

Effect of kopyor coconut water on early-onset preeclampsia-like impairments in rats induced by L-nitro-arginine methyl ester

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Abstract

Preeclampsia (PE) is a severe pregnancy disorder posing significant health risks to both the mother and the fetus without available preventive measures or treatments. More specifically, the main pathological feature of early-onset PE (EO-PE) is the incomplete transformation of the spiral artery. Meanwhile, aspirin has proven effective for the treatment and prevention of PE. Previous studies indicated a relationship between the phytochemical compound of kopyor coconut water (KCW) and nutritional treatment for PE. Therefore, the objective of this study was to determine the efficacy of KCW as a potential preventive treatment for PE. An experimental laboratory method was used with pre-test and post-test control group designs. The samples comprised 35 pregnant Wistar rats, divided into five groups with seven members each. Rats were then randomized into control and groups exposed to L-NAME, L-NAME and aspirin, L-NAME and KCW 2 ml/200 gBW, as well as L-Name and KCW 2 ml/200 gBW for GD4 to 19. The results showed that after administering L-NAME to induce EO-PE, mean arterial pressure (MAP), proteinuria, placental hypoxia, oxidative stress, and endothelial dysfunction decreased due to KCW nutritional treatment. Specifically, KCW nutrition prevented the uterine spiral artery from expanding and reduced the number of neutrophils. The decreased survival rate caused by L-NAME-induced PE was reversed by providing KCW nutrition. Moreover, results indicated that KCW was a potential alternative for the prevention and treatment of EO-PE even at doses of 2 or 3 ml/200 gBW, offering insights for the community and clinical practitioners in treating PE as a therapeutic option.

Keywords

artery spiralis uteri, early onset, eNOS, HbF, HIF1 α , MAP, L-NAME, preeclampsia, proteinuria

Introduction

Preeclampsia (PE) is one of the main causes of morbidity and mortality in mothers and fetuses (Bouter and Duvekot 2020). According to reports, 500,000 fetuses and 76,000 pregnant women pass away from PE annually (Poon et al. 2021). This condition is characterized by systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg on at least two measurements taken 4 hours apart in previously normotensive women, which begin 20 weeks into pregnancy (ACOG 2020). PE is classified into two categories, early-onset (EO) and late-onset (LO), which have unique effects on the fetus and neonate. Perinatal mortality increases approximately 10-fold higher in EO and twice in LO (Poon et al. 2021).

Early onset Preeclampsia (EO-PE) is a condition that can endanger the lives of the mother and the fetus (Staff et al. 2022). The onset of the condition is characterized by trophoblast invasion disorders, uterine spiral artery transformation, immune maladaptation, and elevated markers of endothelial dysfunction (Staff 2019). Patients with PE, especially those with EO, have significantly higher levels of HIF-1 α in their serum due to placental ischemia and trophoblast hypoxia (Tianthong and Phupong 2021). Moreover, excessive systemic inflammation can increase local oxidative stress responses, leading to decreased antioxidants. Furthermore, the liver and lymphocytes produce alpha-1 microglobulin (A1M), which aids cells in fending off damage from oxidative stress and fetal hemoglobin (HbF) (Kristiansson et al. 2020). Additionally, an imbalance between VEGF and PlGF causes decreased NO and increased eNOS, which in turn causes endothelial damage and vascular dysfunction, ultimately leading to endothelial dysfunction (Pereira et al. 2021). Consequently, endothelial dysfunction leads to increased vascular resistance, heightened glomerular permeability, and other symptoms, including cerebral edema and liver dysfunction, ultimately resulting in PE (Jung et al. 2022).

The management of PE using aspirin has proven to be a cost-effective strategy compared to aspirin prophylaxis for high-risk women. Aspirin, an anti-inflammatory drug, is a non-steroidal molecule that acts as a cyclooxygenase (COX) inhibitor (Shanmugalingam et al. 2020). However, other potential side effects, such as bleeding, are a serious concern (Rolnik et al. 2022). Prevention and treatment interventions for PE are more effective in the early phase before spiral artery remodeling progresses towards worsening effects, leading to hypertension, proteinuria, tissue edema, and multiple systemic organ damage (Lecarpentier et al. 2016). The standard treatment for PE has not been established. Even though, recently, daily low-dose aspirin (150 mg/day) has been reported to reduce the risk of EO-PE (before 34 weeks of pregnancy) if it is initiated before the first trimester of pregnancy. However, aspirin does not reduce the risk of LO-PE (at or after the 34th week of pregnancy). Data reported that there were approximately 90% of cases of LO-PE in developed countries and 40% to 70% in developing countries (Kalafat et al. 2020). In addition, long-term treatment

with aspirin has negative impacts, including bleeding at the last stage of pregnancy (Hastie et al. 2021).

Herbal remedies, such as Extra Virgin Olive Oil (EVOO), which has high levels of monounsaturated fatty acids that can inhibit the angiotensin-converting enzyme (ACE), control blood pressure, and lower urine levels of nitric oxide and 8-isoprostane, are another treatment option for PE (Alcaide-Hidalgo et al. 2020). However, consuming high doses of olive oil may lead to weight gain and nausea in some individuals (Lakshmanan et al. 2020). Previous studies reported that coconut water attenuated blood pressure and mitigated inflammation (Bhagya et al. 2012; Airaodion et al. 2020; Lakshmanan et al. 2020). In addition, it reduces oxidative stress induced by isoproterenol-induced myocardial infarction (Prathapan and Rajamohan 2011).

Several bioactive natural products have been used both as prophylaxis and treatment to prevent or alleviate disorders (Cragg and Pezzuto 2016). The advantage of using natural products is their high tolerability and minimal side effects. The coconut tree, commonly referred to as the “tree of life,” is a tropical plant whose entire part of the plant is beneficial to human life (Rao and Najam 2016). Typically, one coconut has 300 milliliters of water, ranging in pH from 3.5 to 6.1 based on the type, maturity, and climate (Lopez 2023). Young coconut water is rich in minerals and amino acids, and its composition is similar to that of an isotonic fluid, making it ideal for replacing bodily fluids (Yong et al. 2009). L-arginine (Arg), a functional amino acid (AA), is an essential substrate for the production of NO, creatine, polyamines, homoarginine, and agmatine in mammals, including humans, acting as protein building blocks (Wu et al. 2021). Nitric oxide (NO) increases blood flow to tissues, while arginine and its by-products are important for physiology and metabolism (Tuyekar et al. 2021).

Kopyor Coconut Water (KCW) (*Cocos nucifera* L. var. Kopyor), commonly known as Kelapa Kopyor in Indonesia, is a member of the *Cocos nucifera* family and a new substitute for PE due to the high arginine content. A previous study reported that KCW consists of vitamins C and E. The prominent amino acids are glutamic acid, alanine, and arginine. In addition, KCW contains high levels of Mn, Zn, and Mg but low levels of vitamins B1, B2, and C, as well as sucrose (Santoso et al. 1996).

A previous study revealed that tender coconut water (TCW) could lower blood pressure in hypertensive rats by reducing oxidative stress (Bhagya et al. 2012). In addition, another previous study also reported that TCW affects free radicals due to mercury exposure (Zulaikhah and Wibowo 2018). However, the mechanism behind anti-inflammatory properties has not been determined (Radenahmad et al. 2012). TCW regulates the expression of inflammatory mediators and NO production mediated by cytokines, Nos2 mRNA, and iNOS protein expression in the primary hepatocytes of rats (Lakshmanan et al. 2020). This study proposed that KCW would prevent the primary cause of EO-PE, uterine spiral artery remodeling, due to its anti-inflammatory properties.

