Research Article

Non-Monotonic Dose Response of *Eleutherine palmifolia* on Fetal Weight in a Preeclampsia Model: Morphological Safety and Growth Concerns

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Abstract | *Eleutherine palmifolia* (Dayak onion) contains flavonoids and saponins with antioxidant and anti-inflammatory properties that may ameliorate preeclampsia-associated oxidative stress. However, evidence on its developmental safety and fetal outcomes remains limited. This preliminary descriptive study used pregnant BALB/c mice (one dam per group) with preeclampsia induced by anti–Qa-2 antibodies. The extract of *Eleutherine palmifolia* bulb was orally administered at doses of 90, 100, and 125 mg/kg from gestational day 4 to 18. A total of 36 fetuses were evaluated for external morphology, viability, fetal and placental weight, and body length. No external malformations or resorptions were observed across groups. However, the low-dose group exhibited reduced fetal and placental weights compared with controls, while the moderate and high doses produced nearly normal growth outcomes. As each group contained only one dam, these findings are treated as preliminary signals suggesting a possible non-monotonic (hormetic) dose-response, in which suboptimal doses may fail to provide sufficient antioxidant protection against placental oxidative and vascular stress, whereas higher doses restore near-normal fetal development. *Eleutherine palmifolia* extract was not teratogenic morphologically but influenced fetal growth in a dose-dependent and possibly hormetic manner. Due to the use of only one dam per group, these findings should be interpreted descriptively and cautiously.

Keywords | *Eleutherine palmifolia*, Fetal weight, Morphology safety, Morphology growth, Preeclampsia model

Received | November 06, 2025; **Accepted** | November 17, 2025; **Published** | January 10, 2026
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Citation | Asmalinda W, Irwanto, Prasetyo B (2026). Non-monotonic dose response of *Eleutherine palmifolia* on fetal weight in a preeclampsia model: Morphological safety and growth concerns. Adv. Anim. Vet. Sci., 14(1):90-98.
DOI | https://dx.doi.org/10.17582/journal.aavs/2026/14.1.90.98
ISSN (Online) | 2307-8316

INTRODUCTION

Preeclampsia is a serious pregnancy complication and a major cause of maternal and fetal morbidity and mortality worldwide, particularly in developing countries. It is characterized by the onset of hypertension after 20 weeks of gestation (Ives *et al*., 2020; Kornacki, 2024). This condition adversely affects intrauterine development and increases the risk of fetal growth restriction or morphological abnormalities (Gatford *et al*., 2020). Although the precise etiology of preeclampsia remains unclear, inflammation and oxidative stress in the placenta are recognized as key contributors to endothelial dysfunction and impaired placental perfusion (Kornacki, 2024). These pathological changes can create a hypoxic environment that alters the release of placental factors, leading to systemic maternal

vascular dysfunction (Sakowicz *et al*., 2023).

Animal models of preeclampsia are essential for understanding its complex pathophysiology and for evaluating potential therapeutic agents before clinical application (Sakowicz *et al*., 2023; Ożarowski *et al*., 2021). In this context, the search for plant-based compounds that may help alleviate oxidative and inflammatory processes without causing fetal harm is of growing interest. One candidate of traditional origin is *Eleutherine palmifolia* (Dayak onion or Tiwai onion), a bulbous plant widely used in Indonesian ethnomedicine for its purported health benefits (Febrinda *et al*., 2020).

Phytochemical analyses have identified that *Eleutherine palmifolia* contains alkaloids, tannins, saponins, and flavonoids (Prayitno *et al*., 2013; Kamarudin, 2021; Rahmatullah *et al*., 2024). Previous research suggests that its bulb extract exhibits antioxidant, anti-inflammatory, and antihypertensive properties (Ahmad *et al*., 2018; Sari, 2024). These bioactivities may be relevant in managing preeclampsia, which involves oxidative and endothelial stress. Moreover, *in vitro* studies indicate that *eleutherinone*, an active compound isolated from *Eleutherine palmifolia*, can protect endothelial cells from oxidative injury (Kamarudin *et al*., 2021; Poerwosusanto *et al*., 2019).

