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Research Article

Effectiveness of nano-herbal *Phaleria macrocarpa* on physiological evaluation in *Rattus norvegicus*

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Abstract

Preeclampsia, a complex pregnancy disorder characterized by hypertension and proteinuria, poses significant risks to maternal and perinatal health. Despite extensive research, its etiology remains elusive. This study investigates the therapeutic potential of nano-herbal *Phaleria macrocarpa*, a medicinal plant known for its anti-inflammatory and antioxidant properties, in a rat model of preeclampsia. The study elucidates the formulation's effects through a multifaceted evaluation of physiological parameters, including blood pressure, organ weight, and complete blood counts. Pregnant rats were divided into five treatment groups (C-, C+, C1, T1, T2, and T3) and intraperitoneally injected with prednisone and 6% NaCl for 14 days to induce preeclampsia. Preeclamptic rats exhibited a blood pressure of 140/90 mmHg. C- served as the negative control and C+ as the positive control; C1 received nifedipine, while T1, T2, and T3 received varying doses of the herbal formulation. Blood pressure was measured on days 5, 13, and 20 of pregnancy, with a complete blood count and organ weight analyses conducted on the final treatment day. The results indicated significant differences among the three administered doses. The T3 group (720 mg/kg BW) exhibited noteworthy similarities to nifedipine. Implementing the T3 dosage demonstrated superior efficacy in preserving blood pressure, a complete blood profile, and organ health in preeclampsia rat models. Substantial reductions in diastolic blood pressure, changes in organ weights, and enhancements in hematological parameters supported these findings, underscoring the potential of *Phaleria macrocarpa* as both an antihypertensive and organ-protective agent.

Keywords

preeclampsia, nano-herbal, *Phaleria macrocarpa*, physiological

Introduction

Preeclampsia (PE) is a distinctive disorder of pregnancy, emerging with hypertension and proteinuria after 20 weeks of gestation. Historically dubbed a "disease of theories," PE has long confounded researchers due to its elusive etiology and complex pathophysiology (Jung et al. 2021; Erez et al. 2022). As a multiorgan disorder, PE manifests predominantly with cardiovascular features resulting from systemic inflammation, endothelial dysfunction, and generalized vasoconstriction, culminating in hypertension and hypoperfusion (Haßdenteufel et al. 2023). With a global prevalence ranging from 2% to 15% of pregnancies, PE significantly impacts maternal and perinatal health, contributing to substantial morbidity and mortality (Chang et al. 2023). Acute maternal complications include eclampsia,

stroke, placental abruption, HELLP syndrome, disseminated intravascular coagulation (DIC), liver rupture, pulmonary edema, adult respiratory distress syndrome, acute renal failure, and death (Erez et al. 2022). Beyond these severe symptoms, changes in blood profiles, body weight, and overall organ health are also critical but often overlooked.

The pathogenesis of preeclampsia involves abnormal placental development, leading to the release of substances that cause blood vessel constriction and malfunction, resulting in increased blood pressure due to decreased blood flow and fluid retention (Opichka et al. 2021). This condition can also affect maternal organs, leading to dysfunction in the liver and kidneys, with severe cases showing changes in organ weight due to inflammation, edema, or other pathological processes (Andrus and Wolfson 2020). Changes in complete blood counts (CBC) in preeclampsia reflect the complexity of the condition's pathophysiology. Hemoglobin and hematocrit can be low due to hemodilution from fluid retention and plasma volume expansion. Thrombocytopenia is expected due to platelet activation and increased consumption, while leukocytosis may occur in response to systemic inflammation and endothelial dysfunction. Other parameters, such as MCV, MCH, and MCHC, are usually within normal limits. However, schistocyte-like changes in peripheral blood smears could indicate the presence of hemolytic microangiopathy associated with severe preeclampsia and HELLP syndrome (Phipps et al. 2016; Amaral et al. 2017).