Materials and methods

Materials

The KCW was obtained from Puan Kalianda, Tanjung Anom Village, Kalianda Sub-district, South Lampung Regency, Lampung Province, Indonesia. Subsequently, KCW was prepared at PT Saraswati Indo Genetech (SIG), as reported. Female Wistar rats were provided by the Integrated Research and Testing Laboratory (LPPT) at 4 Universitas Gadjah Mada Yogyakarta. L-nitro-arginine methyl ester (Cas. No. 51298-62-5) and aspirin (Cas. No. 50-78-2. 1.2) were purchased from Sigma, St. Louis, Missouri, USA. In addition, proteinuria (Catalog Number FY-RA 4983), fetal hemoglobin (HbF) (Catalog Number FY-RA 4973), hypoxia-inducible factor 1 α (HIF1 α) (Catalog Number RK 03528), and endothelial nitric oxide synthase (eNOS) (Catalog Number RK03528) were acquired from Eiyue Biology Company, China.

Methods

Animal experimental study design

This study was an in vivo experiment with a pre-post-test control group design, except for the levels of HbF, eNOS, and uterine spiral artery diameter (USAD). The number of female rats was calculated using the G-Power statistical tool, downloaded from https://www.gpower.com/Menu/OE_Menu.htm. The 6–8-week-old healthy female Wistar rat weighing 180–200 g was mated with the 8–12-week-old male rat weighing 250–350 g before developing the PE model. Pregnancy was determined by examining the presence of copulation plugs in the vagina or sperm through vaginal swabs. Subsequently, it was observed under a light microscope (Olympus CX32; Olympus, Tokyo, Japan) at 100 \times magnification. Induction was carried out by providing drinking water containing 0.75 mg/mL L-nitro-arginine methyl ester from gestational day 4 to 19.

Furthermore, rats were randomly divided into 5 different groups:

PC	Pregnant control rats (PC) without L-nitro-arginine methyl ester treatment, n = 7.
PE	Pregnant rats were exposed to L-nitro-arginine methyl ester from gestational day 4 to gestational day 19, n = 7.
PE + aspirin 2 ml 1.5 mg/kg BW	Pregnant rats were exposed to L-nitro-arginine methyl ester and aspirin from gestational day 4 to gestational day 19, n = 7.

PE+KCW 2 ml/200 g BW

Pregnant rats were exposed to L-nitro-arginine methyl ester and 2 ml/200 g BW of KCW from gestational day 4 to gestational day 19, n = 7.

PE+KCW 3 ml/200 g BW

Pregnant rats were exposed to L-nitro-arginine methyl ester and 3 ml/200 g BW of KCW from gestational day 4 to gestational day 19, n = 7.

Aspirin, 1.5 mg/kg BW/day (2 ml) or KCW (2 or 3 ml), was administered for 16 consecutive days by oral gavage. At the end of the experimental period, all animals were sacrificed using 100 mg/kg thiopental sodium intraperitoneally (a cervical dislocation was performed to verify the death of mice). The ethics committee of the medical faculty at Universitas Sebelas Maret (UNS) approved the experimental protocol. The ethical code is 164/UN27.06.11/KEP/EC/2022 on 01 November 2022.

Measurements of serum HbF and eNOS levels

A total of 1.5 ml of venous blood was centrifuged at 1,500 rpm for 10 min. Subsequently, the collected serum was stored at -20 °C before further analysis. Levels of HbF and eNOS in the serum were measured using the manufacturer's protocol. Standard and sample absorbance values were measured spectrophotometrically at a wavelength of 450 nm.

Tissue preparation for measurement of USAD and placental tissue HIF1 α levels

The region of the endometrium where the placenta is based is known as the fixed uterine tissue. Briefly, the tissue was processed for grossing and fixation (8–48 hours), followed by dehydration, clearing, and embedding for 24 hours at 58 °C. Subsequently, paraffin block preparation/depaffinization (blocking, sectioning, identification, and incubation) and histopathological observation of the uterus were conducted. Furthermore, the level of HIF1 α in placental tissue was observed. Briefly, a total of 0.1 g of sample was homogenized in a 0.9 ml PBS solution, followed by centrifugation at 5000 rpm for 5 min (4 °C) to collect the supernatant.

Urine analysis and blood pressure measurement

Rats were housed in separate metabolic cages for 24 hours, followed by measurement of urine output on gestational day 4, gestational day 12, and gestational day 19. The BP-2000 Blood Pressure Analysis System (Visitech Systems, Inc., Apex, NC, USA) was used to perform a non-invasive tail-cuff to measure mean arterial pressure (MAP) on gestational day 7, gestational day 13, and gestational day 18. Subsequently, all female rats were warmed to a temperature of 38 °C for further analysis.

Statistical analysis

A paired t-test was used to assess differences in normally distributed variables between pre- and post-test groups. All data were presented as means \pm SD. The post hoc Tukey test was used alongside a one-way analysis of variance (ANOVA) to assess group comparisons. In addition, ANOVA and post-hoc Bonferroni tests were used to evaluate group differences over time. The analysis was performed using GraphPad Prism version 9.1.1 software (GraphPad Software, San Diego, CA, USA) ($p < 0.05$).

Results

Characteristics of experimental animals

This study conducted a similar assessment on the gestational day of pregnancy by measuring the body weight, rectal temperature, systolic blood pressure, diastolic blood pressure, MAP, and urine volume of rats, as shown in Table 1. Results revealed those parameters were not significantly different between each group ($p > 0.05$).

KCW nutritional treatment prevented L-nitro-arginine methyl ester-induced vasoconstriction in the uterine spiral artery of PE rats.

USAD was determined at the placental site to reflect the response of spiral artery remodeling. As shown in Fig. 1, KCW nutritional treatment was more effective in reducing spiral artery remodeling failure. USAD in H&E histopathology showed a similar trend, where KCW 3 ml/200 g BW had a stronger effect compared to aspirin and KCW 2 ml/200 g BW, as shown in Fig. 1b. The results suggested that in the PE model, KCW nutritional treatment enhanced aspirin inhibition of vasoconstrictive responses.

KCW nutritional treatment attenuated placental hypoxia in L-nitro-arginine methyl ester -induced PE rats

In order to illustrate hypoxia in the placental tissue of PE rats, the levels of HIF1 α were measured. Results revealed that placental HIF1 α samples decreased after L-nitro-arginine methyl ester induction; it might be due to the presence of aspirin or KCW nutritional treatment. In the KCW group

of 3 ml/200 g BW, results showed that the levels of HIF1 α were higher compared with the aspirin group, as shown in Fig. 2. The results suggested that the reduction in hypoxia regulation induced by L-nitro-arginine methyl ester was influenced by KCW nutritional treatment at 3 ml/200 g BW.

KCW nutritional treatment reduced oxidative stress and serum HbF levels in L-nitro-arginine methyl ester-induced PE rats

Examining markers of systemic oxidative injury revealed a marked rise in the onset of PE. The effects of aspirin were significantly increased in pregnant rats treated with L-nitro-arginine methyl ester, as Fig. 3 illustrates. The findings indicated that a dose of KCW 3 ml/200 g BW and aspirin could decrease the increased activation of NADPH oxidase (NOX2) caused by L-nitro-arginine methyl ester. This dose had a more notable effect than 2 ml/200 g BW.

