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Research Article

Effects of olive leaf extract and nifedipine, alone and in combination, on blood pressure, neutrophil gelatinase-associated lipocalin, malondialdehyde, and creatinine levels in an Nω-nitro-L-arginine methyl ester-induced rat model of preeclampsia

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Abstract

Preeclampsia (PE) is a significant health problem in pregnancy, affecting 6–7% of all gestations and leading to fetal growth retardation, infant morbidity and mortality, premature birth, and maternal death. Currently, effective treatment options for PE are limited. This study aimed to evaluate the therapeutic potential of olive leaf extract (OLE) and nifedipine, alone and in combination, in improving pregnancy outcomes in rats induced with Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME). Rats were treated with single and combination treatments of nifedipine and OLE for four weeks, after being induced with L-NAME for six weeks. Following gestational day assessment and systolic blood pressure testing, the rats were sacrificed, and blood samples were collected for Neutrophil Gelatinase-Associated Lipocalin (NGAL), Malondialdehyde (MDA), proteinuria and Creatinine (SCr) Levels examinations. The results revealed the combination of nifedipine and OLE significantly improved the results of several biomarker analyses associated with PE, including increased NGAL levels, a significant decrease in MDA levels, decrease in proteinuria and elevated creatinine levels. The combination nifedipine and OLE treatment also exhibited a significant antihypertensive effect compared to either nifedipine or OLE monotherapy, as evidenced by the reduction in systolic and diastolic blood pressure levels. This study provides novel evidence for the potential of olive leaf extract as a nutraceutical adjunct for the prevention and treatment of PE. The significant improvements observed in several pathological features associated with PE, including antihypertensive effects, warrant further investigation. Future research should focus on elucidating the underlying mechanisms of action and exploring the clinical applicability of olive leaf extract in human populations with PE.

Keywords

nifedipine, olive leaf extract, preeclampsia, rat model

Introduction

Preeclampsia (PE) is a critical clinical condition that occurs after 20 weeks of pregnancy, affecting 5–10% of all pregnancies (Hayes-Ryan et al. 2021), responsible for over 70,000 maternal deaths and 500,000 fetal deaths worldwide every year. (Mihalceanu et al. 2019). Characterized by hypertension, proteinuria, hematological complications, and uteroplacental disorders (Magee et al. 2014), PE is marked by dysregulation of angiogenic factors like Soluble Fms-Like Tyrosine Kinase-1 (sFlt-1), leading to diminished levels of vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), exacerbating endothelial dysfunction and vascular damage (Mlambo et al. 2023; Velegrakis et al. 2023). Additionally, heightened levels of Neutrophil Gelatinase-associated Lipocalin (NGAL) reflect inflammatory status and epithelial damage (Romejko et al. 2023), while excessive apoptosis in the placenta of PE women inhibits trophoblast invasion into spiral arteries, exacerbating placental ischemia and systemic endothelial damage (Jena et al. 2020).

The etiology of PE involves placental/trophoblast ischemia and hypoxia, oxidative stress, genetic predispositions, immune dysregulation, and vascular endothelial injury, contributing significantly to maternal morbidity and mortality (Abalos et al. 2013; Staff et al. 2013). Oxidative stress, characterized by an imbalance between oxidants and antioxidants, emerges as a significant factor, with lipid peroxide/Malondialdehyde (MDA) levels often elevated in preeclamptic patients (Skytte et al. 2023). Excessive apoptosis in trophoblast villi can damage the placenta and lead to fetal death and developmental disorders. Once caspase-3 is activated, cell death in the form of apoptosis will occur (Suparman et al. 2018).

Effective and safe pharmacological treatment of PE is still lacking. The complex etiology and high demand for safety profiles because of pregnancy are major hurdles for drug development for pre-eclampsia. Currently, due to anti-platelet and anti-inflammatory properties, low-dose aspirin is recommended for preventing or delaying the onset of pre-eclampsia (Mirabito et al. 2020). Unfortunately, recent studies suggest that the efficiency of low-dose aspirin in pre-eclampsia appears only to be modest or even non-responsive (Yang et al. 2022) and high-dose aspirin is prohibited because of potential adverse effects such as birth defects (Muzaffar et al. 2023). This prompted researchers to pursue alternative treatment regimens to improve the clinical management of PE (Bonnet et al. 2021).

