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In vivo effects of ethanol leaf extract of *Pterocarpus santalinoides* on early pregnancy in Wistar rats: a dose-dependent analysis of embryotoxic activities

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Abstract

Background *Pterocarpus santalinoides* is an edible medicinal plant traditionally used by midwives in southeastern Nigeria for fertility management; however, its safety profile during pregnancy remains unclear.

Objective This study investigated the effects of the ethanol leaf extract of *Pterocarpus santalinoides* (ELEPS) on early pregnancy outcomes in Wistar rats.

Methods Thirty pregnant Wistar rats were randomly assigned to five groups (n = 6): control (distilled water 10 ml/kg), positive control (misoprostol 50 µg/kg), and three ELEPS treatment groups (400, 600, and 800 mg/kg). Treatments were administered orally via gavage for seven consecutive days from gestational day 1. Primary outcomes included pregnancy status assessment, hormonal profiles (estradiol, progesterone, and prostaglandin F2α), connexin 43 expression, and uterine histomorphological changes.

Results ELEPS demonstrated significant dose-dependent embryotoxic effects with resorption rates of 66.67%, 83.33%, and 100% at 400, 600, and 800 mg/kg doses, respectively, compared to 0% in controls. One-way Analysis of Variance (ANOVA) revealed significant alterations in estradiol levels ($F = 2.899$, $p = 0.042$) and progesterone concentrations ($F = 4.144$, $p = 0.010$) across the treatment groups. The highest ELEPS dose (800 mg/kg) induced complete embryonic resorption, elevated connexin 43 expression, and pathological uterine changes, including increased leukocytic infiltration and abnormal decidual morphology.

Conclusion These findings provide scientific evidence for the embryolethal properties of *P. santalinoides*, supporting its traditional use as an abortifacient, while raising significant safety concerns for pregnant women consuming this plant.

Keywords *Pterocarpus santalinoides*, Embryotoxicity, Early pregnancy, Connexin 43, Prostaglandin F2α, Wistar rats

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Background of study

Herbal medicines continue to constitute a fundamental element of healthcare systems worldwide, particularly in low- and middle-income countries, where access to conventional pharmacological treatments is often constrained by economic, geographic, or infrastructural barriers. This reliance on traditional remedies is especially pronounced among women of reproductive age, who frequently turn to herbal products for various health needs, including during pregnancy. The preference for "natural" remedies during gestation is largely influenced by concerns about the teratogenic risks associated with synthetic drugs, despite the fact that comprehensive safety data for many botanicals remain limited or inconclusive. The pervasive use of herbal medicines in pregnancy has thus reignited scientific interest in the reproductive toxicology of medicinal plants, as numerous phytochemicals can cross the placental barrier and potentially disrupt critical processes in early embryonic development (Bernstein et al., 2020; Torloni et al., 2020).

Increasingly, experimental studies have provided evidence that a range of medicinal plants exhibit embryotoxic, teratogenic, or abortifacient effects. These adverse outcomes may arise through several mechanisms, including endocrine disruption that alters hormonal homeostasis, direct uterotonic activity that stimulates uterine contractions, or induction of oxidative stress and inflammatory responses within the uterine microenvironment. Rodent models, particularly Wistar rats, are extensively utilized in this field of research because their reproductive physiology shares key similarities with that of humans, including hemochorial placentation, hormonal regulation of gestation, and early pregnancy dynamics. The relatively short gestation period of these animals facilitates controlled dose–response studies and mechanistic investigations. Furthermore, these models enable the integration of multiple investigative endpoints, endocrine, histomorphological, and molecular, to elucidate the pathways through which plant extracts influence implantation success and pregnancy maintenance (Aguilera et al., 2022a, 2022b; Sakowicz et al., 2022).

Within this broader framework, *Pterocarpus santalinoides* (Fabaceae) has emerged as an edible medicinal plant with significant ethnomedicinal importance in West and Central Africa. It is traditionally used for a wide array of health conditions, including inflammatory diseases and infections, and is incorporated into local diets as a vegetable or soup thickener in certain cultural contexts (Ayéna et al., 2021a, 2021b). Pharmacological investigations of various plant parts have revealed a spectrum of bioactivities, such as antimicrobial, anti-inflammatory, antioxidant, and hepatoprotective effects, which collectively support its continued use in traditional medicine and suggest a complex phytochemical composition. Toxicological assessments, including acute toxicity tests in rodents, have indicated a relatively wide safety margin for hydroethanolic extracts of *P. santalinoides* when administered in single high doses (Ayéna et al., 2021a, 2021b).

Beyond these general systemic effects, *P. santalinoides* holds a notable place in ethnobotanical practices related to reproductive health in parts of Nigeria and neighboring regions. Traditional birth attendants and midwives frequently use leaf preparations of this plant for fertility regulation, menstruation induction, and early pregnancy termination (Ayéna et al., 2021a, 2021b; Brahmi et al., 2025). Consequently, pregnant women or those seeking to prevent conception often consume this plant without standardized dosing regimens or medical oversight. This practice raises critical safety concerns, especially given the well-documented ability of phytoestrogens, saponins, and other bioactive constituents present in medicinal plants to modulate reproductive hormone levels, stimulate uterine contractility, and compromise decidual integrity. These bioactivities have the potential to adversely affect maternal and foetal health (Ayéna et al., 2021a, 2021b).

Despite the extensive ethnomedicinal utilization and growing pharmacological profiling of *P. santalinoides,* there remains a conspicuous lack of systematic studies specifically addressing its reproductive safety during pregnancy. The majority of existing research has focused on its antimicrobial and organ-protective properties, leaving significant gaps in the knowledge regarding its embryotoxic potential, effects on hormonal balance, uterine histomorphology, and molecular markers indicative of uterine activation during gestation (Bernstein et al., 2020). This gap is particularly alarming in contexts where *P. santalinoides* serves dual roles as a food source and medicinal agent, potentially exposing pregnant women and their developing embryos to pharmacologically active compounds without the benefit of evidence-based risk evaluation (Bernstein et al., 2020).