KCW nutritional treatment attenuated endothelial dysfunction in L-nitro-arginine methyl ester serum-induced PE rats

In order to illustrate endothelial dysfunction in the blood of PE rats, eNOS levels were measured in this study. The findings showed that L-nitro-arginine methyl ester induction reduced eNOS levels in serum samples, which could be raised by taking quercetin or aspirin. Preventive treatment groups receiving doses of 2 ml/200 g BW, 3 ml/200 g BW, and aspirin had eNOS levels that were significantly higher than those of the PE group, as shown in Fig. 4. As seen in Fig. 3, similar outcomes were achieved on the fourth day; however, on gestational day 20, serum eNOS levels were elevated by KCW doses of 2 ml/200 g BW, 3 ml/200 g BW, and aspirin. The findings indicated that KCW nutritional therapy enhanced endothelial vascular function in PE induced by L-nitroarginine methyl ester.

KCW nutritional treatment reduced MAP and urinary protein levels in L-nitro-arginine methyl ester-induced PE rats

MAP of pregnant rats was measured on gestational day 4, gestational day 13, and gestational day 19, as shown in Fig. 5a, b. On gestational day 0, baseline MAP and proteinuria in each group did not show significant differences, as illustrated in Table 1. Compared to the PC group, the MAP of rats significantly increased after L-nitro-arginine methyl

Table 1. Characteristics of experimental animals.

Variable	PC	PE	PE+Asp	PE+KCW 2 ml/200 g BW		PE+KCW 3 ml/200 g BW	p-value
				Mean	SD		
Rats weight (g)	186.19 \pm 9.88	187.71 \pm 6.84	183.60 \pm 7.52	180.23 \pm 2.33	187.90 \pm 8.35		0.358
Temperature ($^{\circ}$ C)	36.99 \pm 0.5	37.48 \pm 0.70	37.28 \pm 0.2	37.10 \pm 0.1	37.20 \pm 0.2		0.358
SBP (mmHg)	104.86 \pm 11.16	98.00 \pm 7.5	97.86 \pm 17.28	102.29 \pm 10.01	105.29 \pm 9.52		0.615
DBP (mmHg)	72.58 \pm 7.1	70.00 \pm 7.02	70.29 \pm 11.11	71.71 \pm 10.73	70.00 \pm 8.02		0.164
MAP (mmHg)	81.34 \pm 7.12	80.33 \pm 5.33	79.48 \pm 12.62	81.90 \pm 8.65	81.76 \pm 7.13		0.453
Proteinuria (ml)	11.71 \pm 1.38	11.43 \pm 1.51	12.57 \pm 2.37	12.09 \pm 0.75	11.86 \pm 1.34		0.328

Note: PC = pregnant control rats, PE = preeclampsia, PE+ASP = preeclampsia+aspirin, PE+KCW 2 ml/200 g BW = preeclampsia+KCW 2 ml/200 g BW, PE+KCW 3 ml/200 g BW, p-value = Anova test $p < 0.05$ = significantly different, SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial pressure.

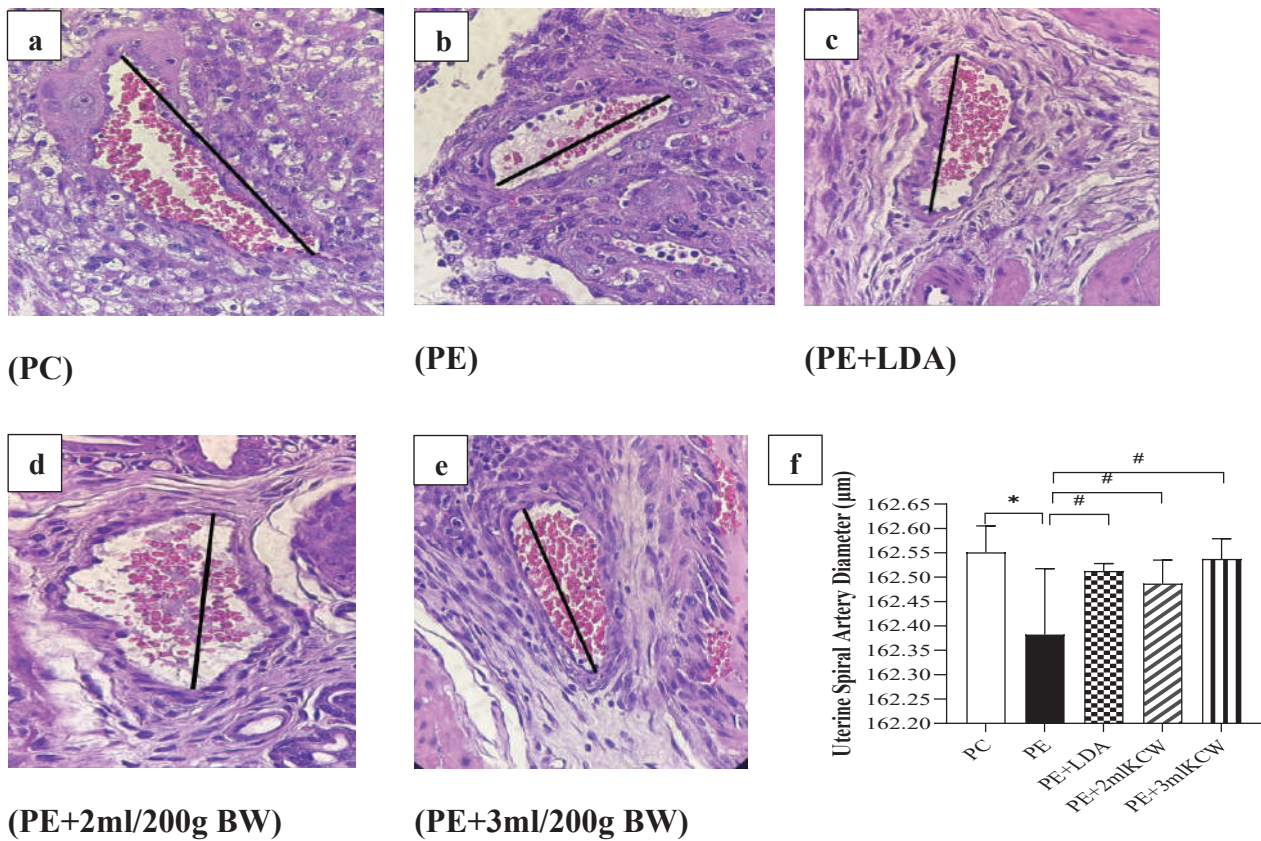


Figure 1. USAD in rats PE model in PC, PE, PE+aspirin, PE+KCW 2 ml/200 g BW, and PE+KCW 3 ml/200 g BW was measured using HE staining. USAD was measured from 5 fields from outer to outer using a microscope with NA 0.65 and a magnification of 400×. Measurements were performed using Touplite and manual applications converted to micrometer units. In L-NAME-induced PE rats, the nutritional treatment effect of KCW increased the vasodilation of the spiral artery. On GD 20, USAD was measured in several groups. Data are presented as means ± SD. ANOVA and post hoc Tukey test were significant **p* < 0.05 compared to the PC group; # *p* < 0.05 compared to the PE group.

