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ORIGINAL ARTICLE

Effect of *Ravenala madagascariensis* Sonnerat (Strelitziaceae) Administration During Pregnancy on Fetal Growth and Development: A Morphological Study in a Murine Model

Efecto sobre el crecimiento y desarrollo fetal de la administración de *Ravenala madagascariensis* Sonnerat (Strelitziaceae) durante la gestación: estudio morfológico en un modelo murino

Efeito da administração de *Ravenala madagascariensis* Sonnerat (Strelitziaceae) durante a gestação sobre o crescimento e desenvolvimento fetal: Estudo morfológico em modelo murino

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Abstract
The objective of this study was to evaluate the effects of administering an ethanolic extract from the leaves of *Ravenala madagascariensis* Sonnerat (Strelitziaceae) on fetal growth and development during gestation in Wistar rats, through external, skeletal, and visceral morphological analyses. Pregnant rats were divided into two groups (n = 10 per group): a control group (water) and a treatment group (dry ethanolic extract of *R. madagascariensis*, 400 mg/kg), administered in drinking water from gestational day 5 to 15. On gestational day 20, cesarean section and euthanasia under anesthesia were performed. The fetuses and placentas were removed for immediate external morphological examination. Selected fetuses were subjected to skeletal and visceral examinations. The extract did not alter fetal glycemia levels. Although fetal size was not changed in the treatment group, fetal weight and placental efficiency were increased. No small-for-gestational-age fetuses were identified, and an increase in adequate and large-for-gestational-age fetuses was observed. The skeletal examination showed increased ossification of the parietal bones and the first phalanx of the forelimbs. No skeletal or visceral malformations were detected in any of the regions studied. While these findings indicate a favorable safety profile for *R. madagascariensis* under specific experimental conditions, further research is needed to confirm its safety and therapeutic applications. Rev Arg Antrop Biol 28(1), e126, 2026. https://doi.org/10.24215/18536387e126

Keywords: experimental teratology; medicinal plants; morphology; reproductive toxicology; Wistar rats

Resumen
El objetivo de la investigación fue evaluar los efectos sobre el crecimiento y desarrollo fetal de la administración del extracto etanólico de hojas de *Ravenala madagascariensis* Sonnerat (Strelitziaceae) durante la gestación en ratas Wistar, mediante análisis morfológico externo, esquelético y visceral. Ratas gestadas se dividieron en dos grupos (n = 10 por grupo): un grupo control (agua) y un grupo tratado (extracto etanólico seco de *R. madagascariensis*, 400 mg/kg), administrado en el agua de beber del día 5 al 15 de gestación. El día 20 de gestación, se realizó cesárea y eutanasia bajo anestesia. Se extrajeron los fetos y placentas para el examen morfológico externo inmediato. Los fetos seleccionados se sometieron a exámenes esqueléticos y viscerales. El extracto no alteró los niveles de glucemia fetal. En el grupo tratado, aunque no se modificó el tamaño fetal, se observó un aumento del peso fetal y de la eficiencia placentaria. No se identificaron fetos pequeños para la edad gestacional y se evidenció un incremento de la cantidad de fetos adecuados y grandes para la edad gestacional. El examen esquelético mostró un aumento del proceso de osificación de los parietales y de la primera falange de las patas anteriores. No se detectaron malformaciones esqueléticas o viscerales en ninguna de las regiones estudiadas. Aunque estos hallazgos indican un perfil de seguridad favorable para *R. madagascariensis* bajo estas condiciones experimentales específicas, se requieren más investigaciones para confirmar su seguridad y aplicaciones terapéuticas. Rev Arg Antrop Biol 28(1), e126, 2026. https://doi.org/10.24215/18536387e126

Palabras Clave: morfología; plantas medicinales; ratas Wistar; teratología experimental; toxicología reproductiva

Resumo
O objetivo desta pesquisa foi avaliar os efeitos do extrato etanólico das folhas de *Ravenala madagascariensis* Sonnerat (Strelitziaceae) no crescimento e desenvolvimento fetal durante a gestação em ratas Wistar, por meio de análises morfológicas externa, esquelética e visceral. Ratas grávidas foram divididas em dois grupos (n = 10 por grupo): um grupo controle (água) e um grupo tratado (extrato etanólico seco

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de *R. madagascariensis*, 400 mg/kg), administrado na água de beber do 5º ao 15º dia gestacional. No 20º dia gestacional, foram realizadas cesárea e eutanásia sob anestesia. Fetos e placentas foram extraídos para exame morfológico externo imediato. Fetos selecionados foram submetidos a exames esqueléticos e viscerais. O extrato não alterou os níveis de glicemia fetal. Embora o tamanho fetal não tenha sido alterado no grupo tratado, o peso fetal e a eficiência placentária foram aumentados. Não foram identificados fetos pequenos para a idade gestacional, e observou-se um aumento no número de fetos adequados e grandes para a idade gestacional. O exame esquelético demonstrou aumento do processo de ossificação dos ossos parietais e da primeira falange dos membros anteriores. Não foram detectadas malformações esqueléticas ou viscerais em nenhuma das regiões estudadas. Embora esses achados indiquem um perfil de segurança favorável para *R. madagascariensis* em condições experimentais específicas, pesquisas adicionais são necessárias para confirmar sua segurança e aplicações terapêuticas. Rev Arg Antrop Biol 28(1), e126, 2026. https://doi.org/10.24215/18536387e126

Palavras-chave: morfologia; plantas medicinais; ratos Wistar; teratologia experimental; toxicologia reprodutiva

Over the past few decades, numerous epidemiological and experimental studies have demonstrated that maternal nutritional status is a crucial determinant of fetal growth and development. Maternal health during pregnancy not only impacts fetal well-being in the short term but also has long-lasting repercussions on the health of the offspring (Karimi *et al.*, 2021; Kathuria, 2023; Moreno-Fernandez *et al.*, 2020). This phenomenon, known as fetal programming or early programming, suggests that exposure to specific environmental factors during intrauterine development can induce permanent changes in the organism, increasing the predisposition to chronic diseases in adulthood, such as obesity, type 2 diabetes, and cardiovascular diseases (Godfrey & Barker, 2000; Mukoyama, 2024). Studies in biological and evolutionary anthropology also support this theory. In this context, the relationship between the environment and phenotypic plasticity, particularly during the developmental period, is a focus of discussion and debate (Gowland, 2015).

Among these factors, maternal conditions such as malnutrition, overweight, obesity, and gestational diabetes stand out due to their capacity to increase the risk of metabolic disorders and chronic diseases in the offspring (Karimi *et al.*, 2021; Lecoutre *et al.*, 2021; Parrettini *et al.*, 2020). Additionally, these conditions are associated with congenital abnormalities, delayed neurocognitive development, intrauterine growth restriction (IUGR), a higher risk of fetal macrosomia, and perinatal morbidity (Cortés-Albornoz *et al.*, 2021; Kankowski *et al.*, 2022; Thornburg & Valent, 2024).