To address this critical deficiency in the literature, the present study was designed to investigate the effects of ethanol leaf extract of *P. santalinoides* on early pregnancy outcomes in Wistar rats. This study integrates multiple investigative approaches, including assessment of pregnancy status, profiling of reproductive hormone levels, detailed uterine histomorphological analysis, and evaluation of connexin 43 expression, a key molecular marker implicated in uterine contractility and embryo implantation (Challis et al., 2000). By employing a validated rodent model of early gestation, this study aimed to elucidate the potential embryotoxic and abortifacient actions of the extract. The findings are intended to provide a scientific basis for clinical counselling and public health guidance, particularly for women who consume this plant

during the peri-conceptional period, thereby contributing to safer traditional medicine practices and improved maternal-foetal health outcomes.

Materials and methods

Plant material collection and authentication

Fresh leaves of *Pterocarpus santalinoides* L'Hérit ex DC (Fabaceae) were collected from Umudike, Abia State, Nigeria (5°28′60″ N, 7°32′60″ E) during the dry season (November 2023) to ensure optimal phytochemical content and minimal moisture interference. Plant authentication was performed by a qualified taxonomist at the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, following the standard botanical identification protocol described by Chase et al. (2016). A voucher specimen (MOUAU-PSL-2023-11) was deposited in the university herbarium for future reference and taxonomic verification.

Extract preparation

The plant material was processed following standardized ethnopharmaceutical protocols adapted from Bharathi et al. (2025). The collected leaves were thoroughly washed with distilled water to remove surface contaminants, air-dried under shade at ambient temperature (25 ± 2 °C) for 14 d to preserve thermolabile compounds, and subsequently pulverized using electric mill (Thomas Wiley Mill, Model 4) (Thomas Scientific®) to achieve a uniform particle size (40–60 mesh). The pulverized material (500 g) was subjected to cold maceration using 80% ethanol (1:5 w/v ratio) for 72 h with intermittent mechanical agitation every 12 h to enhance the extraction efficiency. This solvent concentration was selected to optimize the extraction of both polar and semi-polar bioactive compounds, while minimizing the degradation of heat-sensitive metabolites.

The mixture was filtered through Whatman No. 1 filter paper (pore size 11 µm) to remove particulate matter, and the filtrate was concentrated using a rotary evaporator (Heidolph, Germany) at 45 °C under reduced pressure to prevent thermal degradation of the bioactive compounds. The resulting crude ethanol leaf extract of *Pterocarpus santalinoides* (ELEPS) was stored at – 20 °C in amber vials under a nitrogen atmosphere until use. The extraction yield was calculated to be 12.4% w/w based on the dry weight of the starting material (Fig. 1).

Acute toxicity assessment

Acute toxicity assessment was carried out as outlined by OECD (2002b), involved determining the adverse effects that occurred within a short period following exposure to ELEPS. This evaluation typically measured the lethal dose (LD50) that caused death in 50% of the test population, thus providing critical data on the potential hazard of ELEPs.

Experimental animals and ethical considerations

Thirty sexually mature virgin female Wistar rats (*Rattus norvegicus*), weighing 140–223 g, were obtained from the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike. Animals were housed in standard polypropylene cages (43 × 27 × 15 cm) under controlled environmental conditions following the international guidelines for laboratory animal care (National Research Council, 2011). The housing conditions included a 12:12 h light–dark photoperiod, a temperature of 25 ± 2 °C, a relative humidity of 55 ± 10%, and adequate ventilation (15–20 air changes per hour).

The animals received a standard pelleted laboratory diet (Ladokun Farms Ltd., Nigeria) containing crude protein (18%), crude fiber (5%), fat (4%), and metabolizable energy (2500 kcal/kg), and water was provided ad libitum from automatic drinking systems. All animals underwent a 14-day acclimatization period to minimize stress-induced physiological variations that could interfere with reproductive parameters. The procedures of laboratory animal care according to NIH Publication (2011) were employed. The Institutional Animal Care and Use Committee (IACUC) of the Micheal Okpara University of



Fig. 1 Close-up of *Pterocarpus santalinoides* leaves showing broad green foliage

Agriculture, Umudike's College of Veterinary Medicine, examined and approved the study protocol (MOUAU/CVM/REC/202123).

Estrous cycle monitoring and mating procedures

Estrous cycle synchronization was performed as described by Ekambaram et al. (2017). Daily vaginal cytological examinations were conducted using a non-invasive vaginal lavage technique with 200 µL of sterile normal saline (0.9% NaCl) administered via sterile plastic pipettes. Cytological smears were stained with methylene blue and examined under a light microscope (×400 magnification) to identify estrous cycle phases based on cellular morphology and differential cell counts (Cora et al., 2015).

Females displaying regular 4-day estrous cycles for two consecutive cycles were selected for mating studies. At the proestrus stage, identified by the presence of nucleated epithelial cells and the absence of leukocytes, females were paired with proven fertile males at a 2:1 ratio overnight (18:00-06:00 h) in standard mating cages (Cora et al., 2015). Successful mating was confirmed the following morning by the presence of vaginal plugs or sperm in vaginal smears, designated as gestational day 1 (GD1), according to established protocols (Behringer et al., 2016; Umamageswari et al., 2020).

Experimental design and treatment protocol

A randomized controlled experimental design was employed, with 30 confirmed pregnant rats randomly allocated into five groups (n = 6 each) using computer-generated random numbers to minimize selection bias. The treatment groups were as follows:

- Group A (Negative Control): Distilled water 10 ml/kg body weight
- Group B (Positive Control): Misoprostol 50 µg/kg body weight (Saucedo et al., 2024)
- Group C (Low Dose): ELEPS 400 mg/kg body weight
- Group D (Medium Dose): ELEPS 600 mg/kg body weight
- Group E (High Dose): ELEPS 800 mg/kg body weight

All treatments were administered once daily via oral gavage from GD1 to GD7, using graduated syringes with blunt-tipped feeding needles to ensure accurate dosing. Dose selection was based on acute toxicity results, literature review of related plant species, and preliminary pilot studies (Ani & Christopher, 2021; Ayéna et al., 2022). Misoprostol was selected as a positive control because of its well-established abortifacient properties (Chatsis & Frey, 2018) and similar mechanism of action to plant-derived uterotonic compounds (Allen & O'Brien, 2009).

Pregnancy status evaluation

On GD8, approximately 24 h after the final treatment, the animals were sacrificed by cervical dislocation under light ether anesthesia to minimize stress-induced hormonal fluctuations. This timing was selected to capture early pregnancy effects, while allowing sufficient exposure duration for treatment-induced changes to manifest. (Carbone et al., 2012).