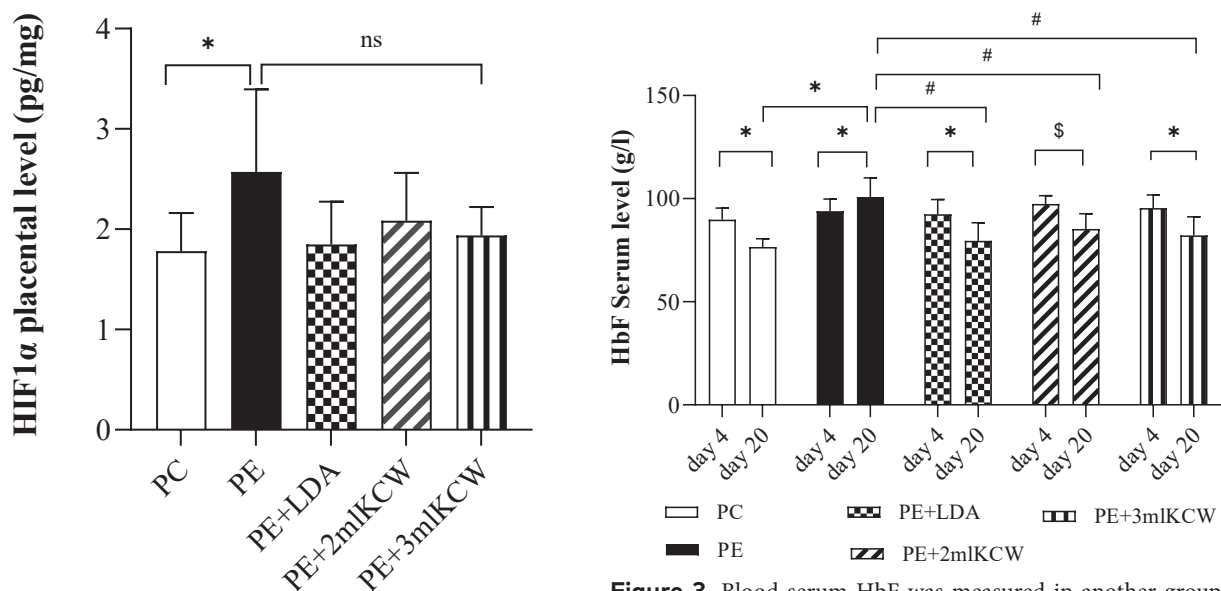


Figure 2. Placental hypoxia in L-NAME-induced PE rats was reduced by KCW nutritional treatment. On GD 20, placental HIF1α was evaluated in several groups. Data are presented as means ± SD. *ANOVA and post hoc Tukey test were significant at *p* < 0.05 compared to the PC group. Ns *p* > 0.05 compared to the PE group.

Figure 3. Blood serum HbF was measured in another group on GD 4 and 20. Serum HbF level data are presented as means ± SD. *ANOVA test is significant, and post hoc Tukey test **p* < 0.05, compared to PC group. # *p* < 0.05 compared to the PE group. Serum HbF data *paired t-test is significant *p* < 0.05 and \$ Wilcoxon test *p* < 0.05.

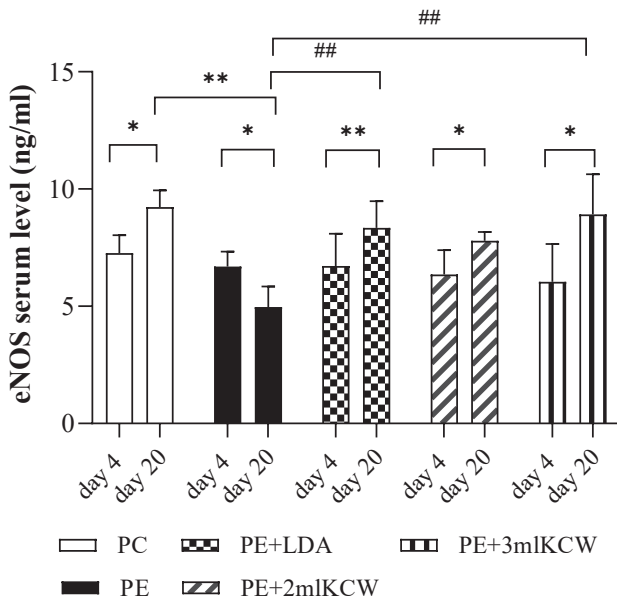


Figure 4. KCW nutritional treatment reduced endothelial dysfunction in the serum of L-NAME-induced PE rats. Blood serum eNOS was measured in another group on GD 4 and 20. Data are presented as means \pm SD. Paired t-test significant * $p < 0.05$ and significant Wilcoxon test * $p < 0.05$ and Turkey test * $p < 0.05$ compared to PC group, post hoc Tukey test # $p < 0.05$ compared to the PC group, ## $p < 0.001$ compared to PE.

ester treatment on gestational day 13, which was re-regulated by KCW and aspirin. The results indicated that 3 mg/kg KCW affected MAP and proteinuria in PE rats. More precisely, KCW nutritional therapy showed a higher capacity

to lower MAP. Proteinuria in each group was also detected on gestational day 4, gestational day 12, and gestational day 19, as shown in Fig. 5a, b. After receiving L-nitro-arginine methyl ester treatment, the rise in proteinuria levels was re-regulated with aspirin and KCW 3 ml/200 g BW. In line with MAP, KCW nutritional treatment and aspirin also showed a stronger ability to reduce proteinuria, as depicted in Fig. 1b. Therefore, it was concluded that KCW nutritional treatment enhanced the effects of reducing MAP and proteinuria in L-nitro-arginine methyl ester-induced PE rats.

Impact of KCW nutritional treatment on the course of pregnancy in L-nitro-arginine methyl ester-induced PE rats

Significant variations were observed in the number of viable fetuses among PC, PE, KCW nutritional treatment, and aspirin (Fig. 6). However, in normal pregnant rats receiving KCW nutritional treatment and aspirin or not, there were no differences in placental weight. Rats given L-nitro-arginine methyl ester showed no discernible decrease in average fetal weight, as shown in Fig. 6. The findings indicated that KCW improved the number of viable fetuses and the outcomes of the pregnancy.

Discussion

According to the results, the administration of 75 mg/kg BW/day of L-nitro-arginine methyl ester inhibited NO synthesis in the endothelium, which mimics the effects of PE