Medicinal plants have become a promising sources for drug development (Abdallah et al. 2023), Olive leaf extract (OLE/ *Olea europaea* L.), renowned for its antioxidant (Akbari et al. 2022), anti-inflammatory (Tasneem et al. 2019), and vasodilatory properties(McNeill and Jurgens 2006), has garnered attention for its potential in mitigating PE pathogenesis (Silvani et al. 2020). OLE contains bioactive compounds like oleuropein that may mitigate placental ischemia, oxidative stress, and apoptosis in PE (Yang et al. 2022). Moreover, study on the effects of OLE at a single dose and in combination with common medication such as nifedipine for the treatment of PE are still limited. Nifedipine, a calcium channel blocker, has shown promise in managing hypertension and oxidative stress in various clinical and experimental settings (Ding et al. 2017; Yang et al. 2022). However, their efficacy in treating PE warrants further investigation, particularly in preclinical models.

This study aimed to evaluate the therapeutic potential of olive leaf extract (OLE) to improve clinical outcomes (systolic and diastolic) and biomarkers in pre-eclampsia Rats model induced by Nω-nitro-l-arginine methyl ester hydrochloride (L-NAME). L-NAME inhibits nitric oxide synthase (NOS), reducing nitric oxide (NO) production and leading to vasoconstriction (narrowing of blood vessels) and increased blood pressure, which are characteristic features of preeclampsia. It is considered a standard model for inducing preeclampsia-like symptoms in rats.

Methods

Rat pre-eclampsia models and treatment procedures

We housed female Wistar rats (*Rattus norvegicus*), 12 weeks old and weighing 200–250 g, in a light-controlled and humidity-controlled environment with unrestricted access to food and water. After a one-week acclimatization, female Wistar rats were mated overnight with healthy male Wistar rats at a 2:1 ratio. Successful pregnancy was confirmed by the presence of vaginal spermatozoa, with designated gestational day (GD) 0 marking the date of pregnancy. Subsequently, pregnant rats were divided into five groups, each receiving specific interventions: (1) the negative control group (NC; normal pregnancy) which received no interventions (n = 7); (2) the positive control (PC), rats received PE induction only (n = 7); (3) pre-eclampsia plus nifedipine group (PE+NIF), where rats received only nifedipine, at a dose of 0.54 mg/kg body weight (n = 7); (4) pre-eclampsia plus olive leaf ethanol extract group (PE+OLE), in which pre-eclampsia rats received OLE treatment only at the dose of 200 mg/kg body weight (n = 7); (5) pre-eclampsia plus combination nifedipine + olive leaf ethanol extract (PE+NIF+OLE), in which pre-eclampsia rats received both nifedipine 0.54 mg/kg body weight and olive leaf ethanol extract 200 mg/kg body weight (n = 7). The induction of pre-eclampsia (PE) and treated-PE groups, L- NAME (50 mg/kg body weight; Sigma) was orally administered on the first day of pregnancy (GD0) until the onset of PE, detected from the presence of vaginal plugs. Treatment with NIF at 0.54 mg/kg body weight and OLE at 200 mg/kg body weight, occurred from days 7 to 20 of pregnancy. On GD21, the rats were sacrificed using ketamine 100 mg/kg body weight + xylazine 15 mg/kg body weight anesthesia, and the kidney organ tissue and blood

specimens were collected. All experimental protocols were approved by the ethics committee of Faculty Medicine Universitas Sebelas Maret, Indonesia (Reference No. 07/UN27.06.11/KEP/EC/2023).

