborating our findings. Due to the elevated synthesis of free radicals and/or defects in antioxidant defenses in an oxidative stress state, there was a significant implication for the mother organism, placental function, and fetal welfare (Lappas et al. 2011, Sinzato et al. 2022).

In the diabetic rats presents biological data that there was a similar outcome in women with uncontrolled diabetes with increased rates of embryonic losses before implantation in the maternal endometrium (Eriksson et al. 2003, Afiune et al. 2017, Bueno et al. 2020). *B. holophylla* treatment did not interfere with the development these complications in diabetic rats. Furthermore, this plant increased the pre-implantation loss in nondiabetic rats. These findings might be explained due to concentrations of biogenic amines such as spermine, spermidine, and putrescine found in our extract, which might be a limiting factor for adequate blastocyst formation and implantation (Lefèvre et al. 2011, Cruz et al. 2022).

These results show the extract may not be safe for maternal outcomes, especially in a normal pregnancy, due to toxic biological features to embryos and/or fetuses because of high circulating levels of some biogenic amines. Our results demonstrated that *B. holophylla* administration caused intrauterine growth retardation. Fetuses explain this finding from the Nondiabetic group treated with the *B. holophylla* aqueous extract showed a lower fetal and placental weight, a lower proportion of

fetal ossification sites, and a higher percentage of SGA fetuses, suggesting that the extract may play in fetal growth since the fetal body weight is positively correlated with the placental weight (Fowden et al. 2009). An essential factor to be considered is the elevated serotonin concentrations, one of the significant amines in the leaves of *B. holophylla*. Serotonin production is regulated by the activity and availability of tryptophan and 5-hydroxytryptophan, which was also abundant in phytochemical analyses. Excess serotonin can induce hyperglycemia mediated by norepinephrine release, which may inhibit insulin release and consequently increase inflammation-related oxidative stress (Nguyen et al. 2018), damaging fetal development. Besides that, hyperserotonemia also affects the pituitary growth hormone secretion and limits stimulation in the liver to produce insulin-like growth factor-I (IGF-I) (Salas et al. 2007, Castrogiovanni et al. 2014), associated with a vasoconstrictor effect on umbilical and chorionic arteries that reduces uteroplacental blood flow, and consequently damages fetal growth (Salas et al. 2007).

The reduction of the data related to fetal body weight and a higher value SGA fetus in the Diabetic group corroborate other experimental studies performed in our group, which showed that severe diabetes in experimental rats also presents its fetuses with intrauterine growth restriction (Corvino et al. 2015, Cruz et al. 2023). This study showed the placental weight, and its index was increased in diabetic group rats. Nevertheless, the higher placental weight was insufficient for fetal nutrition, confirmed by the lower fetal body weight, which might be related to physiological alterations in placental-fetal exchanges (Sinzato et al. 2019, 2022). Nonetheless, the *B. holophilla* treatment increased the rates of AGA fetuses and decreased the rates of SGA fetuses, contributing to improved fetal body weight in diabetic animals. However, the action mechanism of these constituents needs to be further evaluated; these initial results are very encouraging for further follow-up studies.

Many congenital disabilities in the gestational period complicated by diabetes are related to environmental hyperglycemia during embryogenesis (Bueno et al. 2020). These anomalies can often affect the development of the axial skeleton kidneys, large vessels, heart, and central nervous system (Schaefer-Graf et al. 2000). In diabetic rats, there are more fetuses with data related to skeletal and visceral anomalies (Afiune et al. 2017, Soares et al. 2021). The same abnormalities were observed in our study. *B. holophylla* treatment did not alter the high incidence of skeletal and visceral anomalies, perhaps because the plant has not changed the oxidative stress biomarkers. However, the plant administration in the Nondiabetic group increased the frequency of fetal skeletal anomalies, and this fact can be related to the toxic effect of the plant due to flavonoids since the most frequent causes of changes and malformations in the fetal bone structure are caused by their metabolites (Lesser et al. 2015).

The most critical limitations of this study are the absence of an evaluation of *B. holophylla* extract in other maternal biomarkers involved in regulating glucose metabolism and fetal development (insulin, cytokines and reproductive hormones), and the treatment before with continuation during pregnancy. As a strong point, this study is original since it is the first to explore the effects of *B. holophylla* leaves during Diabetic and Nondiabetic pregnancy. Furthermore, the results of the phytochemical analysis of biogenic amines obtained from the present study, and yet to be no described before for this plant, are promising that other studies with other solvents, bioassay-guided isolation and

mechanism of action of bioactive constituents should be used. Further investigations aimed at the isolation and biological evaluation of isolated compounds represent an interesting pathway to better understand the mechanism of action of the extract, as the outcome will help to prevent any unknown maternal or fetal complications.

In conclusion, our results showed that an aqueous extract of *B. holophylla* leaves caused lipid-lowering effects, while it did not control decompensated maternal hyperglycemia in diabetic rats. In addition, the plant treatment caused no changes in the reproductive outcomes and promoted fetal development in the offspring of diabetic animals. These effects could be associated with the plant's phenol, flavonoids, and biogenic amines. However, these compounds present in *B. holophylla* leaves can cause reproductive abnormalities, fetal growth restriction, and teratogenicity in nondiabetic rats due to the toxic effect, showing that a normal metabolism might be altered by unnecessary treatments indiscriminately used.

Acknowledgments

The authors are thankful to the research team of the Laboratório de Fisiologia de Sistemas e Toxicologia Reprodutiva (FisioTox). The authors are thankful the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) [Finance Code 001] for Financial Support.

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How to cite

SAVAZZI K ET AL. 2024. Phytochemical characterization and antidiabetic analysis of *Bauhinia holophylla* extract on the maternal-fetal outcomes of rats. An Acad Bras Cienc 96: e20230604. DOI 10.1590/0001-3765202420230604.

Manuscript received on September 18, 2024; accepted for publication on April 4, 2024

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