However, despite these potential benefits, certain phytochemicals present in *Eleutherine palmifolia*, such as alkaloids and flavonoids, are known to cross the placental barrier and may interfere with fetal organogenesis, particularly under pathological or high-dose conditions (Mulyani *et al*., 2020; Adamski *et al*., 2020; Tang and Zhang, 2022). Alkaloids have been associated with neurotoxic and endocrine-disrupting effects in animal studies, while flavonoids can exert reproductive and developmental toxicity at elevated concentrations (Bugel *et al*., 2016; Nawab *et al*., 2020). These findings underscore the importance of assessing both the efficacy and safety of plant-derived compounds during pregnancy.

Teratogenicity testing forms a critical component of preclinical safety evaluation, aiming to identify potential fetal toxicities, such as growth restriction, external malformations, or organ abnormalities, resulting from maternal exposure during organogenesis (Mulyani *et al*., 2020; BPOM, 2022; State Gazette, 2022). To date, limited data exist regarding the teratogenic potential of *Eleutherine palmifolia* bulb extract, particularly in pregnancies complicated by preeclampsia.

Given the traditional use of *Eleutherine palmifolia* and the common assumption that herbal remedies are inherently safe during pregnancy, systematic safety evaluation is required. Therefore, this preliminary study aimed to observe whether administration of *Eleutherine palmifolia* extract to preeclampti pregnant mice produces any external fetal malformations or affects fetal growth parameters. While the mechanistic aspects of its action were beyond the scope of this exploratory work, the results are expected to serve as an initial safety profile and to generate hypotheses for further studies employing larger sample sizes and molecular assessments. The novelty of this study is that it provides the first investigation of the teratogenic effects of *Eleutherine palmifolia* extract in a preeclamptic pregnancy model, offering previously unavailable data on its fetal safety and presenting a new assessment of its potential impact on fetal development under pathological pregnancy conditions.

MATERIALS AND METHODS

Study period and location

Herbal material preparation was conducted at the Herbal Material Medica Laboratory in Batu, Malang. Extraction and phytochemical testing were carried out at the Testing Service Unit Laboratory, Faculty of Pharmacy, Airlangga University. Teratogenic (morphology) testing was carried out at the Embryology Laboratory, Faculty of Veterinary Medicine, Airlangga University. The study took place over four months, from October 18, 2024, to January 14, 2025.

Experimental design

This study used 2- to 3-month-old female BALB/c mice weighing 25–30 grams. Teratogenicity (morphology) testing involved four pregnant mice divided into three treatment groups and one control group, yielding a total of 36 fetuses for observation. Each treatment group consisted of one dam, which represents a major limitation that restricts the inferential validity of the findings. Group allocation was performed using a simple random assignment process. However, with only one dam per group, true randomization and replication could not be achieved. It is acknowledged that, in developmental and reproductive toxicology studies, the appropriate experimental unit is the dam, as fetuses from the same litter are not statistically independent observations. Therefore, this study should be regarded as a preliminary descriptive investigation, designed to explore potential morphological trends rather than to establish statistically significant effects. The limited number of dams was due to ethical and logistical constraints. Consequently, it was not possible to compute litter-based means or perform statistical comparisons across groups. The analysis was therefore focused on descriptive evaluation of fetal parameters, including the number of viable and resorbed fetuses, external malformations, fetal number, fetal condition, body weight, body length, and placental weight. These observations provide initial insights for hypothesis generation and for guiding future studies employing adequate biological replication.

Animal care

All experimental procedures involving animals were conducted in accordance with national and institutional ethical guidelines for care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee and the Airlangga University Animal Research Ethics Committee. The animals were housed in standard polypropylene cages (maximum five animals per cage) with sterilized paddy husk bedding. They were maintained under controlled environmental conditions with a temperature of 22 ± 2 °C, relative humidity of 50-60% and a 12 h light/12 h dark cycle. Standard laboratory chow and filtered tap water were provided *ad libitum*. Veterinary oversight was ensured throughout the study. A licensed veterinarian conducted routine health checks at least twice per week and supervised all humane handling and experimental procedures. Any signs of illness, pain, or distress were immediately reported and assessed by the attending veterinarian. Human endpoints were predefined before the experiment. Animals showing weight loss greater than 20% severe lethargy, inability to eat or drink, or labored breathing were humanely euthanized to minimize suffering. Animal well-being was monitored daily by trained personnel. All efforts were made to minimize the number of animals used and reduce pain or discomfort in accordance with ARRIVE 2.0 guidelines (National Research Council, 2011; Noor *et al*., 2022).