Despite the recognized importance of adequate nutrition during pregnancy, achieving optimal nutritional and health status remains particularly challenging for women in poverty or developing countries. Economic and social barriers limit access to nutritious foods and safe treatments, leading to the use of medicinal plants and natural extracts as accessible alternatives. In various communities, plant infusions and extracts are traditionally used as a source of nutrients and to treat gastrointestinal disorders, infections, nausea, vomiting, and also to induce labor (Ahmed *et al.*, 2021).

However, although plant consumption is generally regarded as safe, its use during pregnancy may pose significant risks. Certain plant species contain teratogenic, embryotoxic, or abortifacient compounds (Campos *et al.*, 2023), yet the precise thresholds for

safe consumption remain largely undefined. For instance, culinary spices such as garlic (*Allium sativum*) and ginger (*Zingiber officinale*), while beneficial at moderate doses, may exhibit dose-dependent toxicity when consumed in excessive amounts (Campos *et al*., 2023; Sarecka-Hujar & Szulc-Musioł, 2022).

Given these concerns, the administration of herbal products during pregnancy must be supported by rigorous scientific studies to establish safe dosage parameters and assess their potential effects on fetal development. However, the number of herbal preparations recognized as safe for use during pregnancy remains limited (Sarecka-Hujar & Szulc-Musioł, 2022). Ethical constraints surrounding clinical research in pregnant women necessitate the use of animal models as indispensable tools for evaluating the safety and teratogenic potential of medicinal plants.

Animal models allow the evaluation of the effects of plant extracts and other agents on fetal development. In particular, murine models have proven valuable in reproductive toxicology and teratology studies, as they provide relevant data on the safety and toxicity of compounds administered during gestation (Gonçalves *et al*., 2020; Leal-Silva et al., 2023; Lopes Cruz *et al.*, 2022). For example, recent studies have evaluated the effects of plant extracts during gestation using rats as animal models, such as *Phyllanthus niruri* L. (da Costa Alves *et al*., 2025), *Thymelaea hirsuta* L. (El-Hak *et al*., 2025), and *Cyanthillium cinereum* L. (Kerdsuknirund *et al*., 2025). Furthermore, studies using experimental models of obesity and gestational diabetes in rats have demonstrated that these conditions can lead to significant alterations in fetal growth and development, including skeletal and visceral malformations (Araujo-Silva *et al.*, 2021; Bequer *et al.*, 2023; Gómez *et al.*, 2021). These observations underscore the importance of employing animal models to assess the impact of treatments during gestation and their influence on fetal growth and development.

In this context, *Ravenala madagascariensis* Sonnerat, commonly known as the "traveler's tree" and belonging to the Strelitziaceae family, has gained relevance in recent years due to its potential therapeutic properties (Suroowan & Mahomoodally, 2020). While this plant is endemic to Madagascar, where it is considered an iconic symbol (Rakotoarivelo *et al.*, 2014), it has spread worldwide as an ornamental species in various regions of North America, as well as in many tropical and subtropical areas. In Mauritius, for example, it is considered an invasive species that represents a constant threat to native forests (Suroowan & Mahomoodally, 2020).

Despite its wide distribution and popularity as a medicinal plant in Madagascar, India, and several African countries (Ramiarantsoa *et al.*, 2014), its traditional use is best known in rural areas of Madagascar, such as the Ambalabe community, a remote region in the east of the island with limited access to modern healthcare (Rakotoarivelo *et al*., 2014; Rakotoarivelo *et al.*, 2015). In these communities, the plant's heart, rich in minerals, is cooked as a vegetable, especially during times of scarcity. It is also used to regulate albumin levels. A decoction of the young leaves is consumed to relieve stomach aches and dizziness (Rakotoarivelo *et al*., 2014). The seeds are used for their antiseptic properties to relieve coughs and urinary retention. In addition, in Madagascar, this plant is used to treat diarrhea, edema, kidney stones, hypertension, and diabetes mellitus (Suroowan & Mahomoodally, 2020). Although no evidence of its use during pregnancy has been found, its widespread consumption—particularly in communities with limited access to medical care—suggests that pregnant women should also consume it.

The widespread ethnomedicinal use of *R. madagascariensis*, coupled with its invasive nature, easy availability, and the limited scientific research available, motivated investigations to evaluate its biological activities and discover low-cost extracts with therapeutic potential (Suroowan *et al*., 2023). In this regard, the *in vitro*, *in silico* (Suroowan *et al.*, 2023), and *in vivo* activity of various extracts has been evaluated using different

extraction methods (Priyadarsini *et al.*, 2010a; Priyadarsini *et al.*, 2010b; Priyadarsini *et al*., 2013). In preclinical animal studies, the ethanolic extract has consistently shown favorable results in terms of biological activity and low toxicity, displaying protective effects on several organs. Specifically, the ethanolic extract of the plant's leaves demonstrated greater antidiabetic efficacy compared to the aqueous extract, significantly reducing blood glucose levels in alloxan-induced diabetic rats, both in acute and prolonged treatments (Priyadarsini *et al.*, 2010b). In subsequent studies, the ethanolic extract also exhibited superior hypolipidemic, renoprotective (Priyadarsini *et al.*, 2010a), and antioxidant activity (Priyadarsini *et al*., 2013).

These effects could be related to the ability of the ethanolic extract of this plant to decrease lipid peroxidation and restore levels of reduced glutathione (GSH), which plays a vital role in protecting cells against free radicals. These mechanisms could exert a beneficial effect by counteracting the pathological alterations induced by oxidative stress at the organ and tissue level (Priyadarsini *et al*., 2013). This could be especially important during gestation, as it is considered a state of heightened oxidative stress (Nacka-Aleksić *et al*., 2022). The therapeutic effects found in this extract could be attributed to the greater extraction capacity of ethanol in extracting phytoconstituents (Priyadarsini *et al.*, 2010b), such as alkaloids, flavonoids, saponins, tannins, glycosides, proteins, phenols, and steroids present in the leaves (Priyadarsini *et al.*, 2010c). For instance, phenols may act through specific biological mechanisms that promote fetal growth, tissue development, and glucose metabolism, important indicators of maternal-fetal health. However, there is still a lack of evidence-based data on their safety during pregnancy (Nacka-Aleksić *et al.*, 2022).

Acute oral toxicity studies of ethanolic extracts of the plant's leaves showed no symptoms indicative of toxicity at a dose of 2,000 mg/kg body weight (Priyadarsini *et al.*, 2010a; Priyadarsini *et al.*, 2010b; Priyadarsini *et al*., 2013). Additionally, preliminary trials conducted by this research group on the plant cultivated in Cuba, using Wistar rats, demonstrated that the administration of the ethanolic extract leaves did not produce significant morphological alterations in the organs evaluated (liver, pancreas, and kidneys). It also did not modify physiological parameters indicative of metabolic and systemic balance, such as blood glucose, heart rate, or blood pressure, and no signs of toxicity were observed (Freire-Gómez *et al.*, 2023).