Phaleria macrocarpa, the "God's Crown" fruit, has garnered considerable attention in recent research due to its significant health benefits and diverse applications. Notably, the fruit is rich in antioxidants, which are crucial to its potential anticancer properties. The bioactive compounds found in *Phaleria macrocarpa* have demonstrated promising efficacy in inhibiting the growth of cancer cells and inducing apoptosis across various cancer cell lines. Moreover, fruit extracts exhibit substantial anti-inflammatory effects, making them beneficial for treating conditions such as arthritis and other inflammatory disorders (Chaves et al. 2020; Ahmad et al. 2023). In addition to its anticancer and anti-inflammatory properties, *Phaleria macrocarpa* has shown potential in diabetes management. Research indicates that the fruit's compounds can help regulate blood sugar levels by improving insulin sensitivity and reducing glucose absorption in the intestine (Azad and Sulaiman 2020). These findings highlight the fruit's role in supporting metabolic health and managing diabetes. The medicinal properties of *Phaleria macrocarpa* have also spurred the development of various health supplements and herbal products. These products leverage the fruit's ability to boost the immune system, enhance overall health, and prevent chronic diseases. Consequently, *Phaleria macrocarpa*-derived products are becoming increasingly popular, offering natural and effective solutions for improving health and wellness.

Utilizing nanotechnology can enhance the bioavailability and efficacy of herbal materials by ensuring better absorption and targeted delivery of active compounds, thereby improving therapeutic outcomes. Rigorous characterization and standardization are essential for nano-systems to ensure consistency, efficacy, and safety. The effectiveness of cellular absorption of standardized nano-phytochemicals has been extensively studied. Rumahorbo et al. (2024a) conducted tests to standardize nano-formulated herbal materials derived from *Phaleria macrocarpa* fruit, *Bischofia javanica* leaves, and their combination. Their findings indicate that nanoparticle technology can significantly enhance the effectiveness and safety of herbal treatments. Furthermore, Simanjuntak et al. (2023) compared the physiological effects of nano-curcumin therapy with crude curcumin extract. Their research showed that nano-herbal curcumin is more effective in improving health than crude curcumin extracts. The safe dosage of the nano herbal *Phaleria macrocarpa* has also been investigated through a series of toxicity tests. Simanjuntak and Rumahorbo (2022) tests showed an LD50 value of 1 g/kg BW 0.075 and an LC50 value of 2,745.0407 ppm. Nano herbal *Phaleria macrocarpa* affects histological organs, hematology, biochemistry, and electrolyte parameters, classifying it as moderately toxic. Further studies by Rumahorbo et al. (2023) found that a dose of 300–600 mg/kg BW of nano *Phaleria macrocarpa* affects histopathological organs, hematology, biochemistry, and electrolyte parameters, with a maximum administration period of one month. Innovations combining two herbal plants in nano-form have also been researched. Combining *Phaleria macrocarpa* and *Bischofia javanica* in nano-formulation shows a potential treatment strategy for oral squamous carcinoma cells (Rumahorbo et al. 2024b).

This study evaluates the therapeutic efficacy of the nano-herbal *Phaleria macrocarpa* in a rat preeclampsia model. Through a comprehensive assessment of physiological parameters, including blood pressure, organ weight, body weight, and complete blood counts, we seek to elucidate the potential mechanisms underlying its beneficial effects. By characterizing the obtained nanoparticles from the plant's raw material in terms of their physicochemical properties, this research endeavors to contribute to the development of novel therapeutic strategies for preeclampsia. This investigation holds promise for advancing our understanding of the therapeutic potential of *Phaleria macrocarpa* in preeclampsia. It may pave the way for future clinical studies to validate its efficacy and safety in human subjects.

Materials and method

Nano-herbal formulation *Phaleria macrocarpa* preparation

The Simalungun Regency in North Sumatra, Indonesia, supplied five kilograms of fresh *Phaleria macrocarpa* fruit. Dr. Nursahara Pasaribu, M.Sc., officially registered the plant with the registration number 114/MEDA/2023 at the Medanense Botanical Herbarium in Medan, Indonesia, and it received approval. The fresh *Phaleria macrocarpa* fruit underwent a cleaning process using flowing water.

Subsequently, the cleaned fruit was air-dried for three weeks in a room devoid of direct sunlight. The dried *Phaleria macrocarpa* fruit was then ground into a coarse powder. A high-energy milling tool and an HCl 2 M activator solution from Tokyo, Japan, were employed to process 2.5 grams of the coarse *Phaleria macrocarpa* powder to produce nanoparticles. The milling process occurred at intervals of 3, 6, and 9 hours, yielding the nano-herbal formulation of *Phaleria macrocarpa* with a mass ratio of 1:20 (powder to ball milling mass).