Uteri were carefully removed via midline laparotomy, and the pregnancy status was examined following established protocols (Fischer et al., 2008). Implantation sites were identified as distinct swellings along the uterine horns, while resorption sites appeared as dark, hemorrhagic lesions or collapsed decidual tissue.

The parameters recorded included the total number of implantation sites, resorption sites, number of retained viable fetuses, and overall uterine morphology. The resorption percentage was calculated using the following formula: (number of resorption sites/total implantation sites) × 100 (Rodrigues et al., 2023).

$$\begin{matrix} Resorption \\ percentage \end{matrix} = \frac{Number\ of\ resorption\ sites}{Total\ implantation\ sites} \times 100$$

Hormonal analysis

Blood samples (3–5 ml) were collected via cardiac puncture immediately following sacrifice using sterile 5 ml syringes with 21-gauge needles to minimize hemolysis. The samples were transferred to plain tubes and allowed to clot at room temperature for 30 min before centrifugation at 3000 rpm for 15 min at 4 °C. Serum was separated, aliquoted into sterile microcentrifuge tubes, and stored at − 80 °C until analysis to preserve hormone stability. Hormonal concentrations were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Biochemical Systems International, RM 2060) according to the following specifications:

- Estradiol (E2): Sensitivity 1.0 pg/ml, intra-assay CV < 8%, inter-assay CV < 10%
- Progesterone (P4): Sensitivity 0.1 ng/ml, intra-assay CV < 6%; inter-assay CV < 12%
- Prostaglandin F2α (PGF2α): Sensitivity 5.0 pg/ml, intra-assay CV < 9%, inter-assay CV < 15%

All assays were performed in duplicate, according to the manufacturer's protocols, with quality control samples included in each run to ensure analytical accuracy. Optical density was measured using a microplate reader (BioTek Instruments, United States of America) at 450 nm with 630 nm reference wavelength (Darwish and Alzoman 2023).

Histomorphological analysis

Representative uterine tissue samples were collected from the mid-uterine horn region to ensure standardized sampling. Tissues were immediately fixed in 10% neutral buffered formalin (pH 7.4) for 24 h, processed using ascending grades of ethanol (70%, 80%, 90%, and 100%) for dehydration, cleared in xylene, and embedded in paraffin wax blocks.

Serial Sects. (4-5 µm thickness) were cut using a rotary microtome (Leica RM2125RT, Germany) and stained with hematoxylin and eosin (H&E) following standard protocols (Fischer et al., 2008). Histomorphological examination was conducted using a light microscope (Olympus BX41, Japan) at various magnifications (× 100, × 200, and × 400). The evaluated parameters included epithelial proliferation, endometrial gland morphology and proliferation, stromal cellularity, neovascularization, inflammatory cell infiltration, and hemosiderin deposition.

Histological changes were graded using a semi-quantitative scoring system: (-) absent, (+) mild, (++) moderate, and (+++) marked, based on the established criteria for reproductive toxicology studies (Schafer et al., 2018). All evaluations were performed by experienced histopathologists blinded to the treatment groups to ensure objective assessment.

Immunohistochemical analysis

Connexin 43 (Cx43) expression was evaluated using standard immunohistochemical techniques adapted from established protocols (Savic et al., 2022). Briefly, deparaffinized tissue sections were subjected to antigen retrieval in citrate buffer (pH 6.0) using microwave heating (750 W for 10 min) to expose masked epitopes. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide in methanol for 10 min at room temperature.

Non-specific binding was prevented by incubation with normal goat serum (5%) in phosphate-buffered saline containing 0.1% bovine serum albumin for 30 min. Primary antibody against Cx43 (monoclonal, 1:200 dilution, Abcam) was applied overnight at 4 °C in a humidified chamber Binder GmbH, Germany (Tuttlingen). Following Phosphate Buffered Saline washes, the sections were incubated with biotinylated goat anti-mouse secondary antibody (1:300) for 30 min at room temperature.

Detection was performed using the avidin–biotin-peroxidase complex method with 3, 3'-diaminobenzidine (DAB) as a chromogen, producing brown coloration at sites of protein expression. The sections were counterstained with hematoxylin, dehydrated, cleared, and mounted with permanent mounting medium. Expression intensity was graded as: (-) absent, (±) very weak, (+) mild, () moderate, and (+) intense, based on established scoring criteria for gap junction proteins (Gibson-Corley et al., 2013).

Statistical analysis

The gathered data were subjected to one-way analysis of variance (ANOVA) using the Statistical Package for Social Science (SPSS) version 23. The least significant difference (LSD) post-hoc test separated the varying means. The probability significance was set ($p < 0.05$) was then considered significant. Results are presented as the mean ± standard error of the mean (SEM) in tabular and graphic forms.

Results

Effects of ethanol leaf extract of *Pterocarpus santalinoides* on pregnancy status

Administration of ELEPS resulted in significant dose-dependent embryotoxic effects across all treatment groups compared with the positive and negative controls (Table 1). The control group administered distilled water at 10 ml/kg, exhibited typical pregnancy outcomes. On average, these subjects displayed 11 implantation sites and no resorption sites, resulting in a 0% resorption rate. In contrast, all ELEPS-treated groups demonstrated substantial embryonic loss with marked pregnancy disruption.

The positive control group (misoprostol 50 µg/kg) exhibited 10 implantation sites with four resorption sites, resulting in a 66.67% resorption rate, which served as the expected abortifacient response. The group administered a low dose of ELEPS (400 mg/kg) exhibited embryotoxicity comparable to that of the control, with 10 implantation sites and six resorption sites observed. This resulted

Table 1 Effects of *Pterocarpus santalinoides* on pregnancy status in Wistar rats during early gestation

Treatment groups	Average number of implantation sites	Average number of resorption sites	Average number of retained fetuses	Percentage resorption (%)	Classification
A (Control—Distilled water 10 ml/kg)	11	0	11	0.00	NR
B (Positive Control—Misoprostol 50 µg/kg)	10	4	6	66.67	IR
C (Low Dose—ELEPS 400 mg/kg)	10	6	4	66.67	IR
D (Medium Dose—ELEPS 600 mg/kg)	11	6	5	83.33	IR
E (High Dose—ELEPS 800 mg/kg)	13	13	0	100.00	CR

ELEPS Ethanol Leaf Extract of *Pterocarpus santalinoides*, *NR* No Resorption, *IR* Incomplete Resorption, *CR* Complete Resorption

in a resorption rate of 66.67%, identical to that of the control group. The medium-dose ELEPS group (600 mg/kg) demonstrated increased embryotoxicity with 11 implantation sites and six resorption sites, producing a higher resorption rate of 83.33%. Most significantly, the high-dose ELEPS group (800 mg/kg) exhibited complete pregnancy failure, with all 13 implantation sites undergoing resorption, achieving a 100% resorption rate and indicating total embryolethality (Table 1).