Despite these advances, *in vivo* studies on *R. madagascariensis* remain limited, and no research has been found evaluating its safety or its potential effects—whether beneficial or harmful—during pregnancy, which is concerning given its increasing use. The described biological properties and low documented toxicity could suggest favorable effects. However, as previously mentioned, the administration of this plant during pregnancy must be supported by scientific evidence, since substances that are generally safe can cause significant alterations during this period. Hence, it is hypothesized that *R. madagascariensis* administration during pregnancy might influence fetal growth and development either positively or negatively. Therefore, this study aims to evaluate the effects of the ethanolic extract of *R. madagascariensis* leaves on fetal growth and development during gestation in a Wistar rat model.

MATERIALS AND METHODS

Ethanolic extract preparation

Fresh, undamaged leaves of *Ravenala madagascariensis* were collected from areas within the Central University "Martha Abreu" of Las Villas, Santa Clara, Cuba. The plant was

taxonomically identified, and a specimen was deposited in the Herbarium of the Botanical Garden of Villa Clara (R.A. Pérez Obregón No. 11942 ULV). The dried and pulverized plant material (maximum particle size of 0.5 mm) was mixed with absolute ethanol as a solvent at a 1:6 ratio (w/v).

Subsequently, ultrasound-assisted extraction was performed using an ultrasonic bath (Branson Ultrasonic Corporation, USA) for 20 minutes, followed by filtration under vacuum (Liuyi, China). The solvent was evaporated under air to dryness using an extraction hood (Siemens, Germany).

Experimental animals, environmental conditions, and ethical considerations

Virgin adult Wistar rats of both sexes (90-110 days old), weighing approximately 200 g, were obtained from the Laboratory Animal Production Center (CENPALAB), Havana, Cuba, with a corresponding quality certificate. The animals were provided with water and a complete diet for conventional rats (formula CMO 1000). They were housed under controlled conditions of temperature (19-25 °C), relative humidity (45-65%), and light (12-hour light/dark cycles).

The animals were handled according to the recommendations of Decree-Law 31/2021, On Animal Welfare (GOC-2021-331-EX25), enacted by the Ministerio de Justicia de la República de Cuba (MINJUS)[1], and the "Guide for the Care and Use of Laboratory Animals" by the National Research Council (NRC) (2011). The minimum number of animals required was used, and every effort was made to minimize their suffering (MINJUS, 2021; NRC, 2011). The remains of the euthanized animals were disposed of in the institutional crematorium under established biosafety standards. The Scientific Council and the Biomedical Research Unit Ethics Committee at the University of Medical Sciences of Villa Clara approved the study under Resolution number 7/2020.

Experimental design

After an acclimatization period, the animals were paired at a ratio of three females to one male. Mating took place over 15 consecutive days (three estrous cycles) to ensure pregnancy. Daily vaginal smear tests were performed, and pregnancy was confirmed by the presence of sperm in the vaginal contents, with this designated as gestation day zero (Sharp & Villano, 2012).

Pregnant females were housed individually in gestation boxes and randomly assigned to two experimental groups (n = 10 per group): Control Group = received water (vehicle); Treated Group = received *R. madagascariensis* dry ethanolic extract (400 mg/kg body weight/day) dissolved in water. The dose of 400 mg/kg body weight was selected based on previous murine studies, where this dose showed no toxicity and was more effective than the 200 mg/kg body weight dose in evaluating the antidiabetic (Priyadarsini *et al.*, 2010b), hypolipidemic, renoprotective (Priyadarsini *et al.*, 2010a), and antioxidant (Priyadarsini *et al.*, 2013) properties of *R. madagascariensis.* Additionally, this dose was also used by our research group in a previous study, showing no alterations in biochemical or morphophysiological parameters in healthy Wistar rats (Freire-Gómez *et al.*, 2023).

The extract was administered daily in the drinking water, individually for each animal, for 10 consecutive days (from gestation day 5 to day 15). This period was chosen as it corresponds to the embryonic phase (organogenesis) of intrauterine development in rats

(York *et al.*, 2014), which has been identified by several authors as the most sensitive stage to system abnormalities induced by teratogenic agents (Hill, 2007; York *et al.*, 2014).

To avoid deviations in the administered doses, the amount of water consumed by each rat was measured during the five days prior to day zero of the experiment. Since all animals exhibited a daily water intake exceeding 25 mL, which may increase during gestation, the extract was dissolved in a fixed volume of 25 mL of water provided during the administration period (8:00 a.m. to 4:00 p.m.) at a concentration of approximately 4 mg of dry extract per mL of water to achieve a dose of 400 mg/kg body weight/day. After this period, untreated water was provided *ad libitum*. This approach ensured complete consumption of the calculated dose while maintaining the extract at a concentration that did not compromise palatability or alter normal voluntary water intake.

On gestation day 20, cesarean sections were performed under anesthesia with 50-60 mg/kg of sodium thiopental (Farmahealth, India). Both uterine horns were extracted, and the surrounding fat and periuterine tissue were removed. Euthanasia was performed by exsanguination via intracardiac puncture (Sharp & Villano, 2012). Fetuses and placentas were extracted and placed individually in Petri dishes with 0.9% NaCl for immediate morphological examination. In addition, mean litter size was calculated based on the total number of offspring per mother in each group.

Fetuses were numbered sequentially from the right uterine horn to the left uterine horn. Their viability was checked through tactile stimulation, and deceased fetuses were excluded from the study. Live fetuses were randomly allocated for different biochemical and morphological analyses, ensuring the representation of fetuses from both uterine horns and various positions within the horn. The first fetus was selected for biochemical analysis (blood glucose and other determinations not included in this study), the second for skeletal examination and ossification site counting, the third for visceral examination, and so on until all offspring were accounted for.

Data collection was conducted under blinded conditions to minimize observer bias. Measurements were performed by a single highly experienced researcher, ensuring methodological consistency and reliability throughout the analyses.

Fetal parameter assessment

A total of 255 live fetuses and their placentas (143 from the Control Group and 112 from the Treated Group), corresponding to the total number of fetuses obtained from all litters, were analyzed individually, and various external morphological parameters were assessed (Table 1). Measurements were taken using a Vernier caliper (Vernier Caliper, China), and weights were determined with a digital scale (Kern, Germany).

Fetuses were classified according to fetal weight percentiles established in the Control Group, based on the modified method of Soulimane-Mokhtari *et al.* (2005). The classification was as follows: small for gestational age —SGA— (fetal weight below the 5th percentile), appropriate for gestational age —AGA— (fetal weight between the 5th and 95th percentiles), and large for gestational age —LGA— (fetal weight above the 95th percentile).

Following the external morphological examination, a drop of blood was obtained by puncturing the aorta with a lancet. Blood glucose levels (mmol/L) were measured in approximately one-third of the fetuses (37 from the Control Group and 42 from the Treated Group) using a glucometer.

TABLE 1. External morphological parameters of fetuses and placentas in the offspring of pregnant Wistar rats administered water (Control Group) and *Ravenala madagascariensis* ethanolic Extract (400 mg/kg body weight) dissolved in water (Treated Group) for 10 days (gestation days 5 to 15).