Preparation of plant material and extract

A total of 29.06 kg of Dayak onion bulbs were washed three times under running water and dried in a drying chamber at 40°C, yielding 9.6 kg of dried material. Organoleptic testing and drying loss analysis (recorded at 8.6%) were used to assess the quality of the material (Poerwosusanta, 2019; BPOM, 2022).

Extraction process

Eleutherine palmifolia bulbs were dried in an oven at 50°C, ground to a fine powder, and weighed. Maceration was performed with 96% ethanol for 72 h (three consecutive 24h periods). The mixture was filtered three times to separate the filtrate from the residue. The combined filtrates were concentrated using a rotary evaporator to obtain thick extracts. The extraction process involves soaking in a 1:2 ratio (1000 grams of the crude drug is extracted with 2000 mL of 96% ethanol). Cover the container tightly to prevent the solvent from evaporating. Store in a cool, dark place, away from direct sunlight. Allow to stand for 24 hours, stirring occasionally (gently shaking) to ensure the active compounds are evenly dissolved. After 24 hours, filter the mixture using filter paper. The extract solution is separated, leaving a solid residue (weighing 700 grams). The residue is then dried in an oven at 60°C until the moisture content is <10% (for 6-12 hours). The remaining dry solid residue is weighed to 630 grams, which is then allowed to cool to room temperature. The next step is to grind the dry residue into a fine powder using a blender and then filter the powder through a 40-60 mesh sieve. The mixed filtrate is then collected and evaporated using a rotary evaporator at 55-60°C with a vacuum pressure of 200 mbar and a rotation speed of 100 rpm to obtain a thick extract of 170 grams. The extract is stored in a closed vial at 4°C. A 96% ethanol extract solution of *Eleutherine palmifolia* bulbs is prepared by adding a 0.5% solution of sodium carboxymethylcellulose (CMC Na+), with a composition of 500 mg of CMC Na+ in 1000 mL of distilled water (Wahdaningsi *et al*., 2023).

Phytochemical testing

Phytochemical analysis included: Alkaloids and Saponins were tested using the gravimetric method. Flavonoids, Polyphenols, and Tannins were analyzed using the spectrophotometric method. This included the creation of a standard curve, sample processing, and determination of compound concentrations (Sirojudin *et al*., 2024).

Pregnancy induction

Estrus was induced by intraperitoneal injection of 5 IU of Pregnant Mare Serum Gonadotropin (PMSG) during the diestrus phase. 48 hours later, 5 IU of Human Chorionic Gonadotropin (hCG) was administered intraperitoneally. Each female mice was then paired with a male mouse at a ratio of 2 males: 5 females for 7 days. Pregnancy was confirmed by observing a minimum weight gain of 10% of the initial weight gain and abdominal palpation, which was performed on the 10th day after mating (10th gestational day). The first day of pregnancy was calculated based on the discovery of Pregnancy was confirmed 17 hours after mating by detecting the presence of a copulatory plug through a vaginal swab examination (Nooranizadeh *et al*., 2018; Tarin *et al*., 2002; Jackson Laboratory, 2006).

Preeclampsia model induction

The anti-mouse anti–Qa-2 monoclonal antibody, catalog no. 121711 from BioLegend, clone 695H1-9-9, RRID is AB_2650759. was used for the induction of preeclampsia in mice. The preeclampsia model was created using anti-Qa-2 antibodies injected intraperitoneally starting on the first day of pregnancy at a daily dose of 10 ng/day, administered at 8 a.m. for four days, for a total dose of 40 ng/kg body weight. Administration of anti-Qa-2 antibodies in pregnant mice induces a preeclampsia-like condition by disrupting key immune-regulatory mechanisms essential for a healthy pregnancy. Qa-2 is a non-classical MHC class Ib molecule in mice, functionally similar to human HLA-G, and plays a critical role in promoting immune tolerance at the maternal-fetal interface. When anti-Qa-2 antibodies are administered, they block the function of Qa-2, leading to a