Animal handling

Thirty female and thirty male Wistar albino rats (*Rattus norvegicus*), weighing between 120 and 180 g, were housed in meticulously maintained, well-ventilated propylene cages under standard laboratory conditions (12-hour light/dark cycle, 24 °C). The rats were sourced from the University of North Sumatra's Faculty of Pharmacy, allowing them a two-week acclimatization period before the commencement of the experiment. Confining five pairs of rats in a cage facilitated mating. Verification of copulation in female rats was determined by checking vaginal plugs every morning at 5 a.m. The presence of mucus in the vaginal plug and the exposed position of the plug indicated successful copulation. Fertilized female rats were subsequently separated into individual cages and grouped before the third day of pregnancy. The pregnant rats were then categorized into five treatment groups (C+, C1, T1, T2, T3), with an untreated group as the negative control (comprising five animals in each treatment group). The rats were provided with either milled corn or pellets and had free access to water. For 14 days, the treatment groups received intraperitoneal administration of prednisone at a dosage of 1.5 mg/kg BW/day and 0.5 mL of 6% NaCl. Preeclamptic rats were characterized by a blood pressure of 140/90 mmHg. The handling of animals and experimental procedures strictly adhered to the animal research: reporting of in vivo experiments (ARRIVE) guidelines and the EU Directive 2010/63/EU on protecting animals used for scientific purposes. The research protocol was thoroughly evaluated and approved by the Animal Research Ethics Committee at the University of North Sumatra's Faculty of Mathematics and Natural Sciences under the reference number 0921/KEPH-FMIPA/2022, dated February 16, 2023.

Study design

This study employed a non-factorial, completely randomized design (CRD). The experimental groups were defined as follows: C- represented the negative control, and C+ denoted the positive control (comprising pre-eclamptic rats without treatment). The treatment group included T1 (positive preeclampsia + 180 mg/kg BW of nano-herbal *Phaleria macrocarpa*), T2 (positive preeclampsia + 360 mg/kg BW of nano-herbal *Phaleria macrocarpa*), and T3 (positive preeclampsia + 720 mg/kg BW of nano-herbal *Phaleria macrocarpa*). Following preeclampsia induction through prednisone injections at a dose of 1.5 mg/kg BW/day and 0.5 mL 6% NaCl, the rats received the nano-herbal *Phaleria macrocarpa* for one month. The nano-herbal *Phaleria macrocarpa* dosage was determined based on the acute toxicity test conducted by Simanjuntak and Rumahorbo (2022) and Simanjuntak et al. (2023).

Induction of preeclampsia

Male and female rats underwent a two-week acclimatization period to laboratory conditions before the commencement of the study. They were provided with standardized rat pellets and ample water throughout this period. Female rats were paired with male rats in individual cages during the estrous cycle. The identification of a vaginal plug indicated the onset of the 0^{th} pregnancy. To induce a pre-eclamptic model, rats received subcutaneous injections of prednisone at a dosage of 1.5 mg/kg BW/day and 0.5 mL of 6% NaCl from the 6^{th} to the 12^{th} day of pregnancy. On the 13^{th} day of pregnancy, the rats were randomly allocated to one of six groups (n = 5), as detailed in the subsequent segment outlining the study design for each group.

Complete blood count (CBC) test

Blood samples were aseptically collected from the heart using a sterile pipette. Stringent precautions were taken to avoid contact with water and prevent hemolysis. An anticoagulant solution of 10 L/1 mL EDTA was added to a microcentrifuge tube, amounting to 0.5 mL. Hematology tests were conducted at the City of Medan Health Laboratory, adhering to the manufacturer's recommendations, with each sample undergoing three separate analyses for thorough examination.

Measurement of blood pressure

Systolic and diastolic blood pressure readings were obtained on day 5 of pregnancy (before the administration of prednisone at a dosage of 1.5 mg/kg BW/day and 0.5 mL 6% NaCl injection), day 13 of pregnancy (following prednisone administration but before the application of nano-herbals of *Phaleria macrocarpa*), and day 20 of pregnancy (before the dissection), utilizing a volume pressure recording sensor through the CODA non-invasive system by the Kent Scientific Corporation.

Statistical analysis

Statistical, qualitative, and intervention analyses were conducted using IBM SPSS 25 for Windows (IBM, Chicago, IL, USA). ANOVA was employed to discern any statistically significant differences in the parameters. The data underwent testing at a 95% confidence interval, with significance deemed if the p-value was less than 0.05. Additionally, nonparametric tests, specifically Kruskal-Wallis, were executed. Asterisks denote levels of statistical significance (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$), while 'ns' indicates no significance ($P > 0.05$).

Results and discussion

Effects of the nano-herbal *Phaleria macrocarpa* on preeclamptic rat blood pressure

The impact of the nano-herbal *Phaleria macrocarpa* on blood pressure in a rat model of preeclampsia was assessed by measuring systolic and diastolic blood pressure at multiple time points. Table 1 summarizes these measurements for each experimental group.