Visual examination of uterine specimens revealed distinct morphological changes associated with embryonic resorption (Fig. 2). Control uteri displayed normal pregnancy characteristics, with viable embryonic sites appearing as distinct swellings along the uterine horns. In contrast, ELEPS-treated uteri showed dose-dependent abnormalities, including irregular decidual development, hemorrhagic areas, and loss of normal embryonic structures, with more severe effects observed at higher doses.

Hormonal profile analysis

Comprehensive statistical analysis revealed significant alterations in maternal reproductive hormone concentrations across treatment groups, providing mechanistic insights into ELEPS-induced pregnancy disruption (Table 2).

Estradiol concentrations

One-way ANOVA demonstrated statistically significant differences in estradiol levels between treatment groups ($F = 2.899$, $P = 0.042$). The control group maintained baseline estradiol concentrations of 39.16 ± 1.41 pg/ml. Misoprostol treatment resulted in elevated estradiol levels (42.56 ± 2.28 pg/ml), while low-dose (42.60 ± 2.48 pg/ml) and medium-dose (41.86 ± 0.08 pg/ml) ELEPS groups showed similar significant elevations above control values. Notably, the high-dose ELEPS group exhibited significantly reduced estradiol concentrations (34.50 ± 0.63 pg/

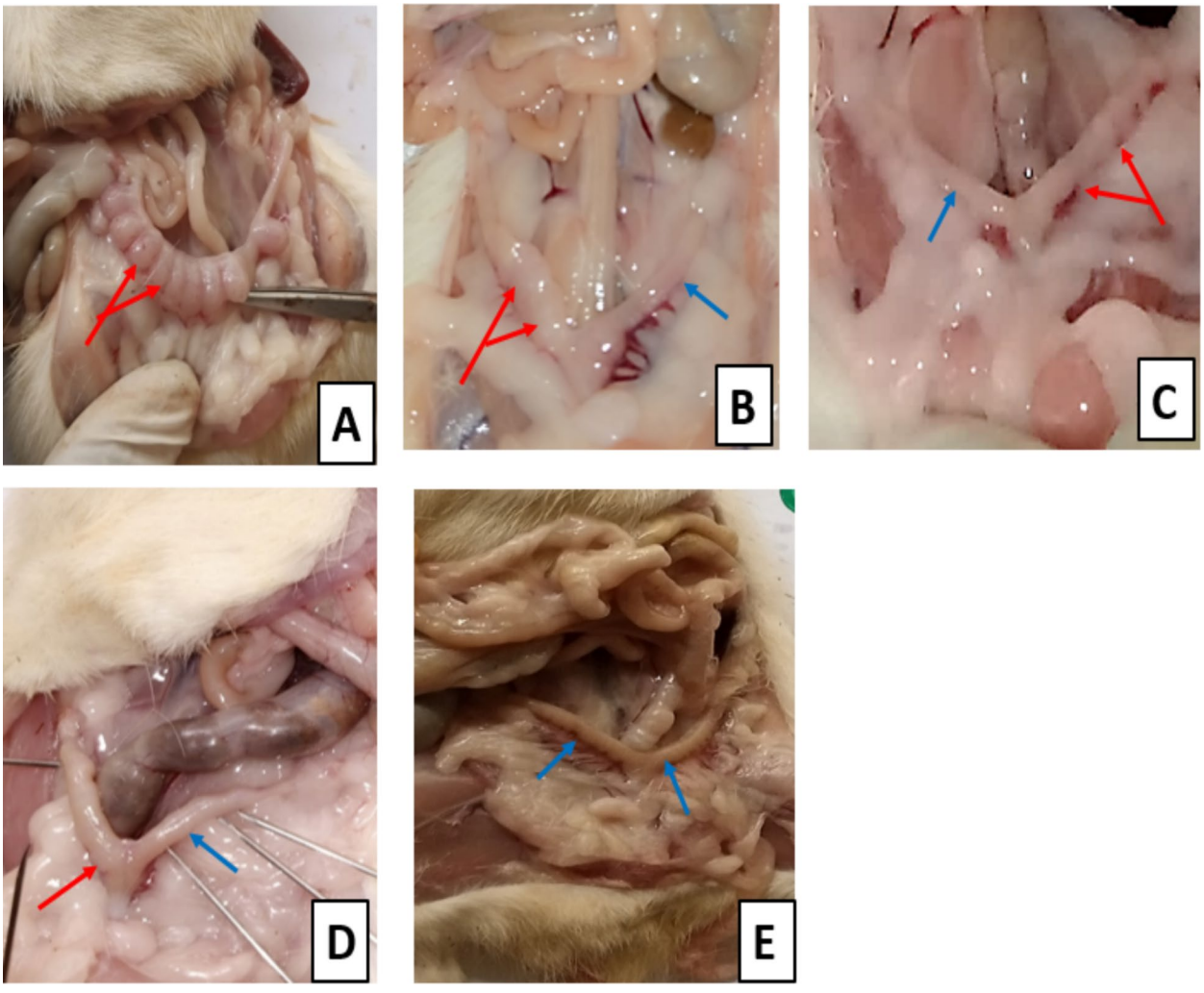


Fig. 2 Effect of ethanol leaf extract of *Pterocarpus santalinoides* (ELEPS) on the pregnancy status of wistar rats at early pregnancy. **A** Uterus from Group A (Distiled water 10 ml/kg) rats, **B** Uterus from Group B (misoprostol 50µ/kg) rats, **C** Uterus from Group C (ELEPS 400 mg/kg) rats, **D** Uterus from Group D (ELEPS 600 mg/kg) rats, **E** Uterus from Group E (ELEPS 800 mg/kg) rats. Red arrow—sites with retained embryo, Blue arrow—resorption sites