Parameter	Description
	Fetuses
External Anomaly Analysis	Anterior and posterior limbs: Absence or excess of digits; limb size and positioning abnormalities
	Thoracic, abdominal, and dorsal regions: Presence of hemorrhage or hematoma; absence of abdominal wall
	Tail: Tail size and shape abnormalities
Craniofacial structures	A partial or total absence of cranial bones; abnormalities in biparietal and anteroposterior diameters; the presence of facial clefts
	Absence of whiskers; low ear implantation; ear deformities (anotia, microtia, dysmorphia)
	Nasal deformities (depressed bridge, enlarged hump, anterograde nose); absence or abnormal positioning of oral structures
Weight (g)	Fetal weight at 20 days of gestation, excluding membranes and amniotic fluid
Crown-to-rump length (cm)	Length from the top of the head to the base of the tail
Tail length (cm)	Length from the base to the tip of the tail
	Plancentas
Weight (g)	Weight of the placenta, excluding fat and umbilical cord remnants
Placental efficiency	It is calculated as fetal weight/placental weight. This measure assesses the placental capacity to ensure maternal-fetal exchanges and nutrient supply to the developing fetus

Skeletal morphology study

The skeletal morphology study was performed on one-third of the fetuses from each litter. A total of 63 fetuses (28 from the Control Group and 35 from the Treated Group) were processed using the specimen clearing technique and stained with alizarin red solution, following the method described by Staples & Schnell (1964). This method enables optimal skeletal visualization by rendering the fetuses transparent and staining calcium deposits in the bones, which appear in a distinctive red hue.

Skeletal abnormalities were diagnosed under a stereomicroscope (BMS, USA), following the staining protocol of Staples & Schnell (1964). A total of 144 bones were examined from head to tail, including the skull, vertebrae (cervical, thoracic, lumbar, sacral, and caudal), ribs, sternum (manubrium, sternebrae, and xiphoid process), shoulder girdle with anterior limbs, and pelvic girdle with posterior limbs. The number, shape, size, position, addition, absence, or fusion of bones, as well as the degree of ossification, were evaluated (Damasceno *et al.*, 2008).

Skeletal ossification was assessed according to the classification categories proposed by Burdan *et al.* (2005): complete ossification (intense alizarin red staining covering the entire bone surface), incomplete ossification (reduced alizarin red staining in the observed bone), or non-ossification (no alizarin red staining, bone not visible). Additionally, ossification sites (sternal, anterior phalanges, metacarpals, posterior phalanges, metatarsals, and caudal vertebrae) were counted as indicators of fetal development, following the methodology described by Aliverti *et al.* (1979).

Visceral morphology study

The visceral morphology study was also performed on one-third of the fetuses from each litter (Fig. 1). A total of 68 fetuses (31 from the Control Group and 37 from the Trea-

ted Group) were processed using Bouin's fixative technique, as described by Damasceno *et al.* (2008). This procedure facilitates the reduction of fine tissues and decalcification of bones. The examination of each fetus's viscera was performed under a stereomicroscope (BMS, USA), following a protocol specifically developed and standardized by our research team (Freire-Gómez *et al.*, 2021) to ensure consistency and precision across samples.

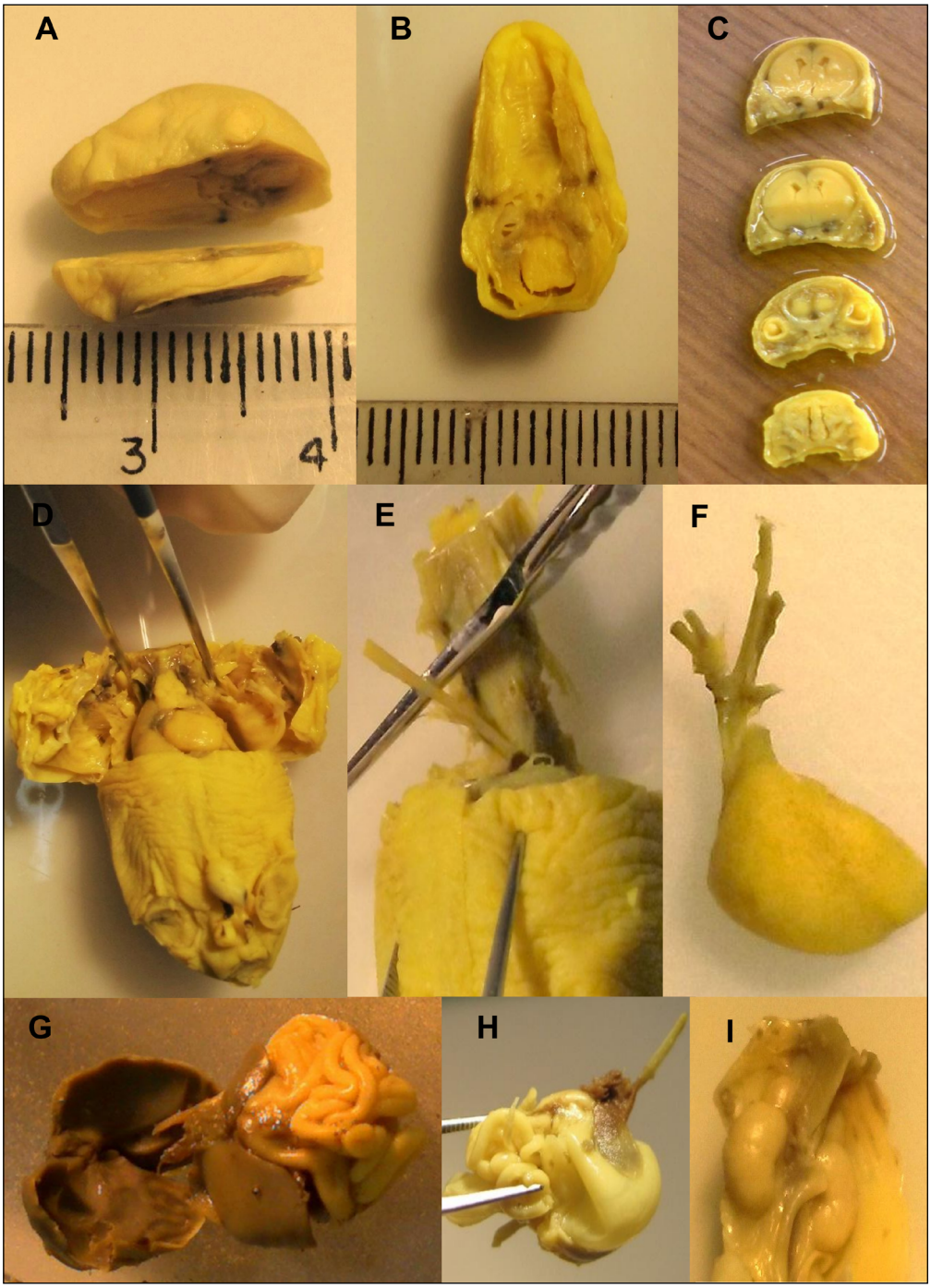

FIGURE 1. Visceral morphology study. **A-C)** Wilson's sectioning technique in the cranial region. **A)** Transverse Wilson section at the level of the mouth opening and ears. **B)** Internal view of the cranial base in the transverse section through the mouth. **C)** A series of vertical Wilson sections cuts through the skull. **D-E)** Anterior thoracotomy technique in the thoracic cavity. **D)** Anterior view of the thoracic viscera. **E)** Examination of the trachea and esophagus. **F)** Examination of the heart. **G-H)** Microdissection technique in the abdominal cavity. **G)** Anterior view of the abdominal viscera following the removal of the hepatic lobes. **H)** Lateral view of the stomach, intestinal loops, and the distal portion of the esophagus. **I)** Examination of the urogenital system. Images taken by the authors, adapted from Freire-Gómez *et al.*, 2021.