The selected biomarkers were chosen based on their established associations with the pathophysiology of PE and their relevance to clinical outcomes. Malondialdehyde (MDA) was selected as a marker of oxidative stress and lipid peroxidation, which are elevated in PE. Soluble Fms-like tyrosine kinase-1 (sFlt-1) was chosen due to its association with endothelial dysfunction and hypertension in PE. Caspase-3 was included as a marker of apoptosis, which is increased in PE. Neutrophil gelatinase-associated lipocalin (NGAL) was selected as a marker of kidney injury and inflammation, common features of PE. Additionally, monitoring of blood pressure, proteinuria, and creatinine levels was included, as these are crucial markers of the clinical progression of PE.

ELISA and measurement of proteinuria, creatinine and blood pressure

Blood pressure (systole and diastole), proteinuria, and creatinine levels were assessed at key time points—GD-0, GD-7, GD-13, and GD-20 (before the rats were sacrificed). Non-invasive blood pressure measurements were conducted using the BP-2000 Blood Pressure Analysis System (Visitech Systems, Inc., Apex, NC, USA). The Coomassie brilliant blue kit (Jiancheng Institute of Biotechnology, Nanjing, China) was employed to detect proteinuria and creatinine under the instructions from the manufacturer. Upon collection of kidney tissue and blood specimens, MDA and sFlt-1 were analyzed from blood samples using enzyme-linked immunosorbent assay (ELISA) kits (Cambridge, MA, USA). Kidney tissue was utilized for the assessment of caspase 3 and Neutrophil gelatinase-associated lipocalin (NGAL) levels, also determined through ELISA.

Statistical analysis

Data analysis employed statistical product and service solutions (SPSS) program version 24.0 for Windows. Statistical analysis was conducted using Statistical Product and Service Solutions (SPSS) program version 24.0 for Windows. Preliminary assessments for homogeneity and normality were performed on each obtained dataset. Homogeneity was assessed using the Levene Statistic, and parametric statistical analysis (One-Way ANOVA) was applied to data with normal distribution and homogeneous variance. For non-normally distributed and non-homogeneous variance data, Kruskal-Wallis analysis was employed. Repeated ANOVA was utilized on datasets with normal distribution to examine proteinuria, blood pressure, and creatinine levels on days GD-0, GD-7, GD-13, and GD-20. In the case of non-normally distributed data, the Friedman Test (Two-way ANOVA based on Rank) was utilized.

Results

Determination of olive leaf plants

Olive leaf plants utilized in this study have been tested in the Botany Laboratory of the Biology Department, Faculty of mathematics and life sciences, Lampung University. This plants i dentified by the scientific name Olea Europaea L, conforming to the Cronquist clarification system (1981) and APG II (2003). The entire olive leaf plant originated from the Yogyakarta Herbal Center Sukoharjo Sleman Regency, Yogyakarta. Ethanol extraction was employed using 96% ethanol solvent through a maceration process.

Olive leaf ethanol extract test

The 1,1-Diphenyl-2-picryl Hydrazyl (DPPH) assay showed the free radical scavenging activity resulting in IC% of 2.322 mg/ml. Total flavanoids were 1.17%, and total phenol equivalents of gallic acid were 4.15%. Olive leaf ethanol extract also contains Zn (Zinc) of 5.72%, and Mg (Magnesium) of 393.88%. GCMS analysis revealed the presence of C19H3602 (Methyl Oleate), C17H3402 (Isopropyl Myristate), and C19H3802 (Ethyl Heptadecanoate) in the extract. LCMS-MS analysis identified oleuropein, kaempferol, and luteolin as compounds within the olive leaf extract.

Malondialdehyde (MDA) levels test

The Malondialdehyde (MDA) levels, indicative of oxidative stress, demonstrated notable variations among treatment groups (Fig. 1). The control group without any experimental manipulation (NC) had a mean MDA level of 1.29 ± 0.15, which indicates a baseline level. In contrast, the positive control group (PC) showed significantly higher oxidative stress, with an average MDA level of 10.00 ± 0.26. The group of patients with pre-eclampsia who received nifedipine therapy (PE+NIF) had an average MDA level of 3.16 ± 0.46, but the group treated with olive leaf extract alone (PE+OLE) had an average of 5.01 ± 0.36. The group that received a combination of nifedipine and olive leaf extract therapy (PE+NIF+OLE) had a significantly decreased average MDA level of 2.07 ± 0.23. The results emphasize the possibility of the combination intervention in efficiently reducing oxidative stress linked to pre-eclampsia and significant reduction in MDA levels compared to the other groups.