Table 2 Hormonal profile analysis in pregnant Wistar rats following *Pterocarpus santalinoides* treatment during early gestation

Parameter	A (Control)	B (Misoprostol 50 µg/kg)	C (ELEPS 400 mg/kg)	D (ELEPS 600 mg/kg)	E (ELEPS 800 mg/kg)
Estradiol (pg/ml)	39.16 ± 1.41^{ab}	42.56 ± 2.28^{a}	42.60 ± 2.48^{a}	41.86 ± 0.08^{a}	34.50 ± 0.63^{b}
Progesterone (ng/ml)	36.10 ± 4.79^{a}	17.90 ± 0.69^{c}	33.60 ± 8.31^{a}	25.30 ± 3.34^{b}	30.95 ± 0.95^{ab}
Prostaglandin F2α (pg/ml)	167.70 ± 8.19^{b}	204.96 ± 37.32^{a}	222.30 ± 4.04^{a}	193.06 ± 7.36^{a}	196.20 ± 7.67^{a}

Values are presented as mean ± SEM (n = 6 per group). Different superscript letters, a b c within rows indicate statistically significant differences ($P < 0.05$) using one-way ANOVA, followed by Tukey's post-hoc test. The superscripts indicate the differences in means among the group with a being the highest difference while c is the lowest. *ELEPS* Ethanol Leaf Extract of *Pterocarpus santalinoides*

Table 3 Semi-quantitative analysis of uterine histomorphological changes in early pregnant Wistar rats

Treatment group	Epithelial proliferation	Endometrial gland proliferation	Neovascularization	Leukocytic infiltration	Hemosiderin deposition
A (Control)	-	+	+	-	-
B (Misoprostol 50 µg/kg)	+	+ +	+	+ +	-
C (ELEPS 400 mg/kg)	+ +	+ +	+	+ +	-
D (ELEPS 600 mg/kg)	+ +	+ +	+	+ +	+ +
E (ELEPS 800 mg/kg)	+	+ + +	+ +	+ +	-

Semiquantitative scores for uterine histomorphological characteristics in the different groups at early pregnancy: (-) absent, indicates not present or seen; (+) Mild; (+ +) Moderate; (+ + +) Marked/Intense. *ELEPS* Ethanol Leaf Extract of *Pterocarpus santalinoides*

ml) compared to other treatment groups, suggesting differential mechanisms of action at higher dosage levels (Table 2).

Progesterone concentrations

Significant inter-group differences in progesterone concentrations were detected ($F = 4.144$, $P = 0.010$). Positive control animals maintained normal progesterone levels (36.10 ± 4.79 ng/ml) essential for pregnancy maintenance. Misoprostol treatment induced a marked decrease in progesterone levels (17.90 ± 0.69 ng/ml), consistent with its established abortifacient mechanism. ELEPS treatments showed dose-dependent effects on progesterone metabolism: the low-dose group maintained near-normal concentrations (33.60 ± 8.31 ng/ml), the medium-dose group exhibited a reduction (25.30 ± 3.34 ng/ml), while the high-dose group showed a high level of suppression (30.95 ± 0.95 ng/ml) (Table 2).

Prostaglandin F2α concentrations

Although elevated PGF2α concentrations were observed across all treatment groups compared to controls (167.70 ± 8.19 pg/ml), statistical analysis revealed no significant differences between groups ($F = 1.499$, $P = 0.233$). Elevated PGF2α concentrations, ranging from 193.06 to 222.30 pg/ml, were observed following Misoprostol and ELEPS treatments (Table 2). This increase suggests that both interventions activate the prostaglandin pathway, which appears to be a shared mechanism in their ability to terminate pregnancy.

Uterine histomorphological analysis

Detailed microscopic examination revealed progressive pathological changes in the uterine architecture that are related to the ELEPS dosage and the degree of embryotoxic effects (Table 3, Fig. 3).

Control group histology

The control group exhibited normal early pregnancy histomorphological characteristics, including intact luminal epithelium with appropriate cellular morphology, well-developed endometrial glands displaying active secretory activity, physiological neovascularization patterns, and a complete absence of inflammatory infiltrates or pathological tissue damage.

Treatment group pathological changes

All ELEPS-treated groups demonstrated varying degrees of histopathological alterations compared to the positive controls and negative controls.

Epithelial Changes: Low-dose (400 mg/kg) and medium-dose (600 mg/kg) ELEPS groups induced marked epithelial proliferation (score: + +) characterized by abnormal thickening and hyperplasia of the luminal epithelium. The high-dose group (800 mg/kg) exhibited moderate epithelial proliferation (score: +), possibly reflecting tissue damage and cellular dysfunction at toxic concentrations.

Endometrial Glandular Proliferation: Progressive increases in glandular proliferative activity were observed across the ELEPS treatment groups: low-dose (score: + +), medium-dose (score: + +), and high-dose (score: + + +), indicating disrupted endometrial differentiation and abnormal secretory function.

Vascular Changes: Neovascularization remained generally maintained across treatment groups (score: +), although the high-dose group demonstrated an enhanced