To detect visceral abnormalities in the cephalic region, a series of standardized section cuts was performed according to the protocol proposed by Wilson (1965). A transverse cut through the mouth region was made to observe the palate, inner ear, spinal canal, and trachea (Figs. 1A, B). Three frontal cuts were then made: at the nasal septum, at the level of the eyes, and at the cerebral hemispheres (Fig. 1C). The first cut allowed visualization of the nasal septum separating the nasal cavities. The second cut evaluated the symmetry of the eyes, the lens, and the olfactory bulbs. The lateral ventricles and the third ventricle were observed in the final cut (Damasceno *et al.*, 2008; Wilson, 1965).

For the thoracic and abdominal cavity examinations, the sectioning and dissection techniques proposed by Barrow & Taylor (1969) and York *et al.* (2014) were employed. A transverse cut was made below the diaphragm, and the upper portion of the liver was removed to verify diaphragm integrity and the presence of abdominal viscera herniations in the thoracic cavity (Fig. 1D). An anterior thoracotomy was performed to examine the thoracic organs: thymus, trachea (Fig. 1E), lungs, and heart. The heart's position and dimensions were checked (Fig. 1F), followed by a longitudinal cut to observe the interventricular septum and the exit of the great vessels (aorta and pulmonary artery) (Barrow & Taylor, 1969; Damasceno *et al.*, 2008).

Next, a laparotomy was performed, the hepatic lobes were removed, and the stomach, duodenum, and intestinal loops were examined (Figs. 1G, H). After this, the stomach and intestinal loops were removed to inspect the pancreas, spleen, adrenal glands, kidneys, ureters, bladder, and gonads. Finally, a transverse cut was made at the renal hilum to examine the renal papillae and renal pelvis (Fig. 1I) (Barrow & Taylor, 1969; Damasceno *et al.*, 2008).

Statistical analysis

Statistical analysis of the obtained data was performed using SPSS software (Statistical Package for the Social Sciences), version 20 for Windows (IBM Corp., 2011). In all cases, normality was assessed using the Shapiro-Wilk test, and homogeneity of variance was evaluated with Levene's test. The Mann-Whitney U test was applied for comparisons between independent groups. For categorical variables, proportions were compared using either the Chi-square test or Fisher's exact test, depending on the expected frequencies in each contingency table. Each table or figure details the statistical procedures applied to the evaluated variables, adhering to the verified assumptions.

Statistical analyses were performed considering individual fetuses as experimental units, grouped according to the treatment received by the mother (control or treated group), rather than at the litter level. Since each mother produced a single litter, a total of 10 litters were included per experimental group. All fetuses were included in the evaluation of fetal parameters, while approximately one-third of each litter was randomly selected for skeletal morphology studies and another third for visceral morphology studies. Given the nature of the determinations performed, occasional data loss occurred due to technical or ethical limitations; therefore, the specific n values for each analysis are detailed in the text, tables, or corresponding figures.

RESULTS

No significant differences in mean litter size per dam were observed between the Control and Treated groups (9.60 ± 0.73 vs. 11.20 ± 0.65, respectively; Mann–Whitney U test, $p = .10$). Additionally, no external anomalies were observed in the fetuses from the experimental group. The administration of the ethanolic extract of *Ravenala madagascariensis* during gestation did not significantly affect fetal glucose levels (Control Group: 5.77 ± 0.306

mmol/L vs. Treated Group: 6.06 ± 0.355 mmol/L; p = .11, indicating p > .05, according to the Mann-Whitney U test). However, the treatment led to a statistically significant increase in fetal weight, while fetal size (Cranio-caudal length) remained unchanged (Table 2).

TABLE 2. External morphological examination of fetuses and placentas in the offspring of Wistar rats treated with water (Control Group) and an ethanolic extract of *Ravenala madagascariensis* (400 mg/kg body weight) dissolved in water (Treated Group), administered for 10 days (gestational days 5 to 15). Values are expressed as means ± standard error (SE). Statistical significance was determined using the Mann-Whitney U test (p < .05).

	Control Group (n=143)		Treated Group (n=112)		
	n	Mean ± SE	n	Mean ± SE	p-value
Fetuses					
Weight (g)	143	3.67 ± 0.041	110	3.86 ± 0.044	.00
Cranio-caudal lenght (cm)	143	3.57 ± 0.022	95	3.59 ± 0.051	.29
Tail lenght (cm)	143	1.25 ± 0.011	95	1.26 ± 0.012	3.70
Placentas					
Weight (g)	143	0.47 ± 0.006	109	0.46 ± 0.008	.18
Placental efficiency	143	7.95 ± 0.196	109	8.55 ± 0.144	.00

This increase in fetal weight was also reflected in the offspring classification by fetal weight category (Fig. 2), where no fetuses in the Treated Group were classified as small for gestational age (SGA). Instead, there was a higher proportion of fetuses classified as appropriate or large for gestational age (p = .015, indicating p < .05 according to the Chi-square test for proportion comparison) (Fig. 2). Additionally, placental efficiency significantly improved in the Treated Group (Table 2).