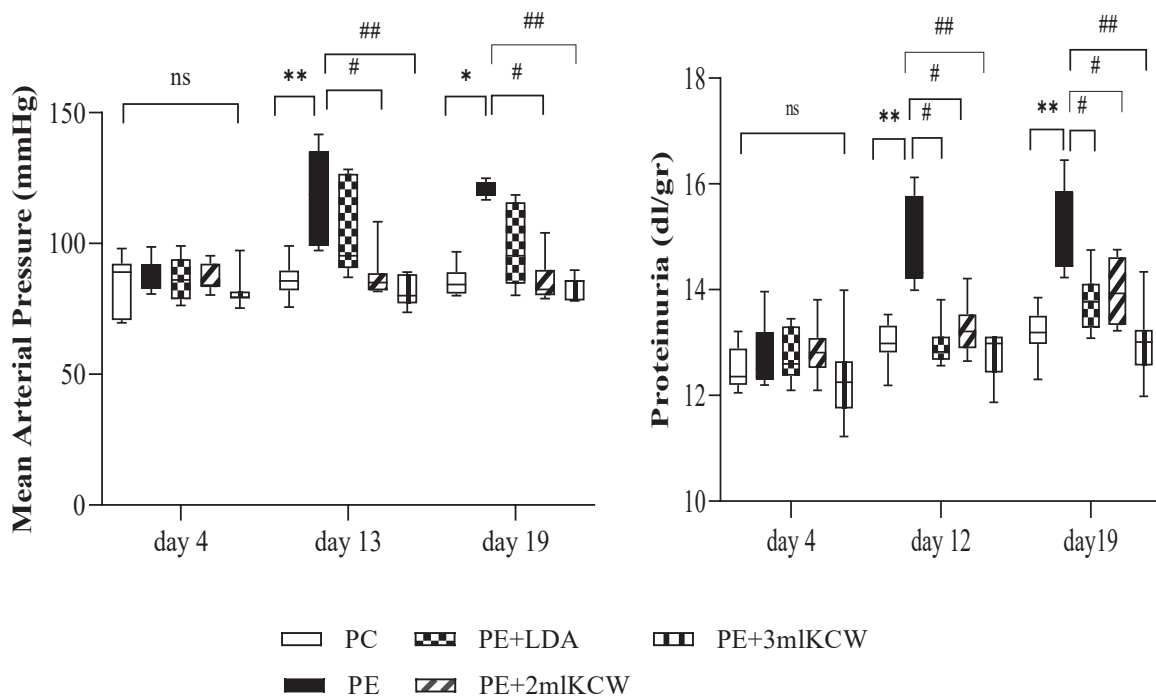


Figure 5. In L-NAME-induced PE rats, KCW nutritional treatment reduced MAP and proteinuria. On GD 4, 13, and 19, each group was measured non-invasively using the tail-cuff method. Data are presented as means \pm SD; significant post hoc Bonferroni test * $p < 0.05$ compared to the PC group; # $p > 0.05$ compared to PE (a). A 24-hour urine protein test. Data are presented as means \pm SD. Significant post hoc Bonferroni test * $p < 0.05$, ** $p < 0.01$ compared to the PC group, # $p < 0.05$, ## $p < 0.001$ compared to PE.

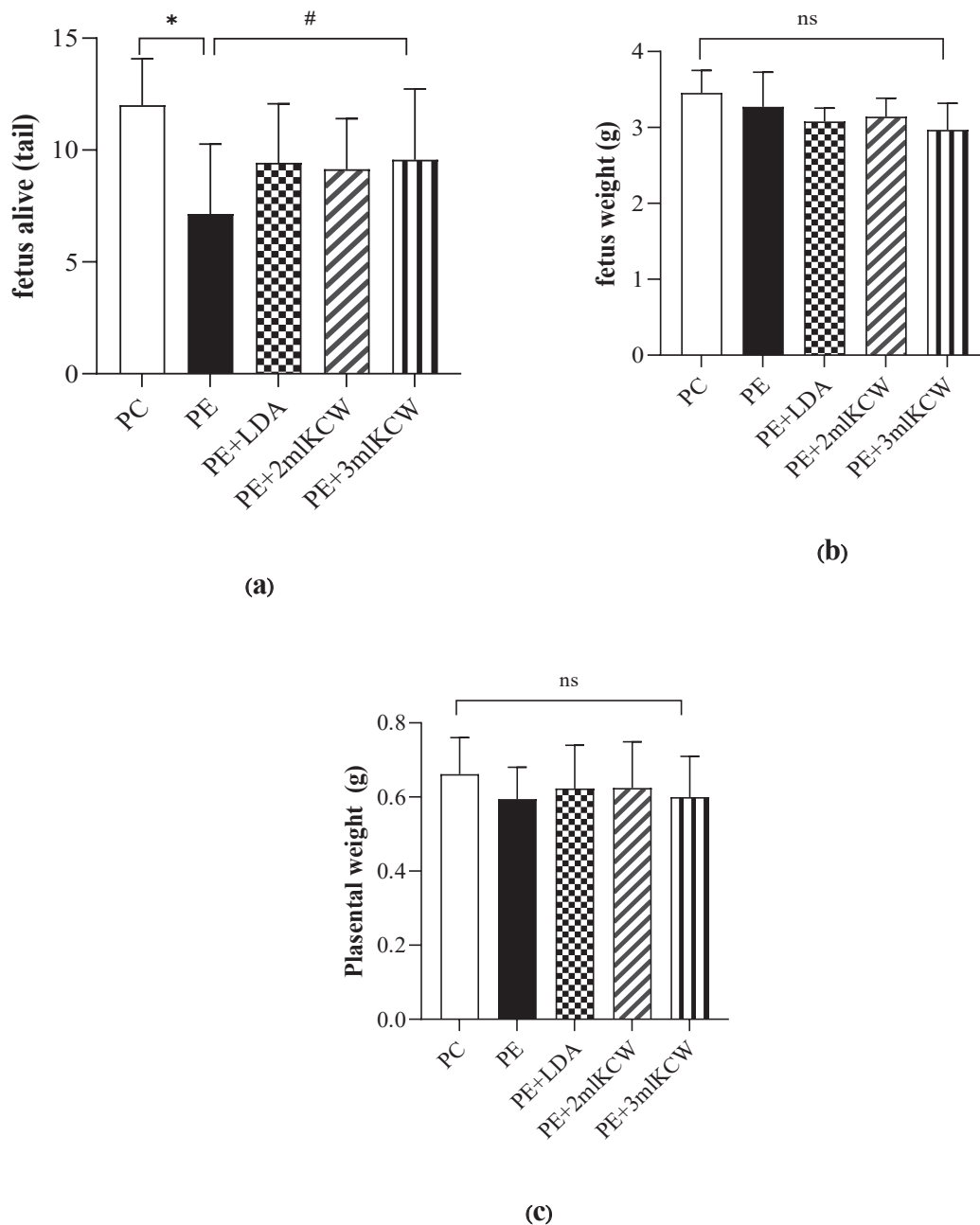


Figure 6. The impact of KCW nutritional treatment on the course of pregnancy in L-NAME-induced PE rats. Surviving rats (a), placental weight (b), and fetal weight (c) were compared. Data are presented as means \pm SD. ANOVA and post hoc Tukey test were significant at * $p < 0.05$ compared to the PC group; # $p < 0.05$ compared to PE; ns $p \geq 0.05$.

and functions in vascular relaxation, followed by the failure of syncytiotrophoblasts to transform from a proliferative epithelial to an invasive endothelial subtype. This resulted in the spiral artery's imperfect remodeling, which is thought to be the cause of EO-PE and is characterized by a narrowing diameter. Failure causes the fetal placenta to become hypoxic, which is indicated by elevated HIF1 levels in placental tissue. Subsequently, placental hypoxia led to increased lipid peroxidation in L-nitro-arginine methyl ester-administered rats, causing elevated free radical production.

KCW is a natural plant product with antioxidant properties in several experimental models and protects the liver from chemical toxin injury (Bhagya et al. 2012). Previous

studies reported that oral kopyor administration reduced oxidative stress caused by isoproterenol myocardial infarction (Prathapan and Rajamohan 2011) and decreased inflammation (Rao and Najam 2016) in rats. However, the mechanism of the vasoconstriction effect is unknown. This study found that KCW activates NO synthesis through eNOS levels and simultaneously affects USAD.

The administration of KCW, both at 2 ml/200 g BW and 3 ml/200 g BW, prevented vasoconstriction in the uterine spiral artery, reduced placental hypoxia, and lowered serum HbF levels and lipid peroxidation. In addition, KCW prevented endothelial dysfunction by reducing eNOS levels, decreasing MAP and urine protein levels,

and preventing a decrease in the number of live fetuses induced by L-nitro-arginine methyl ester. An important pathophysiologic theory pertaining to EO-PE is the partial transformation of the spiral artery, which is vital for the placenta and fetus's nutrition supply (Staff 2019).