Soluble fms-like tyrosine kinase-1 (sFlt-1) level test

Distinctive patterns were seen across the therapy groups when evaluating levels of soluble fms-like tyrosine kinase-1 (sFlt-1), a crucial marker in pre-eclampsia pathology (Fig. 2). The control group (NC) had a baseline average sFlt-1 level of 1.56 ± 0.22. In contrast, the positive

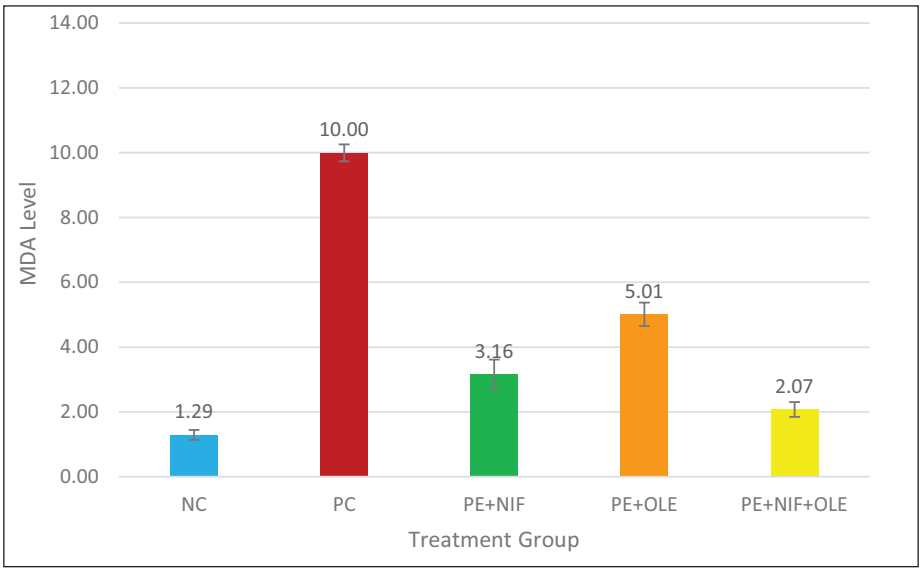

Figure 1. Mean and standard deviation (SD) of MDA levels in various treatment groups: Normal Control (NC), Positive Control (PC), Pre-eclampsia + Nifedipine (PE+NIF), Pre-eclampsia + Olive leaf extract (PE+OLE), and Pre-eclampsia + Nifedipine + Olive leaf extract (PE+NIF+OLE). The error bars represent the standard deviation, highlighting the variability within each group. Significantly reduced MDA levels were observed in the PE+NIF+OLE group (yellow) compared to other groups, indicating the potential efficacy of the combined intervention in mitigating oxidative stress associated with pre-eclampsia.