breakdown of maternal immune tolerance toward the fetus. This immune disturbance impairs trophoblast invasion and spiral artery remodeling in the placenta, resulting in poor placental perfusion and hypoxia. In response to this hypoxic environment, the placenta releases anti-angiogenic factors such as soluble fms-like tyrosine kinase-1 (sFlt-1), which antagonize vascular endothelial growth factor (VEGF) and placental growth factor (PlGF). This imbalance leads to systemic endothelial dysfunction in the mother. As a consequence, the pregnant mice develop clinical features characteristic of preeclampsia, including elevated blood pressure, proteinuria, and intrauterine growth restriction (IUGR). This model effectively mimics several aspects of human preeclampsia and is used to study the disease's pathophysiology and potential therapeutic interventions (Sulistyowati *et al*., 2010).

The European Society of Hypertension (ESH) recommends that hypertension in pregnancy is defined when blood pressure reaches ≥140/90 mmHg, regardless of proteinuria status (Cífková, 2023; Rubio *et al*., 2024). In this study, blood pressure was measured in all groups at two time points: (1) before any intervention and (2) after induction of the preeclampsia model. Hypertension in pregnancy is defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or both (Yemane *et al*., 2021; ACOG, 2020; Ryan *et al*., 2025). In all treatment groups (90, 100, and 125 mg/kg BW), blood pressure measurements showed that each dam experienced an increase that reached the hypertensive threshold following anti–Qa-2 induction. These findings support the successful induction of a hemodynamic response consistent with a preeclampsia-like condition in the experimental animals.

Extract administration

Dayak onion bulb extract was administered orally from day 4 to day 18 of gestation for 14 consecutive days using standard handling techniques. The extract was administered orally. This route was chosen not only because it is standard, but also to minimize stress and discomfort to the mice, in accordance with animal welfare guidelines. Oral administration is less invasive than injection methods and also better reflects the typical human consumption route of similar compounds. The dosage selection was based on prior studies showing blood pressure-lowering effects in hypertensive mice after 14 days of treatment (Yuliandra *et al*., 2018; Sari, 2024).

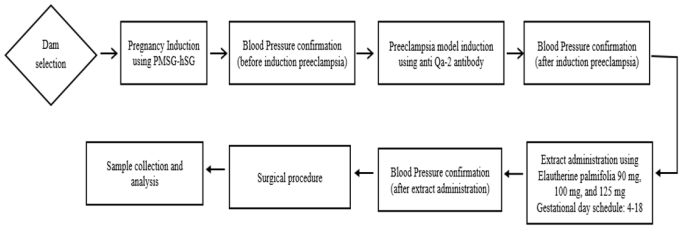
Surgical procedure and fetal evaluation

Anesthesia was induced by intraperitoneal injection of a ketamine (0.025 mL) and xylazine (0.0125 mL) mixture, adjusted to a total volume of 0.1 mL. After the mice showed loss of balance reflex, a laparotomy was performed to remove the uterus. Observations included the number of live and dead fetuses, the incidence of absorption, morphological defects, and measurements of fetal weight and length. The mother mice were cremated after surgery, and the fetuses, placentas, kidneys, and livers were preserved for further analysis (Flecknell, 2009; Norton *et al*., 2016).

Data collection, and analysis

Data on fetal number, fetal condition, body weight, body length, placental weight, and the presence of external morphological abnormalities were recorded for each fetus. Because only one pregnant dam was available per treatment group, statistical inference could not be appropriately conducted. Therefore, all data were analyzed descriptively. Results are presented as individual values and summary measures (range, mean, and standard deviation) to illustrate within-litter and between-group variation. No inferential statistical tests (e.g., *t*-test or ANOVA) were performed, as the limited number of dams precludes valid estimation of variance between biological replicates. Consequently, the findings should be interpreted as preliminary and exploratory, intended to provide descriptive insight into potential patterns or trends that warrant further investigation with adequate biological replication.