The results showed that there was a significant difference between the PE+LDA group, PE 2 ml/200 g BW, and PE 3 ml/200 g BW compared to only the PE group. This was consistent with a study that was conducted by Lalita (2019). The findings demonstrated a significant effect on USAD when 60 *Mus Musculus* were given anti-Qa at a dose of 10 ng from the first to the fourth day of pregnancy. KCW's high arginine content can promote the synthesis of NO. Meanwhile, it has been established that NO, an endothelial vasodilator, is flawed in PE (Sari 2019).

Hypoxia-inducible factor 1-alpha, also known as HIF1 α , is a major transcriptional regulator of the adaptive response to hypoxia (Tal 2012). Based on the results, KCW mitigated hypoxia in the placenta of PE rats. There was a significant difference ($p < 0.05$) between the PC and PE groups. On the other hand, there was no significant difference ($p > 0.05$) between the PE+2 ml/200 g BW and 3 ml/200 g BW groups compared to the PE group. Likewise, a study conducted at the Department of Obstetrics and Gynecology, Safdarjung Hospital, New Delhi, India, on ninety women with EO-PE discovered a significant difference in HIF1 α expression between the PC and PE groups (Rath et al. 2016). The high content of arginine in coconut water is a precursor for the powerful endothelial vasodilator, namely nitric oxide (NO), which plays a role in increasing blood vessel diameter. Moreover, L-arginine decreases malondialdehyde levels and HIF-1 α expression to effectively repair oxidative damage caused by hypoxia (Al-Rasheed et al. 2016).

Hemoglobin fetus (HbF) is the primary oxygen-carrying protein in the human fetus (Perutz et al. 1987). The results showed that KCW reduced oxidative stress levels in serum HbF. On the fourth day of pregnancy, there were no significant differences in any of the groups. However, on day 20, the HbF levels in the PC and PE groups differed significantly. Additionally, compared to PC, there were significant differences between the PC+LDA, PC 2 ml/200 g BW, and PC 3 ml/200 g BW groups. The findings were in line with a study that found that 86 pregnant patients with PE who visited the obstetric unit at St. George Hospital had noticeably higher serum HbF concentrations and went on to develop PE (Anderson et al. 2016). Increased Hb-F leakage into the mother's blood may act as a mediator for systemic effects. Moreover, HbF can also raise blood pressure and improve blood vessel contractility.

Endothelial nitric oxide synthase is an enzyme that regulates the availability of NO (Gambardalle et al. 2020). According to the results, KCW mitigated endothelial dysfunction. On the fourth day of pregnancy, there were no significant differences found in any of the groups. However, the HbF levels of the PC and PE groups differed significantly on day 20. In comparison to PE, significant

differences were also seen between the PE+LDA and PE 3 ml/200 g BW groups. The findings are in line with a study that found that, compared to 143 pregnant women without PE, 63 pregnant women with PE at Bharati Hospital and Gupte Hospital in Pune, Maharashtra, India, had comparatively lower levels of eNOS (Dsouza et al. 2016). In general, eNOS is a crucial mediator for cardiovascular homeostasis, controlling blood vessel diameter and preserving normal levels of cell division and apoptosis. (Heiss et al. 2015). As previously reported, 48 male Sprague-Dawley rats given oral L-arginine supplementation (100 mg/kg/day) for 12 weeks had reduced eNOS gene expression in the abdominal aorta (Adejare et al. 2020). This suggests that the high arginine content in KCW may increase eNOS levels. It might be due to arginine serving as a substrate for eNOS to produce nitric oxide, which in turn plays a crucial role in regulating vascular tone and blood pressure through vasodilation (Elms et al. 2013).

Mean arterial pressure and proteinuria are the primary indicators for diagnosing PE. Results revealed that the administration of KCW reduced MAP and proteinuria levels. There was no significant difference in MAP or proteinuria on the fourth day of pregnancy across any group. However, significant differences between the PE and PC groups as well as the PE, KCW 2 ml/200 g BW, and KCW 3 ml/200 g BW groups were observed on days 13 and 19 (Fig. 6a). The results were consistent with a study conducted by Bhagya et al. (2012), in which six male Sprague-Dawley rats were exposed to 4 mL/100 g BW high-variety TCW for 42 days, then reduced blood pressure from (145 \pm 2) mmHg to (126 \pm 3) mmHg. It might be due to the high content of minerals, amino acids, and vitamins in KCW. Those minerals help to counteract the effects of sodium and regulate fluid balance in the body (Zarean and Tarjan 2017). Moreover, it plays a role in relaxing blood vessels and modulating blood pressure (Xie et al. 2020) by vasodilating and attenuating blood pressure (Li et al. 2022). In addition, an antioxidant may help to improve endothelial function and reduce oxidative stress (Benachi et al. 2020).

Fetal death or distress is another effect of EO-PE, and the number of live fetuses is affected by KCW administration but not placental or fetal body weight. This study may be considered the first report of KCW effects on the number of viable fetuses in rats suffering from PE. The total number of alive and dead offspring at birth is the number of normal fetuses, based on litter size (Eisen 1989). According to Inglis (1980), mice typically have litter sizes of eight to eleven, but Smith and Mangkoewidjojo (1988) reported that litter sizes could reach as high as fifteen. The age of the parents, the time of birth, the food, and the surroundings all affect the number of children (Rosenfeld et al. 2003). The quantity and quality of food provided to the parents, the time of mating, the number of eggs laid, and the degree of embryo mortality are among the environmental factors that have a significant impact on the number of fetuses in a litter (Otto et al. 2015). The number of

live fetuses has a strong influence on placental and fetal body weight, although it is not significant.

The results of this study showed that pregnancy hypertension, L-nitro-arginine methyl ester-induced proteinuria, and adverse pregnancy outcomes like placental weight and fetal growth restriction were all exacerbated by uterine spiral artery narrowing. Followed by the uterine spiral artery constriction, and the attenuation of live pup numbers might be due to placental hypoxia. In addition, results showed the effects of KCW on pregnancy outcomes in L-nitro-arginine methyl ester-induced EO-PE rats. The increased survival ratio was enhanced by KCW nutritional treatment but did not affect placental weight in EO-PE rats for either of the two doses or aspirin treatment. The findings indicate that KCW significantly affects MAP, proteinuria, lipid oxidation, and autophagy, among other PE symptoms. However, placental diameter in EO-PE was not observed. It was established that lesions in the villous and vascular placenta and aberrant trophoblast invasion were closely related to EO-PE. Histopathological characteristics of the placenta of rats given L-nitroarginine methyl ester to induce EO-PE need to be noted in order to further validate the effects of KCW.

Conclusion

In conclusion, KCW improved uterine spiral artery remodeling, prevented placental hypoxia, and raised levels of the antioxidant HbF in plasma in PE Wistar rats, all of which prevented endothelial dysfunction. KCW stimulated the HIF1 α and eNOS pathways, which led to a decrease in HbF and eNOS levels. Additionally, this study showed that in rats induced with L-nitroarginine methyl ester, KCW nutritional treatment could improve the preventive and therapeutic effects of EO-PE. The findings provided a novel strategy for the management and avoidance of PE in people.