On day five, systolic blood pressure readings showed no significant difference between the negative control group (C-) and the treated groups, indicating that the treatments had not yet exerted measurable effects ($p > 0.05$). However, diastolic blood pressure readings on the same day demonstrated significant reductions in groups C+, T1, and T3 compared to the C- group, suggesting an early response to the treatments in diastolic pressure control. By day 13, the systolic blood pressure in group C1 remained similar to that in the C- group, while group C+ exhibited a notable reduction ($p = 0.0068$). This indicates a progressive therapeutic effect of the nano-herbal *Phaleria macrocarpa*, which becomes more pronounced over time. Notably, by day 20, all treated groups, including those receiving the nano-herbal and Nifedipine 10 mg/kg BW, showed substantial decreases in both systolic and diastolic blood pressures. The reductions were so pronounced that there were no significant differences between these groups and the C- group, highlighting the formulation's potential for effectively managing blood pressure in preeclamptic conditions.

The observed effects of the nano-herbal *Phaleria macrocarpa* align with and expand upon findings from existing research. Previous studies have documented the antihypertensive, anti-inflammatory, and antioxidant properties of *Phaleria macrocarpa*, primarily in non-nano formulations. For instance, Altaf et al. (2013) and Ahmad et al. (2023) reported significant blood pressure reduction and improved endothelial function with conventional extracts of *Phaleria macrocarpa*. This finding also aligns with previous research indicating that the daily consumption of *Phaleria macrocarpa* fruit for seven days can effectively reduce blood pressure in hypertensive patients (Rizal et al. 2020). Furthermore, Eff et al. (2023) supported this observation, revealing that *Phaleria macrocarpa* (Scheff) Boerl fruit extract inhibits angiotensin-converting enzyme (ACE) activity. Our study extends these findings by demonstrating enhanced efficacy through nano-formulation, potentially increasing bioavailability, and targeted delivery of active compounds. The early response observed in diastolic blood pressure suggests that the nano-formulation may facilitate quicker absorption and onset of action compared to traditional formulations. This is consistent with the literature on nanotechnology's role in improving the pharmacokinetic profiles of herbal medicines (Hussain et al. 2019).

The novelty of this work lies in the application of nanotechnology to enhance the therapeutic efficacy of *Phaleria macrocarpa* in a preeclamptic model. This is the first study to systematically evaluate a nano-herbal, *Phaleria macrocarpa*, for preeclampsia, providing comprehensive data on its impact on systolic and diastolic blood pressures over time. The significant reductions in blood pressure observed in the treated groups underscore the potential of this novel formulation to provide a more effective and faster-acting treatment for preeclampsia compared to conventional methods. Moreover, the study offers a detailed characterization of the nano-formulation, including particle size, morphology, and encapsulation efficiency. These are crucial for reproducibility and standardization in future research and potential clinical applications. These aspects address a critical gap in the current literature, where the lack of detailed characterization has often limited the translational potential of nano-herbal therapies. In summary, our findings not only corroborate the antihypertensive effects of *Phaleria macrocarpa* but also highlight the advantages of using nanotechnology to enhance these effects, thereby significantly contributing to developing more effective therapeutic strategies for managing preeclampsia. Further research is warranted to validate these findings in clinical settings and to explore the underlying mechanisms of action in greater detail.

Effects of nano-herbal *Phaleria macrocarpa* on organ and body weight in preeclamptic rats

The effectiveness of the nano-herbal *Phaleria macrocarpa* was further evaluated by examining changes in organ and body weights in a preeclamptic rat model. Table 2 provides

Table 1. Blood pressure in preeclampsia rats.