Flow diagram



RESULTS AND DISCUSSION

Changes in systolic/ diastolic blood pressure before and after preeclampsia induction in individual mice

The systolic and diastolic blood pressure measurements showed a consistent pattern across all treatment groups following anti–Qa-2 antibody induction. As illustrated in Figure 1, each animal in the treatment groups demonstrated a clear increase in systolic pressure after induction compared with its own baseline measurement. Although baseline systolic values were similar across groups, the post-induction measurements uniformly exceeded the threshold typically used to define hypertension in pregnant mice. This pattern indicates that the hemodynamic response expected from the preeclampsia model was successfully elicited in each dam. The diastolic pressure profiles (Figure 2) displayed the same within-animal elevation after induction. All treatment animals showed a shift from normotensive baseline values to diastolic levels consistent with hypertensive status following anti–Qa-2 exposure. The

normal control group, which did not undergo induction, showed no meaningful change between its pre- and post-measurement points. Because each bar represents a single dam, these findings are descriptive rather than inferential. Even so, the uniform direction of change across all induced animals suggests that the preeclampsia model produced a reproducible hypertensive effect within each individual, providing physiological confirmation that the model induction was successful before extract administration.

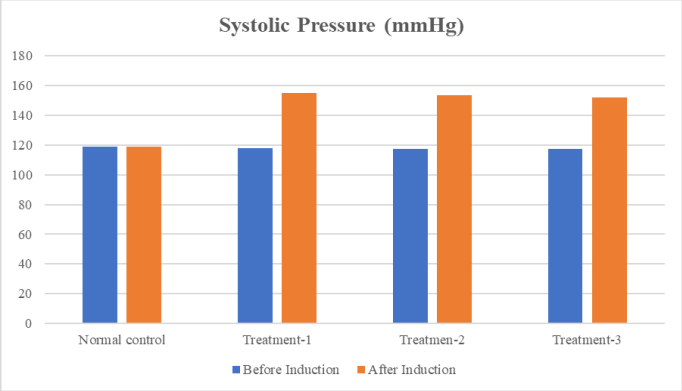


Figure 1: Changes in systolic blood pressure before and after preeclampsia induction in individual mice. Each bar represents a single animal (n = 1 per group). Systolic pressures were measured before and after anti-Qa2 antibody induction.

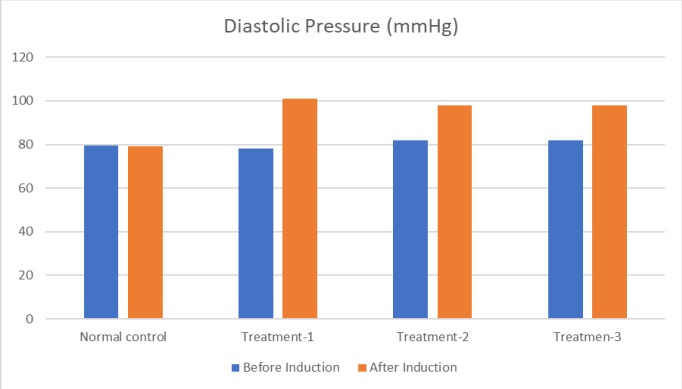


Figure 2: Changes in diastolic blood pressure before and after preeclampsia induction in individual mice. Each bar represents a single animal (n= 1 per group). Diastolic pressures were measured before and after anti-Qa2 antibody induction.

Fetal viability and morphological observation

Observations of fetal number and condition (Table 1) showed that all dams successfully carried their pregnancies to term, producing a total of 36 fetuses across all groups. All fetuses were alive at the time of necropsy, with no evidence of intrauterine death, resorption, or external malformation. Each fetus exhibited complete limb formation with a normal number of digits, symmetrical body shape, and smooth skin surface. There were no visible external defects such as cleft palate, limb deformities. These findings indicate that administration of *Eleutherine palmifolia* extract during gestation did not induce overt teratogenic effects in the preeclamptic mice model.