References

- Adejare A, Oloyo A, Anigbogu C, Jaja S (2020) L-arginine supplementation increased only endothelium-dependent relaxation in Sprague-Dawley rats fed a high-salt diet by enhancing abdominal aorta endothelial nitric oxide synthase gene expression. *Clinical Medicine Insights Cardiology* 14: 1–9. <https://doi.org/10.1177/1179546820902843>
- Airaodion AI, Ekenjoku JA, Megwas AU, Ngwogu KO (2020) Antihypertensive potential of coconut (*Cocos nucifera* L.) water in Wistar rats. *Asian Journal of Research in Cardiovascular Diseases* 1(1): 1–8. <https://journalijrc.com/index.php/AJRCD/article/view/17>
- Al-Rasheed NM, Fadda L, Mohamed AM, Attia HA, Al-Rasheed NM (2016) Regulating effect of carnosine and/or L-arginine on the expression of inflammatory molecules induced nephropathy in the hypoxic rat model. *Brazilian Archives of Biology and Technology* 59: 1–15. <https://doi.org/10.1590/1678-4324-2016150622>
- Alcaide-Hidalgo JM, Margalef M, Bravo FI, Muguerra B, López-Huertás E (2020) Virgin olive oil (unfiltered) extract contains peptides and possesses ACE inhibitory and antihypertensive activity. *Clinical Nutrition* 39(4): 1242–1249. <https://doi.org/10.1016/j.clnu.2019.05.016>
- Anderson UD, Gram M, Ranstam J, Thilaganathan B, Åkerström B, Hansson SR (2016) Fetal hemoglobin, α 1-microglobulin and hemopexin are potential predictive first trimester biomarkers for preeclampsia. *Pregnancy Hypertension* 6(2): 103–109. <https://doi.org/10.1016/j.preghy.2016.02.003>
- Benachi A, Baptiste A, Taieb J, Tsatsaris V, Guibourdenche J, Senat MV, Souberbielle JC (2020) Relationship between vitamin D status in pregnancy and the risk for preeclampsia: A nested case-control study. *Clinical Nutrition* 39(2): 440–446. <https://doi.org/10.1016/j.clnu.2019.02.015>

Author contributions

Conceptualization: F.F, S.S, D.I, S.S, I.N, V.W; Validation: D.I, S.S, S.S, F.F; Methodology: V.W, D.I, F.F; Investigation: S.S, D.I, S.S; Writing original draft preparation: F.F, D.I; Data Curation: D.I, V.W, F.F; Writing-Review and Editing: F.F, S.S, D.I, S.S, I.N, V.W; Visualization: D.I, V.W, F.F; Supervision: S.S, S.S, I.N; Project Administration: D.I, F.F; Funding Acquisition: F.F. All authors have read and agreed to the published version of the manuscript.

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The Research Ethics Committee approved the protocol of this research study, Faculty of Medicine, Universitas Sebelas Maret (UNS), with the 301/UNS/27.06.9.1/TU.00/2022 number and 01 November 2022.

Data availability statement

The corresponding author can provide the datasets created and/or analyzed during this study upon request.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

- Bhagya D, Prema L, Rajamohan T (2012) Therapeutic effects of tender coconut water on oxidative stress in fructose fed insulin resistant hypertensive rats. *Asian Pacific Journal of Tropical Medicine* 5(4): 270–276. [https://doi.org/10.1016/S1995-7645\(12\)60038-8](https://doi.org/10.1016/S1995-7645(12)60038-8)
- Bouter AR, Duvekot JJ (2020) Evaluation of the clinical impact of the revised ISSHP and ACOG definitions on preeclampsia. *Pregnancy Hypertension* 19: 206–211. <https://doi.org/10.1016/j.preghy.2019.11.011>
- Cragg GM, Pezzuto JM (2016) Natural products as a vital source for the discovery of cancer chemotherapeutic and chemopreventive agents. *Medical Principles and Practice* 25(2): 41–59. <https://doi.org/10.1159/000443404>
- D'Souza V, Rani A, Patil V, Pisal H, Randhir K, Mehendale S, Wagh G, Gupte S, Joshi S (2016) Increased oxidative stress from early pregnancy in women who develop preeclampsia. *Clinical and Experimental Hypertension* 38(2): 225–232. <https://doi.org/10.3109/10641963.2015.1081226>
- Eisen EJ (1989) Selection experiments for body composition in mice and rats: A review. *Livestock Production Science* 23(1–2): 17–32. [https://doi.org/10.1016/0301-6226\(89\)90003-1](https://doi.org/10.1016/0301-6226(89)90003-1)
- Elms S, Chen F, Wang Y, Qian J, Askari B, Yu Y, Pandey D, Iddings J, Caldwell RB, Fulton DJ (2013) Insights into the arginine paradox: evidence against the importance of subcellular location of arginase and eNOS. *American Journal of Physiology. Heart and Circulatory Physiology* 305(5): H651–666. <https://doi.org/10.1152/ajpheart.00755.2012>
- Gambardella J, Khondkar W, Morelli MB, Wang X, Santulli G, Trimarco V (2020) Arginine and endothelial function. *Biomedicines* 8(8): 235–242. <https://doi.org/10.3390/biomedicines8080277>
- Hastie R, Tong S, Wikström AK, Sandström A, Hesselman S, Bergman L (2021) Aspirin use during pregnancy and the risk of bleeding complications: a Swedish population-based cohort study. *American Journal of Obstetrics and Gynecology* 224(1): 95.e1–95.e12. <https://doi.org/10.1016/j.ajog.2020.07.023>
- Heiss C, Rodriguez-Mateos A, Kelm M (2015) Central role of eNOS in the maintenance of endothelial homeostasis. *Antioxidants and Redox Signaling* 22(14): 1230–1242. <https://doi.org/10.1089/ars.2014.6158>
- Inglis JM (1980) Reproductive Patterns and Litter Sizes in Laboratory Mice. *Journal of Mammalian Biology* 45(2): 200–210.
- Jung E, Romero R, Yeo L, Gomez-Lopez N, Chaemsathong P, Jaovisidha A, Gotsch F, Erez O (2022) The etiology of preeclampsia. *American Journal of Obstetrics and Gynecology* 226(2): S844–S866. <https://doi.org/10.1016/j.ajog.2021.11.1356>
- Kalafat E, Shirazi A, Thilaganathan B, Khalil A (2020) The role of aspirin in prevention of preeclampsia in twin pregnancies: does the dose matter? *American Journal of Obstetrics and Gynecology* 223(3): 457–458. <https://doi.org/10.1016/j.ajog.2020.03.005>
- Kristiansson A, Gram M, Flygare J, Hansson SR, Åkerström B, Storry JR (2020) The role of α 1-microglobulin (A1M) in erythropoiesis and erythrocyte homeostasis therapeutic opportunities in hemolytic conditions. *International Journal of Molecular Sciences* 21(19): 1–22. <https://doi.org/10.3390/ijms21197234>
- Lakshmanan J, Zhang B, Wright K, Motameni AT, Jaganathan V, Schultz DJ, Klinge CM, Harbrecht BG (2020) Tender coconut water suppresses hepatic inflammation by activating AKT and JNK signaling pathways in an in vitro model of sepsis. *Journal of Functional Foods* 64: 103637. <https://doi.org/10.1016/j.jff.2019.103637>
- Lalita P (2019) Uterine spiral artery diameter and its role in pregnancy. *Journal of Pregnancy and Maternal Health* 12(3): 123–130.
- Lecarpentier E, Fournier T, Guibourdenche J, Tsatsaris V (2016) Physiopathologie de la pré-éclampsie. *Presse Medicale* 45(7–8): 631–637. <https://doi.org/10.1016/j.lpm.2016.04.016>
- Li M, Wu Y, Ye L (2022) The role of amino acids in endothelial biology and function. *Cells* 11(8): 1372. <https://doi.org/10.3390/cells11081372>
- Lopez AS (2023) A concise review on tender and mature coconut water: Its application in food. *The Pharma Innovation Journal* 12(8): 835–848. <https://www.thepharmajournal.com/archives/2023/vol12issue8/PartJ/12-7-452-793.pdf>
- Otto GM, Franklin CL, Clifford CB (2015) Chapter 4 - Biology and diseases of rats. In: Fox JG, Anderson LC, Otto GM, Pritchett-Corning KR, Whary MT (Eds) *Laboratory Animal Medicine* (Third Edition), 17–29. <https://doi.org/10.1016/B978-0-12-409527-4.00004-3>
- Pereira MM, Torrado J, Sosa C, Zócalo Y, Bia D (2021) Role of arterial impairment in preeclampsia: should the paradigm shift? *American Journal of Physiology, Heart and Circulatory Physiology* 320(5): H2011–H2030. <https://doi.org/10.1152/ajpheart.01005.2020>
- Perutz MF, Fermi G, Luisi B, Shaanan B, Liddington RC (1987) Stereochemistry of cooperative mechanisms in hemoglobin. *Cold Spring Harbor Symposia on Quantitative Biology* 52(9): 555–565. <https://doi.org/10.1101/SQB.1987.052.01.063>
- Poon LC, Magee LA, Verlohren S, Shennan A, Von Dadelszen P, Sheiner E, Hadar E, Visser G, Da Silva Costa F, Kapur A, McAuliffe F, Nazareth A, Tahlak M, Kihara AB, Divakar H, McIntyre HD, Berghella V, Yang H, Romero R, Nicolaides KH, Hod M (2021) A literature review and best practice advice for second and third trimester risk stratification, monitoring, and management of pre-eclampsia: Compiled by the Pregnancy and Non-Communicable Diseases Committee of FIGO (the International Federation of Gynecology). *International Journal of Gynecology and Obstetrics* 154(S1): 3–31. <https://doi.org/10.1002/ijgo.13763>
- Prathapan A, Rajamohan T (2011) Antioxidant and antithrombotic activity of tender coconut water in experimental myocardial infarction. *Journal of Food Biochemistry* 35(5): 1501–1507. <https://doi.org/10.1111/j.1745-4514.2010.00471.x>
- Radenahmad N, Saleh F, Sayoh I, Sawangjaroen K, Subhadhirasakul P, Boonyoung P, Rundorn W, Mitranun W (2012) Young coconut juice can accelerate the healing process of cutaneous wounds. *BMC Complementary and Alternative Medicine* 12(252): 1–10. <https://doi.org/10.1186/1472-6882-12-252>
- Rao SS, Najam R (2016) Coconut water of different maturity stages ameliorates inflammatory processes in model of inflammation. *Journal of Intercultural Ethnopharmacology* 5(3): 244–249. <https://doi.org/10.5455/jice.20160402120142>
- Rath G, Aggarwal R, Jawanjal P, Tripathi R, Batra A (2016) HIF-1 α and placental growth factor in pregnancies complicated with preeclampsia: A qualitative and quantitative analysis. *Journal of Clinical Laboratory Analysis* 30(1): 75–83. <https://doi.org/10.1002/jcla.21819>
- Rolnik DL, Nicolaides KH, Poon LC (2022) Prevention of preeclampsia with aspirin. *American Journal of Obstetrics and Gynecology* 226(2): S1108–S1119. <https://doi.org/10.1016/j.ajog.2020.08.045>
- Rosenfeld CS, Grimm KM, Livingston KA, Brokman AM, Lamberson WE, Roberts RM (2003) Striking variation in the sex ratio of pups born to mice according to whether maternal diet is high in fat or carbohydrate. *Proceedings of the National Academy of Sciences of the United States of America* 100(8): 4628–4632. <https://doi.org/10.1073/pnas.0330808100>