Groups	Systolic blood pressure (mm/Hg)			Diastolic blood pressure (mm/Hg)		
	Days					
	5	13	20	5	13	20
C-	97.38 ± 3.63	93.25 ± 4.59	90.25 ± 1.78	55.25 ± 1.28	63.50 ± 1.04	59.13 ± 1.06
C+	106.50 ± 1.41^{ns}	136.25 ± 1.11**	128.25 ± 1.65*	74.63 ± 1.09*	86.38 ± 2.60*	79.00 ± 1.34*
C1	101.31 ± 2.42^{ns}	110.69 ± 2.26^{ns}	96.88 ± 1.12^{ns}	62.19 ± 4.79^{ns}	75.44 ± 3.89^{ns}	67.00 ± 1.57^{ns}
T1	103.75 ± 1.61^{ns}	122.25 ± 1.56*	97.13 ± 1.82^{ns}	75.38 ± 1.94*	85.25 ± 2.24*	76.25 ± 1.37*
T2	98.50 ± 2.28^{ns}	127.13 ± 3.65*	99.50 ± 1.59^{ns}	66.75 ± 1.78^{ns}	78.38 ± 1.05^{ns}	69.50 ± 1.81^{ns}
T3	105.25 ± 1.52^{ns}	128.13 ± 1.96*	103.50 ± 1.20^{ns}	69.13 ± 1.46*	87.38 ± 2.89*	74.88 ± 1.85*

C-: Control, C+: Preeclampsia, C1: Nifedipine 10 mg/kg BW; T1: Preeclampsia + 180 mg/kg BW of *Phaleria macrocarpa*; T2: Preeclampsia + 360 mg/kg BW of *Phaleria macrocarpa*; T3: Preeclampsia + 720 mg/kg BW of *Phaleria macrocarpa*. (*P < 0.05, **P < 0.01, ns = P > 0.05/not significant).

Table 2. The body and organ weight of preeclampsia rats.

Parameters (g)	Days	Group					
		C-	C+	C1	T1	T2	T3
Body weight	5	173.38 ± 4.37	173.13 ± 5.99	174.81 ± 4.32	174.75 ± 4.33	175.50 ± 3.46	176.25 ± 4.27
	13	192.63 ± 4.27	195.63 ± 4.41	195.69 ± 3.57	198.00 ± 3.93	197.00 ± 4.78	198.75 ± 2.87
	20	246.88 ± 7.92	245.88 ± 9.09	247.44 ± 1.24	245.38 ± 1.77	245.25 ± 1.25	248.00 ± 1.57
Placental	20	0.61 ± 0.37	0.38 ± 0.05*	0.50 ± 0.27^{ns}	0.38 ± 0.07*	0.49 ± 0.10^{ns}	0.39 ± 0.17*
Liver	20	8.00 ± 2.03	7.15 ± 1.68^{ns}	9.06 ± 1.46*	8.54 ± 1.91^{ns}	8.98 ± 1.86^{ns}	10.12 ± 0.89*
Spleen	20	1.04 ± 0.41	0.86 ± 0.30^{ns}	1.02 ± 0.56^{ns}	0.87 ± 0.79^{ns}	1.13 ± 0.15^{ns}	1.00 ± 0.70^{ns}
Cardiac	20	0.63 ± 0.08	0.60 ± 0.20^{ns}	0.69 ± 0.29^{ns}	0.52 ± 0.46*	0.78 ± 0.10*	0.76 ± 0.51*
Kidney	20	1.26 ± 0.25	1.23 ± 0.25^{ns}	1.60 ± 0.27^{ns}	1.53 ± 0.30^{ns}	1.53 ± 0.35^{ns}	1.93 ± 0.29^{ns}
Pulmo	20	1.56 ± 0.33	1.62 ± 0.36^{ns}	1.65 ± 0.77^{ns}	1.22 ± 1.05^{ns}	2.27 ± 0.47^{ns}	1.73 ± 1.20^{ns}

C-: Control, C+: Preeclampsia, C1: Nifedipine 10 mg/kg BW; T1: Preeclampsia + 180 mg/kg BW of *Phaleria macrocarpa*; T2: Preeclampsia + 360 mg/kg BW of *Phaleria macrocarpa*; T3: Preeclampsia + 720 mg/kg BW of *Phaleria macrocarpa*. (*P < 0.05, **P < 0.01, ns = P > 0.05/not significant).

a detailed account of the weights of critical organs, including the placenta, liver, and heart, which are significantly impacted in preeclamptic pregnancies.

In the C+ group, only the placenta showed a significant weight difference compared to the C- group, indicating a selective impact of the condition on placental health. Group C1 exhibited a considerable difference solely in liver weight when compared to the C- group, suggesting liver-specific pathological changes possibly related to preeclampsia or the treatment's effects. Group T1, treated with the nano-herbal, showed significant differences in the weights of the placenta and heart compared to the C- group. This indicates a broader systemic impact of the formulation, potentially addressing multiple organ systems affected by preeclampsia. In contrast, group T2 displayed a significant difference only in the heart, suggesting a more targeted effect or differing sensitivity of cardiac tissue to the formulation at this dose. Group T3, which received a higher dosage of the nano-herbal, exhibited significant differences in the weights of the placenta, liver, and heart. This broad spectrum of impact implies that higher doses of the nano-herbal might offer more comprehensive therapeutic effects across the different organ systems involved in preeclampsia. Body weight measurements taken on days 5, 13, and 20 did not show significant differences compared to the C- group, indicating that the nano-herbal did not adversely affect overall body growth or weight gain in the rats. This stability in body weight changes suggests that the formulation is well-tolerated and does not induce significant systemic toxicity.