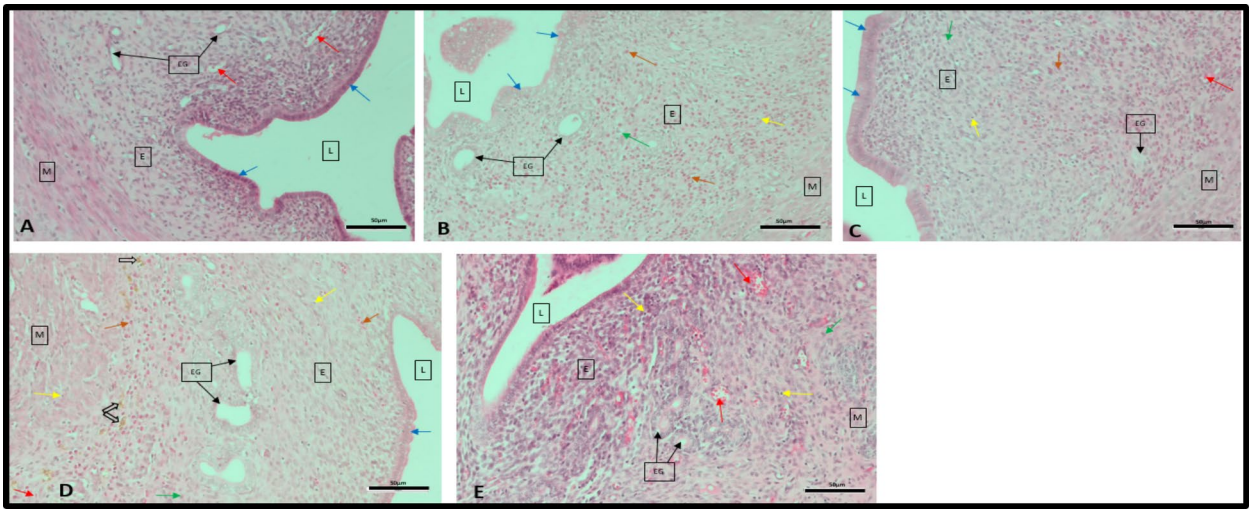


Fig. 3 Photomicrograph showing the effect of *Pterocarpus santalinoides* on uterine histomorphology in pregnant Wistar rats during early pregnancy (H&E). A-Group A (distilled water 10 ml/kg), **B** Group B (Misoprostol 50 µg/kg), C- Group C (ELEPS 400 mg/kg), D—Group D (ELEPS 600 mg/kg), E—Group E (ELEPS 800 mg/kg). *ELEPS* Ethanol Leaf Extract of *Pterocarpus santalinoides*, Blue arrow: Epithelium of the endometrium, Block arrow: Hemosiderin, Brown arrow: Eosinophils, *E* Endometrium, *EG* Endometrial glands, Green arrow: Macrophages, L: Lumen, M: Myometrium, Red arrow: Neovascularization, Yellow arrow: lymphocytes

Table 4 Semi-quantitative analysis of connexin 43 expression in uterine tissues during early pregnancy

Treatment group	A (Control)	B (Misoprostol)	C (ELEPS 400 mg/kg)	D (ELEPS 600 mg/kg)	E (ELEPS 800 mg/kg)
Cx43 expression score	+	+ +	+ + +	+ + +	+ + +

Scoring system: (-) no positive staining; (±) very weak staining in ~ 1% of cells; (+) mild staining; (+) moderate staining; (+) intense staining. *ELEPS* Ethanol Leaf Extract of *Pterocarpus santalinoides*

Elevated Cx43 expression across all ELEPS dosage levels indicates that the plant extract induces premature myometrial activation through enhanced gap junction formation, facilitating coordinated uterine contractions that contribute directly to embryonic resorption and subsequent pregnancy failure

vascular response (score: + +), possibly representing compensatory mechanisms in response to tissue hypoxia.

Inflammatory Response: Marked leukocytic infiltration (score: + +) was consistently observed across all ELEPS-treated groups, indicating significant inflammatory activation and tissue damage.

Hemosiderin Deposition: Only the medium-dose ELEPS group exhibited marked hemosiderin deposition (score: + +), providing evidence of localized hemorrhage and tissue breakdown associated with embryonic resorption.

Connexin 43 expression analysis

Immunohistochemical evaluation of connexin 43 (Cx43) expression revealed significant dose-dependent increases across all ELEPS treatment groups, providing evidence of premature uterine activation (Table 4, Fig. 4).

Control group expression

The control group exhibited minimal Cx43 expression (score: +), consistent with normal early pregnancy physiology, requiring uterine quiescence for successful implantation and embryonic development.

Treatment group expression patterns

Progressive increases in Cx43 expression intensity were observed across treatment groups:

Misoprostol Group: Moderate Cx43 expression (score: + +), confirming gap junction formation characteristic of pharmacologically induced pregnancy termination.

Low-Dose ELEPS (400 mg/kg): Intense Cx43 expression (score: + + +), indicating significant myometrial activation and gap junction upregulation.

Medium-Dose ELEPS (600 mg/kg): Intense Cx43 expression (score: + + +) demonstrating continued gap junction proliferation correlated with increased embryonic resorption.

High-Dose ELEPS (800 mg/kg): Intense Cx43 expression (score: + + +) was directly correlated with complete pregnancy failure and total embryolethality.

Discussion

The present study provides compelling evidence for the dose-dependent embryotoxic effects of ethanol leaf extract of *Pterocarpus santalinoides* (ELEPS) on early pregnancy in Wistar rats. At the highest dose tested