- Santoso U, Kubo K, Ota T, Tadokoro T, Maekawa A (1996) Nutrient composition of kopyor coconuts (*Cocos nucifera* L.). Food Chemistry 57(2): 299–304. [https://doi.org/10.1016/0308-8146\(95\)00237-5](https://doi.org/10.1016/0308-8146(95)00237-5)
- Sari N (2019) Pengaruh Pemberian L-arginine Terhadap Diameter Arteri Spiralis Dan Berat Badan Janin Pada Mencit Bunting Normal Dan Mencit Bunting Model Preeklampsia. Thesis thesis, Universitas Airlangga.
- Shanmugalingam R, Mengesha Z, Notaras S, Liamputtong P, Fulcher I, Lee G, Kumar R, Hennessy A, Makris A (2020) Factors that influence adherence to aspirin therapy in the prevention of preeclampsia amongst high-risk pregnant women: A mixed method analysis. PLOS ONE 15(2): e0229622. <https://doi.org/10.1371/journal.pone.0229622>
- Staff AC (2019) The two-stage placental model of preeclampsia: An update. Journal of Reproductive Immunology 134: 1–10. <https://doi.org/10.1016/j.jri.2019.07.004>
- Staff AC, Fjeldstad HE, Fosheim IK, Moe K, Turowski G, Johnsen GM, Alnaes-Katjavivi P, Sugulle M (2022) Failure of physiological transformation and spiral artery atherosclerosis: their roles in preeclampsia. American Journal of Obstetrics and Gynecology 226(2): S895–S906. <https://doi.org/10.1016/j.ajog.2020.09.026>
- Tal R (2012) The role of hypoxia and hypoxia-inducible factor-1 α in preeclampsia pathogenesis. Biology of Reproduction 87(6): 1–8. <https://doi.org/10.1095/biolreprod.112.102723>
- Tianthong W, Phupong V (2021) Serum hypoxia-inducible factor-1 α and uterine artery Doppler ultrasound during the first trimester for prediction of preeclampsia. Scientific Reports 11(1): 1–7. <https://doi.org/10.1038/s41598-021-86073-w>
- Tuyekar SN, Tawade BS, Singh KS, Wagh VS, Vidhate PK, Yevale RP, Gaikwad S, Kale M (2021) An overview on coconut water: As a multipurpose nutrition. International Journal of Pharmaceutical Sciences Review and Research 68(2): 63–70. <https://doi.org/10.47583/ijpsrr.2021.v68i02.010>
- Wu G, Meininger CJ, McNeal CJ, Bazer FW, Rhoads JM (2021) Role of L-arginine in nitric oxide synthesis and health in humans. Advances in Experimental Medicine and Biology 1332: 167–187. https://doi.org/10.1007/978-3-030-74180-8_10
- Xie K, Li Y, Chen D, Yu B, Luo Y, Mao X, Huang Z, Yu J, Luo J, Zheng P, Yan H, He J (2020) Daidzein supplementation enhances embryo survival by improving hormones, antioxidant capacity, and metabolic profiles of amniotic fluid in sows. Food & Function 11(12): 1–13. <https://doi.org/10.1039/D0FO02472D>
- Yong J W H, Ge L, Ng YF, Tan SN (2009) The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water. Molecules 14(12): 5144–5164. <https://doi.org/10.3390/molecules14125144>
- Zarean E, Tarjan A (2017) Effect of magnesium supplement on pregnancy outcomes: A randomized control trial. Advanced Biomedical Research 6(1): 1–7. <https://doi.org/10.4103/2277-9175.213879>
- Zulaikhah ST, Wibowo JW (2018) The effect of tender coconut water on free radical due to mercury exposure. International Journal of Public Health Science (IJPHS) 7(2): 102–106. <https://doi.org/10.11591/ijphs.v7i2.11849>