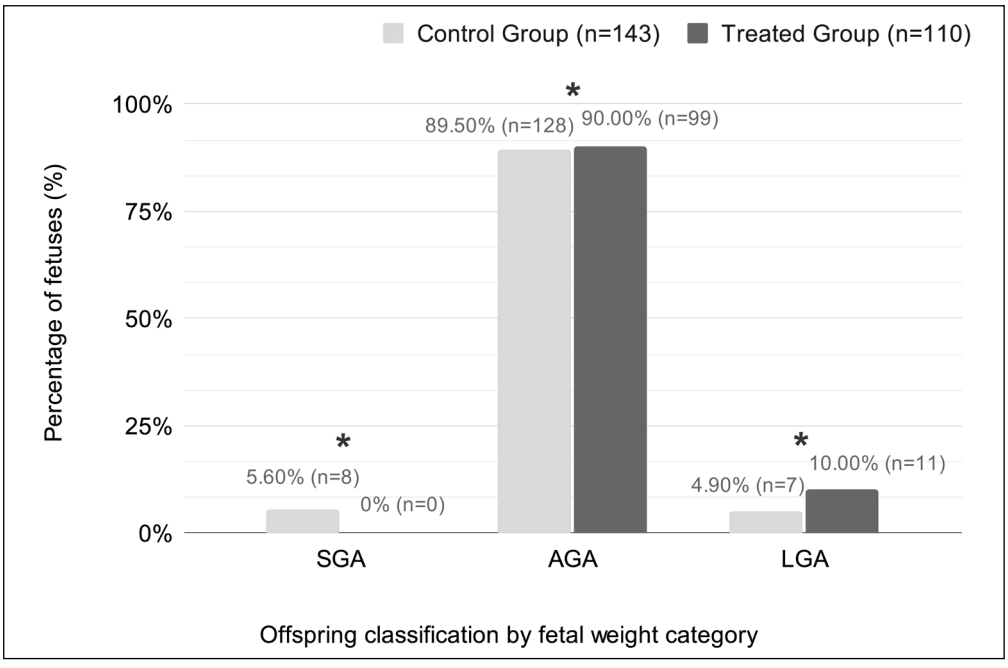

FIGURE 2. Offspring classification by fetal weight category in Wistar rats treated with water (Control Group) and an ethanolic extract of *Ravenala madagascariensis* (400 mg/kg body weight) dissolved in water (Treated Group) administered for 10 days (gestational days 5 to 15). Values are expressed as percentages (%). Statistical significance was assessed using a Chi-square test to compare proportions, * = p < .05. Classification criteria: small for gestational age (SGA) when weight was below the 5th percentile of the Control Group; appropriate for gestational age (AGA) when weight was between the 5th and 95th percentiles of the Control Group; and large for gestational age (LGA) when weight exceeded the 95th percentile of the Control Group.

In the skeletal morphological examination, skeletal alterations were observed in the skull, sternum, caudal region, anterior limbs, and posterior limbs in both experimental groups. The remaining skeletal regions were fully ossified and free of malformations in both experimental groups. The administration of the ethanolic extract of *R. madagascariensis* during gestation increased the ossification process of the parietal bones and the first phalanx (thumb position in humans) of the anterior limbs of the offspring (Table 3 and Fig. 3).

The ossification sites of the sternum decreased significantly in the fetuses from the Treated Group compared to the Control Group (Control Group: 5.89 ± 0.078 vs. Treated Group: 5.51 ± 0.155; p = .033, indicating p < .05 according to the Mann-Whitney U test). However, no significant differences were found in other ossification sites analyzed. Additionally, no visceral malformations were detected in the fetuses studied in either experimental group. Figure 1 shows images of the various organs analyzed.

TABLE 3. Skeletal alterations in the offspring of Wistar rats treated with water (Control Group) and an ethanolic extract of *Ravenala madagascariensis* (400 mg/kg body weight) dissolved in water (Treated Group) administered for 10 days (gestational days 5 to 15). Values are expressed as totals (N) and percentages (%). Statistical significance was assessed using Fisher's exact test (p < .05).

Skeletal alterations	Control Group (n=28) N (%)	Treated Group (n=35) N (%)	p-value
Skull			
Incomplete ossification of the frontal bone	1 (3.60)	0 (0.00)	.44
Incomplete ossification of the parietal bones	4 (14.30)	0 (0.00)	.03
Incomplete ossification of the supraoccipital bone	0 (0.00)	1 (2.90)	.56
Sternum			
Incomplete ossification of the 1st sternebra	0 (0.00)	4 (11.40)	.09
Incomplete ossification of the 4th sternebra	2 (7.10)	1 (2.90)	.14
Non-ossification of the 4th sternebra	0 (0.00)	4 (11.40)	
Incomplete ossification of the xiphoid process	0 (0.00)	1 (2.90)	.09
Non-ossification of the xiphoid process	1 (3.60)	7 (20.00)	
Caudal region			
Incomplete ossification of the 1st caudal vertebra	0 (0.00)	1 (2.90)	.56
Incomplete ossification of the 2nd caudal vertebra	0 (0.00)	3 (8.60)	.17
Incomplete ossification of the 3rd caudal vertebra	0 (0.00)	3 (8.60)	.17
Non-ossification of the 5th caudal vertebra	8 (28.60)	15 (42.90)	.18
Non-ossification of the 6th caudal vertebra	16 (57.10)	20 (57.10)	.60
Anterior limbs			
Incomplete ossification of the 5th metacarpal	0 (0.00)	3 (8.60)	.23
Non-ossification of the 5th metacarpal	3 (10.70)	2 (5.70)	
Non-ossification of the 1st phalanx	28 (100)	4 (11.40)	.00
Non-ossification of the 2nd phalanx	2 (7.10)	2 (5.70)	.55
Non-ossification of the 3rd phalanx	2 (7.10)	1 (2.90)	.50
Non-ossification of the 4th phalanx	2 (7.10)	1 (2.90)	.50
Non-ossification of the 5th phalanx	2 (7.10)	1 (2.90)	.50
Posterior limbs			
Non-ossification of the 1st, 2nd, 3rd, 4th and 5th phalanx	1 (3.60)	4 (11.40)	.33

Note: Incomplete and non-ossification of the same skeletal structures (4th sternebra, xiphoid process, and 5th metacarpal) were analyzed jointly; hence, a single p-value is reported for each.