Fetal growth parameters

As summarized in Table 2, fetal body length, body weight, and placental weight varied slightly among groups. Fetuses from the control group showed normal growth patterns consistent with the expected range for gestational day 18 BALB/c mice. In the group treated with the lowest extract dose (90 mg/kg BW), fetuses tended to exhibit smaller body length and lower body weight compared to those in the control group. Interestingly, at higher doses (100 and 125 mg/kg BW), these parameters appeared to recover toward or slightly exceed control values, suggesting a non-linear or U-shaped dose–response tendency. Placental weights remained relatively consistent among all groups, with no gross abnormalities observed. The placentae appeared intact, rounded, and uniformly colored, without evidence of hemorrhage or necrosis. Minor fluctuations in placental weight were noted, but did not follow a clear dose-related pattern. Overall, oral administration of *Eleutherine palmifolia* extract from gestational day 4 to 18 did not produce any visible structural malformations or fetal deaths. While some variation in fetal growth measures was observed, particularly at the lowest dose, the differences were descriptive in nature and cannot be interpreted statistically due to the use of a single dam per group. These findings provide preliminary descriptive evidence that the extract is not grossly teratogenic but may influence fetal growth in a dose-dependent or non-monotonic manner. Further studies with adequate biological replication are necessary to confirm these observations and clarify the underlying mechanisms.

Table 1: Observation results for number of fetuses and fetal condition.

Group	Number of fetuses	Fetal condition						
		Alive	Dead	Resorption	Defect		Number of fingers	
					Yes	No	Complete	Incomplete
Control	8	8	0	0	0	8	8	0
Treatment 1	9	9	0	0	0	9	9	0
Treatment 2	9	9	0	0		9	9	
Treatment 3	10	10	0	0	0	10	10	0
Total	36	36	0	0	0	36	36	0

Table 2: Fetal body length, body weight, and placental weight between groups.

Group	n	Body length	Body weight	Placental weight
1 (Control)	8	2.48±0.10 2.50 2.30-2.60	1.21±0.05 1.22 1.12-1.26	0.18±0.01 0.18 0.17-0.19
2 (Dayak onion bulb extract 90 mg)	9	2.26±0.43 2.10 1.80-2.90	0.60±0.03 0.59 0.55-0.65	0.20±0.31 0.09 0.08-0.10
3 (Dayak onion bulb extract 100 mg)	9	2.56±0.11 2.60 2.40-2.70	1.24±0.07 1.20 1.13-1.34	0.19±0.01 0.19 0.17-0.20
4 (Dayak onion bulb extract 125 mg)	10	2.73±0.48 2.65 2.00-3.90	1.15±0.11 1.14 1.00-1.39	0.17±0.01 0.17 0.15-0.18

Mean±SD, Median, Min-Max.

This study investigated the effects of *Eleutherine palmifolia* (Dayak onion) bulb extract on fetal growth and placental development in a preeclampsia mice model induced by anti-Qa2 antibodies. The findings demonstrated that administration of the extract did not produce any visible teratogenic or lethal effects, as all fetuses were viable and morphologically normal. However, variations in fetal body length, weight, and placental weight were observed across treatment groups, indicating a possible dose-dependent pattern of physiological response. The lowest dose, both fetal and placental growth appeared reduced compared to control, suggesting an insufficient concentration of active phytochemicals to counteract the oxidative and inflammatory stress characteristic of preeclampsia. *Eleutherine palmifolia* is known to contain flavonoids, quinones, and saponins, compounds with documented antioxidant and vasoprotective properties (Ahmed *et al*., 2015; Wang *et al*., 2022; Prasetya, 2023). When administered at sub-therapeutic levels, these compounds may fail to restore endothelial function and placental perfusion, leading to restricted nutrient and oxygen supply to the fetus. This mechanism aligns with previous reports showing that inadequate antioxidant support under preeclamptic conditions contributes to fetal growth restriction (Rahajeng, 2024; Lathifah *et al*., 2024).