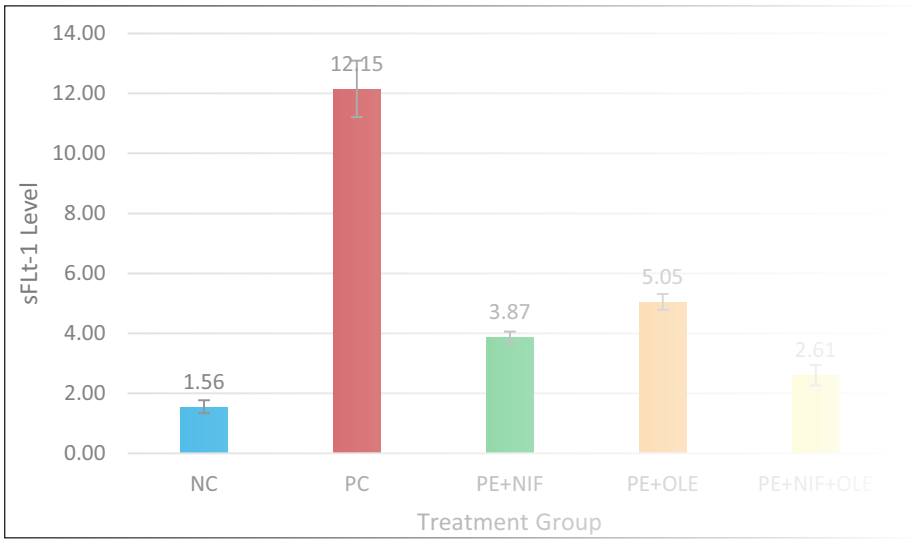

Figure 2. Mean and standard deviation (SD) of sFlt-1 levels in various treatment groups: Normal Control (NC), Positive Control (PC), Pre-eclampsia + Nifedipine (PE+NIF), Pre-eclampsia + Olive leaf extract (PE+OLE), and Pre-eclampsia + Nifedipine + Olive leaf extract (PE+NIF+OLE). The error bars represent the standard deviation, highlighting the variability within each group. Significantly reduced sFlt-1 levels were observed in the PE+NIF+OLE group (yellow) compared to other groups.

control group (PC) showed a considerably higher average sFlt-1 level of 12.15 ± 0.94, suggesting a strong influence on the pathophysiology of pre-eclampsia. The group of patients with pre-eclampsia who were treated with nifedipine (PE+NIF) had an average sFlt-1 level of 3.87 ± 0.19, whereas the group treated with olive leaf extract alone (PE+OLE) had an average level of 5.05 ± 0.26. Remarkably, the group that received a combination of nifedipine and olive leaf extract therapy (PE+NIF+OLE) showed a significantly decreased average sFlt-1 level of 2.61 ± 0.34. These findings highlight the potential effectiveness of the combined intervention (PE+NIF+OLE) in regulating sFlt-1 levels in the setting of pre-eclampsia.

Caspase 3 test

Distinct patterns were identified across the treatment groups (Fig. 3) in evaluating the levels of caspase 3, a critical marker of apoptosis. The control group without any experimental manipulation (NC) had a baseline average level of caspase 3 of 2.07 ± 0.28. In contrast, the positive control group (PC) showed a considerably higher average caspase 3 level of 11.88 ± 0.34, suggesting a significant effect on apoptosis in the context of pre-eclampsia. The group of patients with pre-eclampsia who were treated with nifedipine (PE+NIF) had an average caspase 3 level of 4.07 ± 0.24, but the group treated with olive leaf extract alone (PE+OLE)

had an average of 5.99 ± 0.22. Surprisingly, the group that received a combination of nifedipine and olive leaf extract therapy (PE+NIF+OLE) showed a significantly decreased average caspase 3 level of 2.82 ± 0.17. These data highlight the potential effectiveness of the combined intervention in regulating caspase 3 levels and reducing apoptotic processes in the context of pre-eclampsia.

NGAL level test

The assessment of Neutrophil Gelatinase-Associated Lipocalin (NGAL) levels, a pivotal biomarker for renal injury, revealed distinct patterns across the treatment groups (Fig. 4). The normal control group (NC) displayed a baseline mean NGAL level of 31.18 ± 1.74. In contrast, the positive control group (PC) exhibited a significantly elevated mean NGAL level of 177.63 ± 4.02, indicating substantial renal stress associated with pre-eclampsia. The pre-eclampsia group treated with nifedipine (PE+NIF) demonstrated a mean NGAL level of 47.89 ± 3.18, while the group treated with olive leaf extract alone (PE+OLE) displayed a mean of 59.91 ± 2.83. Remarkably, the group subjected to a combination of nifedipine and olive leaf extract treatment (PE+NIF+OLE) showcased a notably reduced mean NGAL level of 37.77 ± 2.42. These findings underscore the potential efficacy of the combined intervention in mitigating renal injury associated with pre-eclampsia.