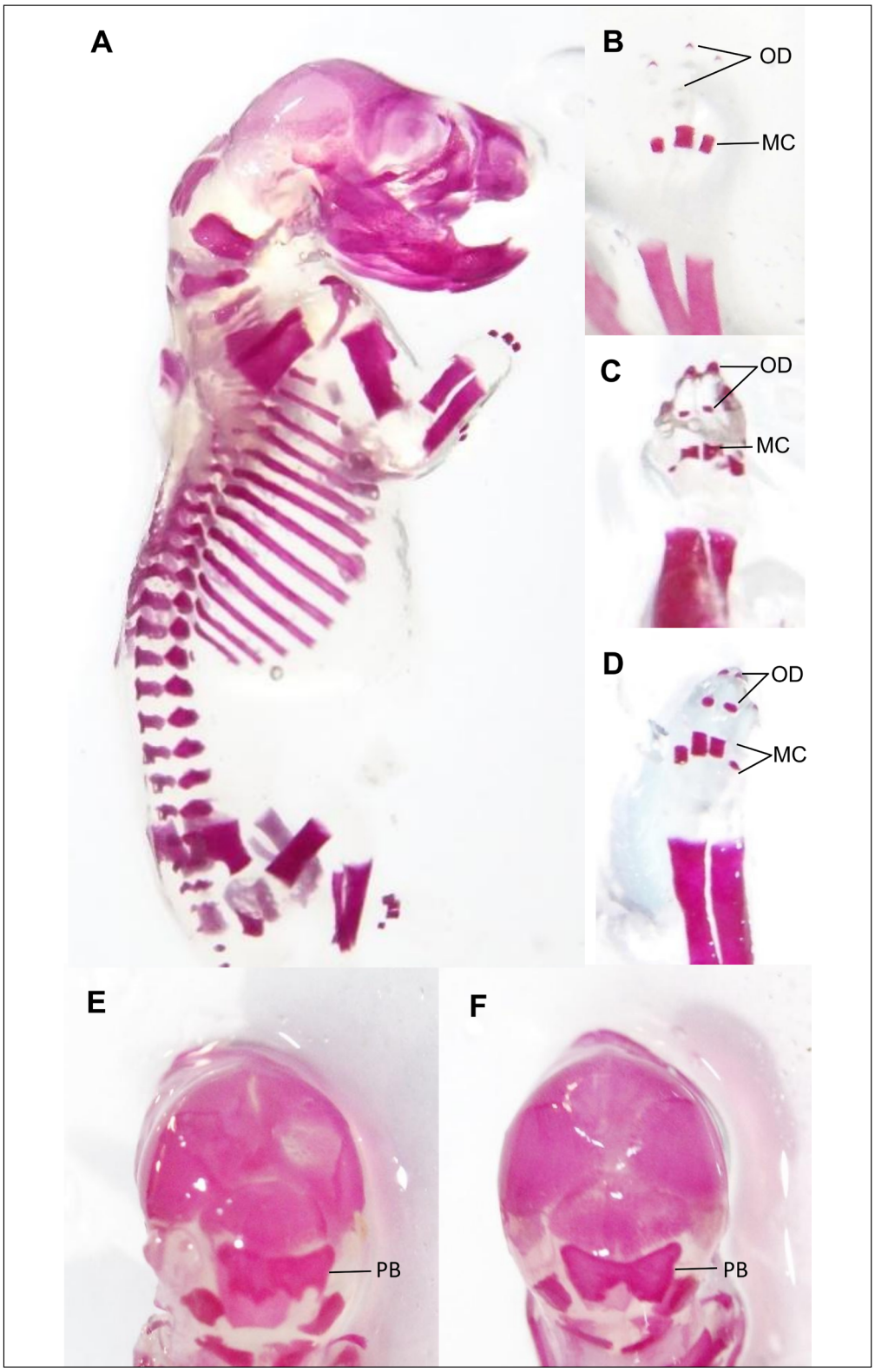

FIGURE 3. Effect of the ethanolic extract of *Ravenala madagascariensis* on fetal ossification. **A)** Lateral view of the fetus at 20 days of gestation during the skeletal morphological examination. **B-D)** Comparison of the ossification process in the anterior limbs between the Control Group and the Treated Group. **B)** Dorsal view of the Control Group's forelimb shows lower ossification of the metacarps (MC) and *ossa digitorum* phalanges (OD). **C)** Ventral view of the Treated Group's forelimb shows greater ossification of the metacarps (MC) and *ossa digitorum* phalanges (OD). **D)** Dorsal view of the Treated Group's forelimb shows greater ossification of the metacarps (MC) and *ossa digitorum* phalanges (OD). **E-F)** Comparison of the parietal bones between the Control Group and the Treated Group. E) Skull of the Control Group with incomplete ossification of the parietal bones (PB). **F)** Skull of the Treated Group, showing complete ossification of the parietal bones (PB).

DISCUSSION

The "fetal origins of adult disease" (FOAD) or "fetal programming" paradigm, more recently replaced by "developmental origins of adult disease" (DOHaD), describes how maternal environments influence offspring health (Gluckman & Hanson, 2006). In biological anthropology, this relationship has been studied through the concept of phenotypic plasticity, defined as "the ability of an organism to react to an internal or external environmental input with a change in form, state, movement, or rate of activity" (Gowland, 2015, p. 530). Biological anthropologists have shown that social, nutritional, demographic, and environmental conditions can trigger phenotypic or developmental plasticity, which could largely explain contemporary human phenotypic variation (Kuzawa & Bragg, 2012). In this context, traditional practices such as the use of medicinal plants during pregnancy take on special relevance, especially in populations with limited access to biomedical care. Evaluating the effects of *Ravenala madagascariensis* in an experimental model provides a controlled analogue for exploring how these cultural practices intersect with maternal biology and influence fetal growth and development.

Taking this context into account, the fact that administering the ethanolic extract of *Ravenala madagascariensis* leaves to pregnant Wistar rats did not alter fetal glycemia levels constitutes a significant finding. Glucose crosses the placenta through facilitated diffusion, and a hyperglycemic state in the mother can induce excessive insulin production in the fetus, potentially leading to metabolic disturbances that impact intrauterine development and predispose the offspring to metabolic diseases later in life (Hufnagel *et al.*, 2022). The fact that the extract did not disturb this balance suggests that, under the conditions of this study, it does not negatively affect fetal glucose metabolism, which is crucial when considering interventions during pregnancy. However, further studies are needed to investigate this effect in altered gestational models, such as diabetic or insulin-resistant rats.

A significant increase in placental efficiency was observed in the treated group, mainly due to increased fetal weight. The placenta plays a crucial role in fetal growth programming (Barker & Thornburg, 2013), as it regulates the transport of nutrients and oxygen. An increase in placental efficiency indicates improved maternal-fetal exchange, suggesting that the extract could promote a more optimal intrauterine environment for fetal development (Bianchi *et al.*, 2021; Ortega *et al.*, 2022). Considering that placental efficiency is calculated as the ratio between fetal and placental weights, and that no significant increase in placental weight was detected, it can be inferred that the improvement in efficiency was primarily due to the increase in fetal weight. Qualitative biochemical analyses have shown that *R. madagascariensis* extracts contain carbohydrates, proteins, and reducing sugars (Handique *et al.*, 2013). These components could contribute to the observed increase in fetal weight by providing additional nutritional support.

However, other phytoconstituents such as flavonoids and phenolic compounds—which, as previously mentioned, have also been identified in the plant (Priyadarsini *et al.*, 2010c)—may act through specific biological mechanisms that promote fetal growth and tissue development. For instance, resveratrol, a natural polyphenol, has been shown to increase average litter size and birth weight in a murine model of gestational diabetes. These effects were associated with enhanced AMPK activation in the liver of both the mothers and their offspring (Yao *et al.*, 2015). Polyphenols can also act as conventional antioxidants by donating hydrogen atoms or electrons, increasing the antioxidant capacity of plasma and reducing oxidative stress parameters in the fetoplacental unit. Further-

more, these compounds can exert modulatory effects at the cellular level by influencing protein and lipid kinase signaling pathways. Polyphenols regulate the activity of a wide range of enzymes and cellular receptors and can also interfere with the function and expression of various membrane transporters. Consequently, the placenta can also be a target of their action, as polyphenols could alter placental uptake of nutrients or other bioactive substances from the maternal circulation, thereby influencing fetal growth and tissue development (Nacka-Aleksić *et al.*, 2022).

However, it remains unclear whether the observed increase in fetal weight is due to an indirect effect of improved placental function or a direct influence of the extract on fetal tissue growth. Further studies are needed, including a detailed phytochemical characterization of this ethanolic extract, as well as determining which specific compounds—or potential synergistic interactions between them—are responsible for the observed effects, and elucidating their mechanisms of action.