In contrast, the moderate dose (100 mg/kg BW) was associated with fetal and placental parameters comparable to the control group, suggesting restoration of near-normal intrauterine growth. This finding supports the hypothesis that, at optimal concentrations, *Eleutherine palmifolia* extract may exert protective effects through modulation of oxidative stress and inflammation. Flavonoids in particular can enhance nitric oxide bioavailability, suppress proinflammatory cytokines, and improve endothelial integrity (Calabrese, 2024), thereby promoting better uteroplacental circulation and nutrient transfer. Interestingly, at the highest dose (125 mg/kg BW), a pattern emerged where fetal body length tended to increase despite relatively smaller placental mass. This could reflect a compensatory mechanism in which improved placental efficiency supports fetal linear growth even with a smaller placental size. Such a biphasic response is characteristic of hormesis, a well-recognized biological phenomenon where low doses are ineffective or inhibitory, whereas higher doses elicit protective effects (Calabrese, 2024). The observed non-linear, possibly U-shaped dose-response relationship underscores the importance of dose optimization when using plant-derived compounds. Saponins, another major component of *E. palmifolia*, may also contribute to these effects. These triterpenoid or steroid glycosides can modulate cell membrane permeability and hormonal signaling, influencing placental nutrient transport (Sharma, 2023). Although no structural abnormalities were observed in any group, it remains plausible that suboptimal saponin exposure could transiently alter placental physiology or vascular resistance, affecting fetal growth (Farida *et al*., 2022). Despite these promising trends, several limitations must be acknowledged. The most critical is the small sample size and absence of biological replication, as only one dam was assigned per group. Consequently, statistical inference is not valid, and the data should be interpreted strictly as descriptive. The analysis at the individual fetal level rather than the litter level introduces pseudoreplication, which may overstate the precision of the findings. Furthermore, biological variability among dams, such as differences in gestational response, metabolism, or immune reactivity could have influenced outcomes. Additionally, molecular and biochemical markers of oxidative stress or vascular function were not assessed, limiting mechanistic interpretation. The results provide preliminary evidence that *E. palmifolia* extract is morphologically safe in this model and may have dose-dependent effects on fetal growth and placental function. This aligns with findings from other phytochemicals such as quercetin and punicalagin, which display similar dose-sensitive effects protective at optimal levels but potentially ineffective or deleterious outside that range (Li *et al*., 2020; Ding *et al*., 2024). These findings highlight the potential of *Eleutherine palmifolia* as a source of bioactive compounds with therapeutic relevance for preeclampsia, while emphasizing the need for careful dose determination. Future research should incorporate larger sample sizes, proper biological replication, and molecular analyses such as measurement of sFlt-1, VEGF, or oxidative stress markers to clarify mechanisms and establish the pharmacodynamic profile of this extract. Understanding these aspects will be essential for ensuring both efficacy and safety, particularly in pregnancy-associated conditions where fetal development is highly sensitive to maternal interventions

CONCLUSION

Eleutherine palmifolia (Dayak onion) extract did not cause fetal malformations, indicating no teratogenic effects within the tested range. The extract affected fetal growth in a dose-dependent manner. Lower doses tended to inhibit development, while moderate doses supported normal growth. Future studies should include larger sample sizes and multiple pregnant dams per group to ensure valid statistical analysis. Further investigations are also needed to assess molecular markers of oxidative stress and placental function, as well as histopathological evaluations, to better understand the mechanisms and safety profile of *E. palmifolia* during pregnancy.

ACKNOWLEDGEMENT

The authors would like to express their sincere gratitude to the Testing Service Unit Laboratory of the Faculty of Pharmacy, the Embryology Laboratory of the Faculty of Veterinary Medicine, Airlangga University, and the Integrated Laboratory of the Faculty of Medicine, Nahdlatul Ulama University for providing the facilities used in this research. Their support is deeply appreciated.

NOVELTY STATEMENT

This study is the first to assess the teratogenic effects and fetal safety of *Eleutherine palmifolia* extract in preeclamptic pregnancy model.

AUTHOR'S CONTRIBUTION

WA and I designed the study, prepared the experimental animals, collected samples, and wrote the manuscript. BP prepared the reagents and supervised the study.

Funding

This research was supported by a scholarship from the Ministry of Health of the Republic of Indonesia.

Data availability

All data supporting the findings of this study are included within the manuscript.

Ethical approval

Ethical Approval for the use of experimental animals was granted by the Research Ethics Committee of the Faculty of Veterinary Medicine Airlangga University (Animal Care and Use Committee), under approval number: 2.KEH.127.09.2024

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflicts of interest

The authors have declared no conflict of interest.

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