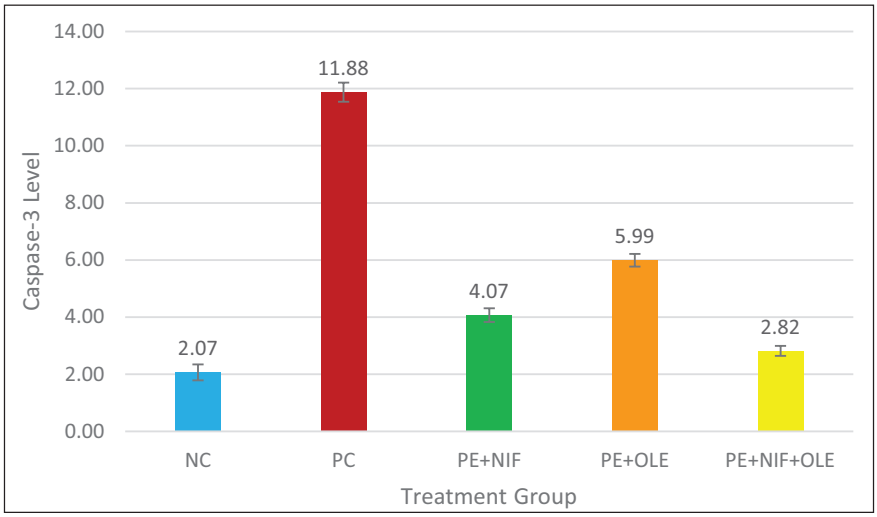

Figure 3. Mean Caspase 3 Levels in Different Treatment Groups. The bar graph illustrates the mean caspase 3 levels (± SD) in the normal control group (NC), positive control group (PC), pre-eclampsia group with nifedipine treatment (PE+NIF), pre-eclampsia group with olive leaf extract treatment (PE+OLE), and pre-eclampsia group with a combination of nifedipine and olive leaf extract treatment (PE+NIF+OLE). The data highlight significant differences in caspase 3 levels among the treatment groups, suggesting the potential of combined interventions in modulating apoptotic processes in pre-eclampsia.

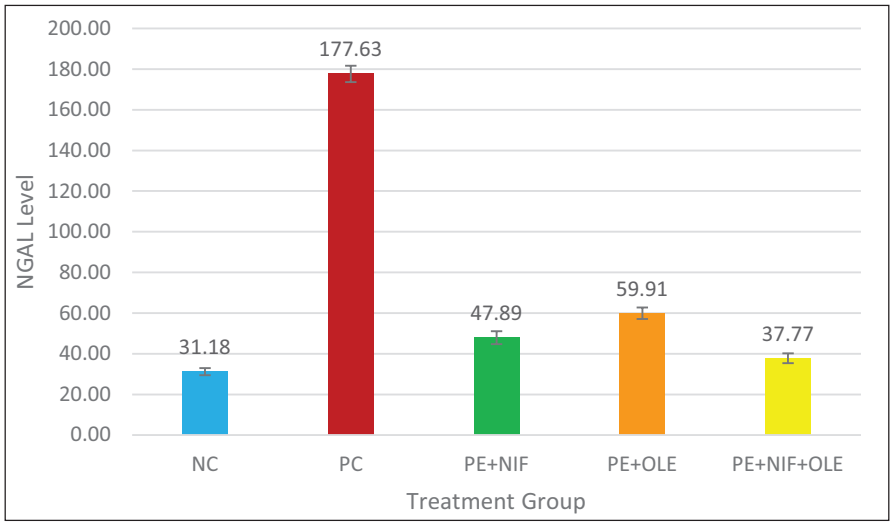

Figure 4. Mean NGAL Levels in Different Treatment Groups. The bar graph illustrates the mean NGAL levels (± SD) in the normal control group (NC), positive control group (PC), pre-eclampsia group with nifedipine treatment (PE+NIF), pre-eclampsia group with olive leaf extract treatment (PE+OLE), and pre-eclampsia group with a combination of nifedipine and olive leaf extract treatment (PE+NIF+OLE). The data reveal significant differences in NGAL levels among the treatment groups, highlighting the potential of combined interventions in mitigating renal injury associated with pre-eclampsia.