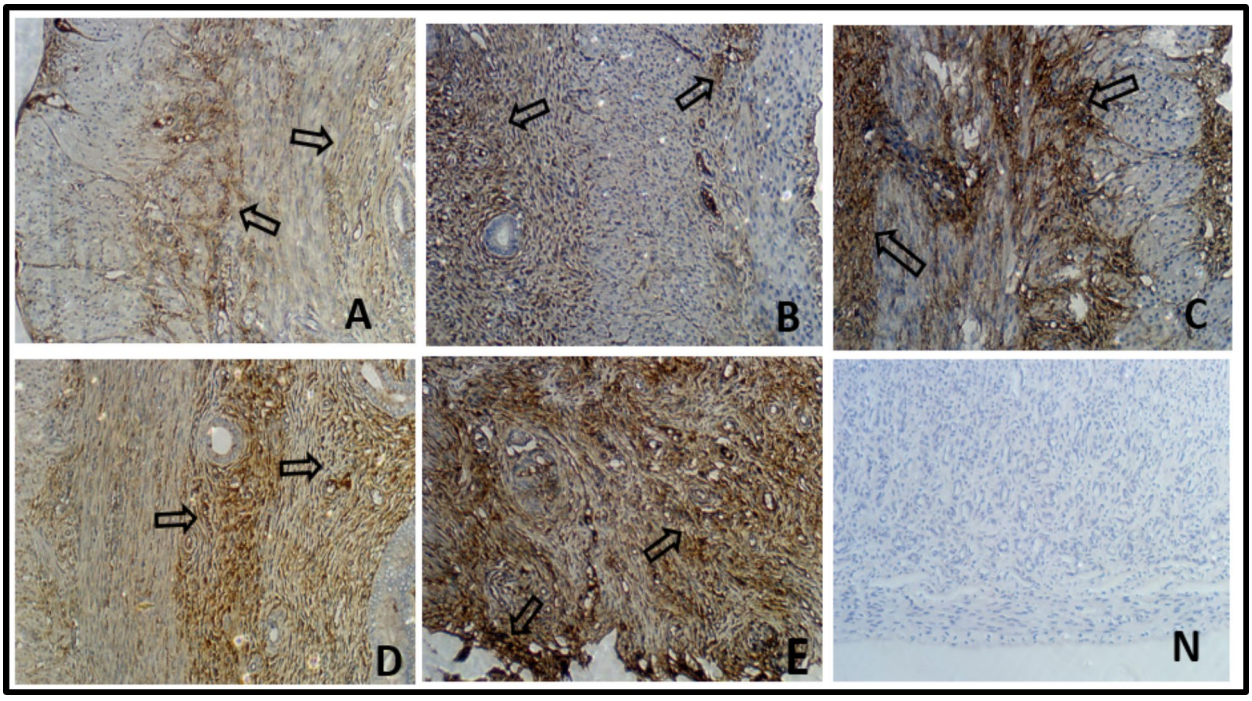


Fig. 4 Effect of *Pterocarpus santalinoides* on the expression of connexin 43 during early pregnancy in Wistar rats. **A** Group A (distilled water 10 ml/kg) rats, **B** Group B (Misoprostol 50 µg/kg) rats, **C** Group C (ELEPS 400 mg/kg) rats, **D** Group D (ELEPS 600 mg/kg) rats, **E** Group E (ELEPS 800 mg/kg) rats. Arrows indicate areas of protein expression. *ELEPS* Ethanol Leaf Extract of *Pterocarpus santalinoides*, *N* Negative control for CX43. (– indicates no positive staining in any cell, ± indicates very weak staining in ~ 1% of the cells, + indicates pale staining, + + indicates marked staining, and + + + indicates intense staining.)

(800 mg/kg), pregnancy did not occur. There were also major hormonal changes and signs of uterine activity in all groups. This shows strong reproductive harm, supporting the plant's traditional use to end pregnancies. It also raises serious safety concerns for pregnant women using this common medicinal plant.

The embryonic resorption rates observed in this study (66.67%, 83.33%, and 100% for 400, 600, and 800 mg/kg doses, respectively) were consistent with the previous investigations on *P. santalinoides* on pregnancy, in which complete embryonic resorption (100%) at a dose of 600 mg/kg also administered during the first week of pregnancy, confirmed the plant's potent anti-implantation and abortifacient properties (Damasceno et al., 2002). The comparable efficacy of misoprostol, a clinically established abortifacient, highlights the significant pharmacological potency of ELEPS in terminating pregnancies.

The observed embryotoxicity likely results from the complex phytochemical composition of *P. santalinoides*, which includes alkaloids, flavonoids, tannins, saponins, steroids, and terpenoids (Ayena et al., 2022). Of particular relevance, the presence of octadeca-9-enoic acid (27.43%), a ricinoleic acid derivative in *P. santalinoides* (Aja et al., 2015; Ikezu & Ikpa, 2024), provided a plausible explanation for the uterotonic effects. Ricinoleic acid, also found in castor oil (*Ricinus communis*), induces labor through prostaglandin EP3 receptor activation and subsequent uterine contractions (Tunaru et al., 2012).

The significant alterations in the reproductive hormone profiles observed in this study provide crucial insights. The elevation of estradiol levels in the low- and medium-dose groups, followed by suppression at the highest dose, suggests a biphasic dose–response relationship, similar to that reported for other phytoestrogenic plants with reproductive effects (Santen et al., 2020). This pattern reflects the biphasic nature of phytoestrogens, where estrogenic effects predominate at lower concentrations, while cytotoxic effects become apparent at higher doses due to the dominance of different phytochemical compounds across concentration ranges.

The estrogen dominance observed in treated groups is particularly significant, as elevated estradiol-to-progesterone ratios are known to induce uterine activation and premature labor (Hamburg-Shields and Mesiano, 2024). Estrogen enhances the expression of proteins associated with uterine contractions, such as connexin 43, while concurrently stimulating the inflammatory processes in the uterus (Andersen, 2000). This dual action of estrogen contributes to complex physiological changes observed in the uterine environment. Mechanism of activity may explain or confirm the ethno botanical knowledge where *P. santalinoides* is specifically used by midwives for labor

induction and fertility control due to its estrogenic activity (Mengue Ngadena et al., 2021).

The significant progesterone suppression observed across treatment groups represents another critical pathway for pregnancy termination. Progesterone is essential for maintaining uterine quiescence and supporting decidual development during early pregnancy (Medina-Laver et al., 2021). The dose-dependent nature of progesterone reduction suggests that ELEPS may interfere with corpus luteum function or enhance progesterone metabolism, leading to withdrawal of pregnancy-supporting hormonal signals (Bulletti et al., 2022).

The upregulation of connexin 43 expression across all ELEPS treatment groups provided molecular evidence for premature myometrial activation. Connexin 43 forms gap junctions between myometrial smooth muscle cells, enabling the electrical coupling necessary for coordinated uterine contractions during labor (Geimonen et al., 1998). During normal early pregnancy, connexin 43 expression remains low to maintain uterine quiescence but increases significantly prior to labor onset in both term and preterm delivery scenarios (Khader et al., 2021).

The upregulation of connexin 43 (scored as + + +) in all ELEPS-treated groups indicated that the extract induced a labor-like state in the myometrium, facilitating the coordinated contractions responsible for embryonic expulsion. This finding is consistent with studies demonstrating that estrogen mediates connexin 43 expression through transcriptional and post-translational mechanisms, whereas progesterone normally suppresses its expression during pregnancy maintenance (Chow & Lye, 1994).

Although the elevation in PGF2α levels was not statistically significant, the observed trend toward increased concentrations across the treatment groups suggests that prostaglandin pathway activation is a contributing mechanism to pregnancy termination. PGF2α functions as a potent luteolytic agent and myometrial stimulant, capable of inducing corpus luteum regression and enhancing uterine contractility (Jungmann et al., 2024). The clinical use of PGF2α analogs for therapeutic pregnancy termination validates the significance of this pathway for abortion induction (Baird, 2000).Estradiol is known to upregulate PGF2α synthesis through the enhanced expression of cyclooxygenase-2 and other prostaglandin synthesis enzymes (Blesson et al., 2012). Concurrent elevation of estradiol and PGF2α levels in ELEPS-treated animals supports this regulatory relationship, suggesting that multiple interconnected pathways contribute to the overall embryotoxic effect (Li et al., 2020).