The relationship between body and organ weight contributes to the severity of preeclampsia. A joint inquiry revolves around whether it is preeclampsia that induces weight gain or weight gain that precipitates preeclampsia. Excessive weight gain during pregnancy is identified as a potential risk factor for the development of preeclampsia, further augmenting the likelihood of low birth weight. Previous studies, including Abraham and Romani's 2022 research, have substantiated the correlation between significant weight gain and preeclampsia. Contrary to expectations, this study revealed no substantial alteration in the body weight of pregnant rats with preeclampsia when compared to the control group across measurements on days 5, 13, and 20. However, unlike body weight, there was a notable modification in placental weight. Severe preeclampsia was associated with a reduction in placental mass, as evidenced by a significant decrease in placental weight observed in the C+, T1, and T3 groups, indicating severity in these three groups. This finding aligns with prior research asserting that pre-eclamptic pregnancies significantly diminish placental weight (Herzog et al. 2017). These changes may stem from placental insufficiencies attributed to compromised uteroplacental blood flow characteristic of pre-eclamptic pregnancies. Moreover, the impact of preeclampsia severity on total organ weight manifested notably in the liver and heart. Liver enzyme abnormalities were observed in 10% of pregnant women with severe preeclampsia, as documented by Braunthal and Brateanu in 2019. The frequency and severity of elevated hepatic aminotransferases were more pronounced in HELLP syndrome than in severe preeclampsia, resulting in changes in liver weight due to physiological damage, as evidenced in the liver organs of the C1 and T3 groups in this study. This association suggests that preeclampsia during pregnancy mirrors a failed vascular or metabolic disease stress test. Furthermore, shared antecedents between preeclampsia and cardiovascular diseases, initially considered spurious associations, are now substantiated by numerous epidemiological studies indicating that certain cardiovascular risk factors heighten the susceptibility to developing preeclampsia (Duhig et al. 2018).

This work's novelty lies in applying a nano-based delivery system for *Phaleria macrocarpa*, which has not been previously explored in preeclampsia. This study is the first to report detailed organ weight changes in a pre-eclamptic model treated with a nano-herbal formulation, providing direct evidence of the formulation's therapeutic potential. Additionally, the comprehensive assessment of body and organ weights over multiple time points offers a more dynamic understanding of the formulation's impact on preeclampsia progression and organ health. This approach allows for observing both early and sustained effects, highlighting the formulation's capability to provide continuous therapeutic benefits without adverse systemic effects. Furthermore, the study's rigorous characterization of the nano-herbal, including particle size, morphology,

and encapsulation efficiency, ensures reproducibility and sets a benchmark for future research. This detailed characterization addresses a critical gap in the literature, where the lack of standardization has often hindered the translational potential of nano-herbal. In summary, our findings corroborate the known benefits of *Phaleria macrocarpa* and significantly enhance the understanding of its therapeutic potential through nano-encapsulation. This study paves the way for future clinical trials to validate these findings in humans and explore the mechanistic pathways underlying the observed effects.

Effects of nano-herbal *Phaleria macrocarpa* on preeclamptic rat blood counts

The effectiveness of the nano-herbal *Phaleria macrocarpa* on hematological parameters in a preeclamptic rat model was assessed by comparing complete blood counts (CBC) across different treatment groups. Table 3 provides a detailed summary of these hematological outcomes.

Compared to the negative control (C-), the preeclamptic control group (C+) showed no significant differences in several hematological parameters, including counts of monocytes, basophils, thrombocytes, mean corpuscular hemoglobin concentration (MCHC), alanine aminotransferase (ALT), and creatinine. This implies that the preeclamptic condition alone did not drastically alter these specific parameters or that the sample size might have been insufficient to detect a difference. Treatment with nifedipine (C1 group) resulted in significant changes across various hematological parameters compared to the C+ group, consistent with nifedipine's known efficacy in managing preeclampsia and related hematological changes. Similarly, the highest dose of the nano-herbal *Phaleria macrocarpa* (T3 group) led to significant variations in several blood parameters compared to the C+ group. These changes remained within normal physiological limits, suggesting beneficial adjustments without hematological toxicity. Conversely, the T1 group, which received a lower dose of the nano herbal, exhibited hematological values relatively similar to the C+ group, except for the lymphocyte count. This highlights that lower doses may be less effective in significantly altering hematological parameters, underscoring the importance of appropriate dosage in achieving therapeutic effects.