In this sense, *R. madagascariensis* could be particularly relevant in maternal malnutrition or metabolic disorders, where placental and fetal development is often compromised, such as in gestational diabetes and maternal obesity (Bianchi *et al.*, 2021; Hufnagel *et al.*, 2022). This finding underscores the importance of further exploring the effect of the extract in conditions where placental and fetal development are at risk.

The impact of the extract on fetal weight is another key outcome of this study. This effect was evident in both the absolute weight values and the offspring classification by fetal weight category. No fetuses in the treated group were classified as small for gestational age (SGA), and there was an observed shift towards a higher proportion of fetuses classified as appropriate or large for gestational age. In both humans and animal models, maternal malnutrition and gestational diabetes are often associated with a high incidence of fetuses born outside normal weight parameters, which can have long-term health implications (Bequer *et al*., 2018; Gómez *et al.*, 2021; Iessi *et al.*, 2010). These results suggest that the administration of the extract could be beneficial in managing pregnancies at risk of intrauterine growth restriction (IUGR) or complications related to maternal undernutrition and metabolic imbalances. Nevertheless, further studies in experimental models are necessary to understand this effect. Several studies have reported reduced fetal weight in offspring from mothers exposed to toxic substances during gestation (Basal *et al*., 2020; Bilir *et al*., 2022; Öztürk *et al*., 2011). In this context, the findings from our study suggest that the extract does not appear to have toxic effects associated with fetal growth under the specific experimental conditions determined. However, future studies are required to confirm the extract's safety.

Fetal development studies frequently report a baseline level of skeletal alterations, as observed in the Control and Treated Groups in this study (El-Nahla *et al.*, 2017; Gómez *et al.*, 2021; Moraes-Souza *et al.*, 2017). However, the significant increase in the ossification of the parietal bones and the first phalanges in the treated group is noteworthy. This finding and the more balanced fetal growth align with the positive correlation between body weight and skeletal ossification reported in various studies (Basal *et al.*, 2020; Bilir *et al.*, 2022; Öztürk *et al.*, 2011). Ossification is a key indicator of fetal development (Öztürk *et al.*, 2011) and is influenced by the availability of essential minerals, such as calcium and phosphorus (DeSesso & Scialli, 2018; Stenhouse *et al.*, 2022) — both present in *R. madagascariensis* extracts (Handique *et al.*, 2013; Priyadarsini *et al.*, 2010c). An adequate supply of these minerals is essential for skeletal mineralization during intrauterine development (Stenhouse *et al*., 2022). However, although these effects of the extract on ossification and

fetal weight may reflect a possible nutritional contribution, the decrease in ossification sites in the sternum highlights the need for cautious interpretation.

It should be noted that reduced ossification sites in the sternum are typically not classified as anomalies or malformations, but rather as variations characteristic of this developmental stage (DeSesso & Scialli, 2018). Similar observations have been reported in fetuses of healthy mothers during several teratogenesis and reproductive toxicology studies. For example, historical control data obtained between 2011 and 2015 in Sprague-Dawley rats and two substrains of Wistar Hanover rats showed variations in the sternebrae on gestational day 20, indicating that these variations can be considered spontaneous and not due to a chemical substance (Kuwagata *et al*., 2019). Similarly, Aliverti *et al*. (1979) reported that not all healthy Wistar rat fetuses had fully ossified sternebrae by gestational day 20, and that even by day 21—when ossification is usually more advanced and homogeneous—, slight variations in the degree of ossification persisted. In turn, Damasceno *et al.* (2016) found that 21.6% of the fetuses in the control group presented incomplete ossification of the sternebrae also on gestational day 21.

Although these results confirm that this variation can occur naturally during normal fetal development, and despite the risks associated with evaluation on gestational day 21—such as imminent parturition and the possible exclusion of animals from the study (El-Nahla *et al*., 2017)—further studies conducted on gestational day 21 are recommended. These studies would allow us to verify whether the differences observed between groups persist or resolve spontaneously and would contribute to a more comprehensive assessment of the extract's safety.

As detailed in the Methods section, the administration period (gestational days 5–15) coincides with the embryonic stage of intrauterine development in rats, a phase widely recognized as the most vulnerable to the teratogenic effects of substances (Hill, 2007; York *et al.*, 2014). However, although organogenesis represents the period of greatest susceptibility to structural malformations, both the preimplantation and fetal stages are also susceptible to toxic insult, potentially leading to growth retardation or functional alterations (York *et al.*, 2014). Consequently, future studies should consider extended or stage-specific administration schedules to determine whether treatment discontinuation may have influenced the observed outcomes and to achieve a more comprehensive understanding of the plant's effects throughout gestation.

Overall, these observations emphasize the need for continued research to fully characterize the safety profile of *Ravenala madagascariensis* and its potential effects on fetal growth and development. Although preliminary, these results contribute to the growing body of evidence evaluating the risk–benefit balance of medicinal plant use on a scientific basis. Moreover, they highlight the value of integrative approaches that connect biological anthropology with biomedicine—a link already emphasized by several authors (Kramer *et al*, 2021; Reiches *et al.,* 2019). The administration of *Ravenala madagascariensis* in a controlled experimental model provides an analogy for understanding how cultural practices involving medicinal plants can influence reproductive outcomes and fetal growth, thereby enriching biological anthropological debates on developmental plasticity, maternal investment, and human adaptation.

CONCLUSION

This study evaluated the effects of the ethanolic leaf extract of *Ravenala madagascariensis* on fetal growth and development during gestation in a Wistar rat experimental

model. Under the conditions tested, observations included indicators consistent with increased fetal weight, enhanced placental efficiency, and more advanced ossification in specific cranial and limb regions. No skeletal or visceral malformations typically associated with teratogenic substances were observed. Considering the traditional and self-administered use of *R. madagascariensis* for various ailments among populations with limited access to healthcare, these preliminary findings provide a basis for future research with more robust experimental designs. Future studies should consider evaluations in larger samples, with different extract concentrations, at different stages of pregnancy and up to day 21 of gestation. This work reinforces the value of integrative approaches that intersect biological anthropology with biomedicine, contributing to current debates on maternal investment, adaptation, and the biocultural determinants of reproductive health.

AUTHOR CONTRIBUTIONS

Cindy Freire-Gómez: conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing (original draft, review & editing). Leticia Bequer Mendoza: conceptualization, data curation, formal analysis, investigation, methodology, project administration, supervision, validation, visualization, writing (original draft, review & editing). Orelvis Portal: conceptualization, data curation, formal analysis, investigation, methodology, project administration, supervision, validation, writing (review & editing). Katia Ojito-Ramos: conceptualization, data curation, formal analysis, investigation, methodology, project administration, supervision, validation, writing (original draft, review & editing). Milagros García Rivero: investigation, methodology, validation. Jose Luis Molina Martínez: investigation, methodology, validation. Carolina Paschetta: writing (review & editing). Tahiry Gómez Hernández: conceptualization, data curation, formal analysis, investigation, methodology, project administration, supervision, validation, visualization, writing (original draft, review & editing).

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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