The progressive histomorphological changes observed with increasing ELEPS doses provided direct evidence of uterine tissue damage and inflammatory activation. Marked epithelial proliferation and abnormal endometrial folding represent disrupted hormonal regulation of normal endometrial differentiation processes (Chou et al., 2024; Fischer et al., 2008; Simitsidellis et al., 2016). During healthy early pregnancy, controlled epithelial growth and secretory transformation are essential for successful implantation and decidual development (Fischer et al., 2008); however, this was not seen in ELEPS-treated rats.

Consistent leukocytic infiltration across the treatment groups indicated significant inflammatory activation within the uterine environment. This inflammatory response may contribute to embryotoxicity through multiple mechanisms, including production of inflammatory cytokines that disrupt maternal–fetal immune tolerance, generation of reactive oxygen species that damage embryonic tissues, and activation of matrix metalloproteinases that degrade extracellular matrix components essential for implantation maintenance (Farine et al., 2017).

The hemosiderin deposition observed in the medium-dose group provides evidence of hemorrhage associated with tissue breakdown during embryonic resorption. This finding suggests that ELEPS-induced pregnancy termination involves mechanical tissue disruption in addition to hormonal pathway modulation, which is consistent with the traditional understanding of the abortifacient mechanism (Prasad et al., 1995).

The observed effects could be attributed to the specific bioactive constituents identified in *P. santalinoides* (Ayena et al., 2021). Flavonoids present in the extract may contribute to the estrogenic effects through direct interaction with estrogen receptors or modulation of steroidogenic enzymes (Nordeen et al., 2025). The documented presence of saponins provides another mechanism for uterine stimulation, as these compounds are known to possess membrane-permeabilizing properties and can affect smooth muscle contractility through calcium mobilization pathways (Watcho et al., 2011).

The tannin fraction may contribute to the observed inflammatory response and tissue damage through protein precipitation and pro-oxidant activity at higher concentrations (Huang et al., 2018). This multi-component mechanism likely explains the complex dose–response relationship observed where different effects predominate at various concentration ranges (Aronson, 2007).

The embryotoxic effects documented in this study align with those of other *Pterocarpus* species and related medicinal plants with traditional abortifacient use (Nwokocha, 2021; Santos et al., 2020). Studies on various herbal abortifacients have consistently demonstrated that pregnancy termination occurs through mechanisms involving hormonal disruption, inflammatory activation, and direct uterotonic effects (Feng et al., 2023). Its efficacy is comparable to that of misoprostol, and therefore,

positions *P. santalinoides* among the more potent plant-derived abortifacients documented in the scientific literature (Raymond et al., 2019).

These findings have immediate clinical relevance for health care providers in regions where *P. santalinoides* is commonly consumed. The demonstrated embryotoxicity at doses potentially achievable through traditional preparation methods necessitates clear contraindications to pregnancy. The dual use of the plant as both a food ingredient and traditional medicine creates particular complexity, requiring targeted public health education approaches (Ayéna et al., 2021a, 2021b).

The complete pregnancy failure observed at high doses, combined with evidence of maternal tissue damage, raises concerns regarding the safety margin for traditional use. Healthcare providers should include *P. santalinoides* consumption in pregnancy history assessments and counsel patients regarding potential reproductive risks (Laelago, 2019).

Although this study provides valuable insights into *P. santalinoides* reproductive effects of *P. santalinoides*, several limitations are acknowledged. The acute exposure protocol (7 days) may not reflect chronic traditional use patterns, where lower doses may be consumed over extended periods. Although well-established for reproductive toxicology, the rodent model may not fully recapitulate human pregnancy physiology, particularly regarding hormone metabolism and placental function.

Future research should focus on identifying the specific bioactive compounds responsible for embryotoxic effects using bioassay-guided fractionation. The investigation of potential protective strategies or antidotes would inform the clinical management of accidental exposure. Long-term studies evaluating the effects on organogenesis and fetal development in pregnancies surviving the initial exposure would provide more comprehensive safety data.

Conclusion

This study demonstrates that the ethanol leaf extract of *P. santalinoides* exerts significant embryotoxic effects during early pregnancy through multiple interrelated mechanisms. The observed combination of hormonal imbalance, premature uterine activation, and inflammatory tissue damage leads to pregnancy loss rates that are comparable to those of conventional pharmaceutical abortifacients. Although these results support traditional ethnobotanical applications, they also highlight critical safety concerns that demand urgent public health intervention and heightened clinical vigilance in regions where this plant is commonly used.

Abbreviations

ANOVA	One-way analysis of variance
CR	Complete Resorption
Cx43	Connexin 43
DAB	Diaminobenzidine
ELEPS	Ethanol leaf extract of *Pterocarpus santalinoides*
ELISA	Enzyme-linked immunosorbent assay
GD	Gestational Day
H&E	Hematoxylin and eosin
IR	Incomplete Resorption
NACL	Sodium Chloride
NR	No Resorption
PGF2α	Prostaglandin F2α
SEM	Standard error of mean
SPSS	Statistical Package for Social Science

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Author contributions

NA and EO conceived and designed experiments. NA, SE, and KR were involved in research supervision, project administration, validation, visualization, drafting, reviewing, and editing of the original manuscript. NA, EO, SE, KR and JO were involved in data curation, formal analysis, investigation, methodology, resources, and software. JO carried out the histological examinations of the organs of interest in this study and was a key contributor in writing the manuscript. All authors have read and approved the final manuscript.

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Data availability

Raw data were generated by the Department of Veterinary Obstetrics and Reproductive Diseases, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Derived data supporting the findings of this study are available from the corresponding authors, Samuel Ekere and Nneka Ani.

Declarations

Ethics approval and consent to participate

The EU Directive 2010/63/EU Ethics Committee's recommendation was adopted to conduct this investigation. The Institutional Animal Care and Use Committee of the Micheal Okpara University of Agriculture Umudike, Faculty of Veterinary Medicine gave its approval to this work (MOUAU/CVM/REC/202123). ARRIVE guidelines were also adopted in carrying out and reporting the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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