Blood pressure (systole and diastole), proteinuria and creatinine levels

The impact of duration of the treatment on key physiological parameters was assessed in pre-eclampsia rats, with results by repated ANOVA was presented in Table 1. Significant variations were observed in systolic and diastolic blood pressure, proteinuria, and creatinine levels across different treatment durations (GD0, GD7, GD13, and GD20).

Table 1. Effect of Treatment by Gestasional Day (GD) on Blood Pressure, Proteinuria, and Creatinine Levels in Pre-eclampsia Rats.

Variabel	Mean ± SD				P value
	GD0	GD7	GD13	GD20	
Systolic	107.83 ± 12.83	119.37 ± 32.01	132.4 ± 44.12	143.17 ± 50.96	<0.001**
Diastolic	83.43 ± 10.16	83.63 ± 9.99	86.38 ± 9.49	86.43 ± 9.65	<0.001**
Proteinuria	57.45 ± 3.87	87.32 ± 2.55	114.61 ± 35.54	120.85 ± 42	<0.001**
Creatinine	0.74 ± 0.02	1.15 ± 0.06	2.31 ± 1.1	2.68 ± 1.38	<0.001**

*Significant at α = 0.05, **Significant at α = 0.001.

Table 1 displays each variable's mean ± SD and p values at different treatment time points. Systolic blood pressure exhibited a steady increase from 107.83 ± 12.83 mmHg on GD0 to 143.17 ± 50.96 mmHg on GD20 ($p < 0.001$**), while diastolic blood pressure followed a similar trend, escalating from 83.43 ± 10.16 mmHg on GD0 to 86.43 ± 9.65 mmHg on GD20 ($p < 0.001$**). Furthermore, proteinuria levels surged significantly from 57.45 ± 3.87 mg/dL on GD0 to 120.85 ± 42 mg/dL on GD20 ($p < 0.001$**). Creatinine levels substantially rose from 0.74 ± 0.02 mg/dL on GD0 to 2.68 ± 1.38 mg/dL on GD20 ($p < 0.001$**).

These findings underscore the progressive deterioration of blood pressure regulation, increasing proteinuria, and renal dysfunction over the course of the treatment period. The results emphasize the severity of pre-eclampsia and the need for timely interventions to mitigate these physiological disruptions. A significant decrease was observed in the treatment group with preeclamptic mice plus the combination of nifedipine + ethanol extract of olive leaves (PE+NIF+OLE), administered at doses of nifedipine 0.54 mg/kg body weight + olive leaf ethanol extract 200 mg/kg on GD0, GD7, GD13, and GD20, further supports the potential efficacy of this combination in managing pre-eclampsia-related physiological changes.

Fig. 5 shows systolic blood pressure changes in pregnant rats within a pre-eclampsia model. The figure reveals a slight reduction in systolic blood pressure in the pre-eclampsia group receiving the combination of nifedipine + olive leaf ethanol extract (PE+NIF+OLE) from GD 0 to GD 7. Moreover, the combination group PE+NIF+OLE statistically significant compared with other groups with < 0,001**. Although all groups experienced an increase from GD 7 onwards, the rise in systolic blood pressure in the combination group PE+NIF+OLE and the NC group (no treatment) was the lowest compared to the other experimental groups. In contrast, the positive control group (PC), consisting of rats with induced PE, exhibited a steep increase throughout the pregnancy period from GD 0 to GD 20. These observations suggest that the combination treatment of PE+NIF+OLE contributed to a milder increase in systolic blood pressure, emphasizing its potential as a therapeutic intervention in managing pre-eclampsia-associated hypertension.