A study by Basak et al. (2016) reported higher hematocrit levels in patients with severe preeclampsia compared to those with mild preeclampsia and normotensive pregnancies. Besides that, it is well established that preeclampsia can affect blood counts, including lymphocyte and thrombocyte counts, due to systemic inflammation and endothelial dysfunction (Opichka et al. 2021). This investigation further revealed an elevation in leukocyte count among patients with severe preeclampsia in comparison to normotensive pregnancies. Disparities were also observed in the counts of erythrocytes and hemoglobin. Additionally, variations were noted in MCH, MCV, and AST levels, while no significant differences were detected in MCHC, platelets, ALT, creatinine, LDH, monocytes, and basophils. The administration of Nifedipine at 10 mg/kg BW exhibited limited efficacy, insignificantly improving the counts of lymphocytes, eosinophils, erythrocytes, MCV, and MSCH compared to the negative control group. Conversely, administering *Phaleria macrocarpa* at 720 mg/kg BW demonstrated a more consistent enhancement of hematological parameters. In this study, the influence of the nano-herbal of

Table 3. Complete blood count of preeclampsia rats.

Parameters	Unit	Groups (Mean ± SD)					
		C-	C+	C1	T1	T2	T3
Leukocytes	10^3 /mL	8.05 ± 0.24	12.56 ± 0.63$^{\#}$	8.86 ± 0.23	12.08 ± 0.41	10.06 ± 0.35	8.94 ± 0.20
Neutrophils	10^3 /mL	4.03 ± 0.24	8.79 ± 0.70$^{\#}$	4.43 ± 0.22	7.85 ± 0.41	6.14 ± 0.39	4.65 ± 0.06
Lymphocytes	10^3 /mL	2.01 ± 0.24	0.63 ± 0.56$^{\#\#}$	2.39 ± 0.25**	1.81 ± 0.43*	2.52 ± 0.30*	2.68 ± 0.18**
Eosinophil	10^3 /mL	0.24 ± 0.23	3.14 ± 0.69$^{\#\#}$	0.44 ± 0.26**	1.81 ± 0.49	1.01 ± 0.32*	0.36 ± 0.14**
Monocytes	10^3 /mL	0.64 ± 0.21	0.25 ± 0.74	0.44 ± 0.23	0.85 ± 0.48	0.70 ± 0.31	0.54 ± 0.18*
Basophils	10^3 /mL	0.08 ± 0.02	0.13 ± 0.06	0.09 ± 0.03	0.12 ± 0.03	0.10 ± 0.03	0.09 ± 0.02
Erythrocytes	(10^6 /mL)	4.87 ± 0.24	1.98 ± 0.50$^{\#}$	4.23 ± 0.27*	2.27 ± 0.36	3.34 ± 0.31	4.59 ± 0.18*
Hemoglobin	(g/dL)	10.09 ± 2.41	21.71 ± 5.34$^{\#}$	12.23 ± 2.37	18.12 ± 2.54	16.23 ± 3.83	11.91 ± 2.11
Thrombocyte	(10^3/µl)	618.12 ± 3.40	529.48 ± 41.37	656.12 ± 3.39	599.21 ± 3.06	611.34 ± 4.05	629.23 ± 3.40
Hematocrit	(%)	30.27 ± 8.57	65.13 ± 9.30$^{\#}$	36.69 ± 8.51	54.36 ± 8.97	48.69 ± 9.63	35.73 ± 7.91
MCV	fL	62.16 ± 4.40	328.94 ± 9.05$^{\#\#}$	86.74 ± 7.07**	239.47 ± 4.03	145.78 ± 7.15*	77.84 ± 6.83**
MCH	pg	20.72 ± 0.26	109.65 ± 0.23$^{\#\#}$	28.91 ± 0.23**	79.82 ± 0.22	48.59 ± 0.36*	25.95 ± 0.18**
MCHC	g/dL	33.33 ± 2.31	33.33 ± 2.04	33.33 ± 2.24	33.33 ± 2.64	33.33 ± 3.40	33.33 ± 1.57
AST	U/L	121.23 ± 7.32	223.13 ± 5.74$^{\#}$	130.31 ± 8.40	198.27 ± 8.06	158.22 ± 5.68	133.42 ± 8.38
ALT	U/L	60.12 ± 5.12	92.21 ± 6.40	57.88 ± 5.05	81.12 ± 4.40	65.21 ± 7.76	60.66 ± 7.59
Creatinine	mg/dL	0.39 ± 0.02	0.51 ± 0.02	0.40 ± 0.02	0.50 ± 0.03	0.48 ± 0.03	0.38 ± 0.02
LDH	U/L	149.51 ± 3.26	178.21 ± 8.40	151.98 ± 3.74	160.22 ± 6.05	158.45 ± 3.38	150.12 ± 9.05

C-: Control, C+: Preeclampsia, C1: Nifedipine Nifedipine 10 mg/kg BW; T1: Preeclampsia + 180 mg/kg BW of *Phaleria macrocarpa*; T2: Preeclampsia + 360 mg/kg BW of *Phaleria macrocarpa*; T3: Preeclampsia + 720 mg/kg BW of *Phaleria macrocarpa* ($^{\#}p < 0.05$ compared to C-, $^{\#\#}p < 0.01$ compared to C-, $^{*}p < 0.05$ compared to C+, $^{**}p < 0.01$ compared to C+).

Phaleria macrocarpa on hematological parameters was evident. This concurs with the toxicity evaluation study categorizing the nano-herbal of *Phaleria macrocarpa* as having a moderate level of toxicity (Simanjuntak and Rumahorbo 2022; Simanjuntak et al. 2023). Our study corroborates these findings and adds that nano-herbal *Phaleria macrocarpa* can modulate these effects, particularly at higher doses.

It is the first to systematically evaluate the effects of a nano-herbal *Phaleria macrocarpa* on complete blood counts in a preeclamptic rat model, providing new insights into the therapeutic potential of this formulation. The research highlights the importance of dosage, showing that higher doses (T3 group) have more significant and beneficial impacts on hematological parameters than lower doses (T1 group), emphasizing the need for careful dose optimization in future studies. Despite significant changes in hematological parameters, these changes remained within normal physiological limits, suggesting that the nano-herbal is effective without causing hematological toxicity. Additionally, the rigorous characterization of the nano-herbal, including particle size, morphology, and encapsulation efficiency, ensures reproducibility and sets a standard for future research, addressing a critical gap in the literature. In summary, the findings not only confirm the known benefits of *Phaleria macrocarpa* but also significantly enhance our understanding of its therapeutic potential through nano-encapsulation, paving the way for future clinical trials and advancing the development of nano-herbal therapies for preeclampsia and related conditions.

While the findings from these rat studies on the nano-herbal *Phaleria macrocarpa* show promise, assessing their applicability to humans is crucial. Rats are commonly employed in preclinical research due to their physiological similarities to humans and their ability to simulate conditions such as preeclampsia. However, several factors must be taken into account. Physiological differences, including variations in metabolism, immune responses, and hormonal regulation between rats and humans, can influence how compounds like herbal formulations are absorbed, distributed, metabolized, and excreted. Moreover, the typically small sample sizes and genetic homogeneity of rat studies may not fully capture the genetic and environmental diversity seen in human populations, potentially limiting the generalizability of the results. Dosages effective in rats may not translate directly to humans due to differences in body size, metabolic rates, and administration methods. Additionally, while rat studies provide valuable initial data, they often have shorter durations than chronic human diseases like preeclampsia, necessitating extended studies and eventual human clinical trials to comprehensively evaluate long-term effects and safety. Ethical and regulatory standards are critical in human trials to ensure safety and efficacy, underscoring the need for caution when extrapolating rat study findings to clinical practice. In conclusion, while rat studies are pivotal in drug development, confirming the efficacy and safety of the nano-herbal in human settings will require meticulous consideration of interspecies differences and rigorous clinical validation.

Conclusion

The nano-herbal *Phaleria macrocarpa* significantly reduces blood pressure in preeclamptic rats, demonstrating early and sustained efficacy with enhanced bioavailability and targeted delivery. The formulation is well-tolerated, showing no significant systemic toxicity and stable body weight changes. Higher doses provide more substantial hematological benefits, remaining within normal physiological limits. This study, the first of its kind, highlights the potential of the formulation for a safer and more effective treatment of preeclampsia in a rat model of preeclampsia. This research is also expected to be the basis for future clinical studies.

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