The results of diastolic blood pressure among treatment groups illustrated in Fig. 6 reveal significant variations in the average reduction of diastolic blood pressure among different treatment groups in the pregnant rat model of pre-eclampsia (PE). The combination PE+NIF+OLE exhibited a slight decrease in diastolic blood pressure throughout the pregnancy period, highlighting the potential synergistic effect of the combined intervention. This result was supported by the post Hoc test which revealed that the PE+NIF+OLE combination treatment group had a significance value of $p < 0.001$** compared to the other groups. Meanwhile, PE+OLE appeared to gradually lower diastolic blood pressure starting from GD 13, although it did not show a significant effect at GD 7. On the other hand, both the NC and PC groups collectively demonstrated an increase in diastolic blood pressure throughout the pregnancy period. However, the PC group exhibited the highest Diastolic

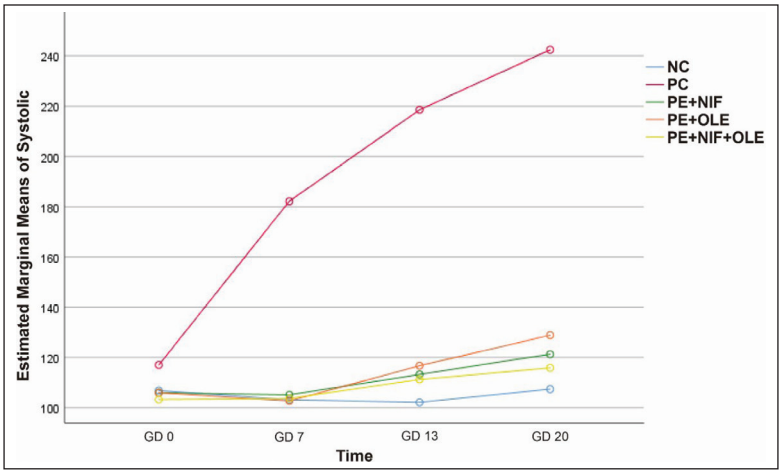

Figure 5. Graph illustrating the Difference in Systolic Blood Pressure Among Treatment Groups.

Blood Pressure among all treatment groups. These findings suggest the promising potential of the combination treatment (PE+NIF+OLE) in mitigating diastolic blood pressure in pre-eclampsia, emphasizing its significance in therapeutic interventions.

The results depicted in Fig. 7 illustrate the examination of proteinuria levels in the preeclampsia (PE) pregnant rat model. A significant reduction in proteinuria levels after GD 13 was consistently observed in the PE+NIF+OLE group, where preeclamptic rats received a combination of nifedipine and olive leaf ethanol extract. These findings suggest a potential beneficial effect of the combined intervention in reducing proteinuria compared to the other groups with statistically significant with $p < 0.001^{**}$. Notably, the positive control group showed a drastic increase in proteinuria levels during pregnancy. On the other hand, the PE+OLE, PE+NIF, and NC groups had a slight increase on days 7, 13, and 20, which tended to stabilize after GD 7, but the results remained below normal levels. This implies that the individual treatments (PE+OLE and PE+NIF) and the normal control group had limited impact on reducing proteinuria, thus emphasizing the need for more effective interventions to bring proteinuria levels within the normal range.

Fig. 8 illustrates the analysis of creatinine levels in the rat model of pre-eclampsia. The findings demonstrate a significant and consistent decline in creatinine levels over time in the combination group (PE+NIF+OLE), commencing on GD 13 and rapidly decreasing by GD 20. This finding was supported by the post Hoc test which revealed that the PE+NIF+OLE combination treatment group had a significance value of $p < 0.001^{**}$ compared to the other groups. This indicates a significant and efficient decrease in creatinine levels with the combined therapy. The PE+OLE group had a modest decline from GD 13 to GD 20, suggesting that the treatment of OLE had a beneficial effect, but not as significant as the combination of PE+NIF+OLE. In contrast, the positive control group (PC), consisting of rats that only underwent PE induction, exhibited a significant rise in creatinine levels. These findings demonstrate the potential effectiveness of using many therapies to successfully reduce creatinine levels. This emphasizes the need of using comprehensive therapy methods to treat renal dysfunction associated with pre-eclampsia.

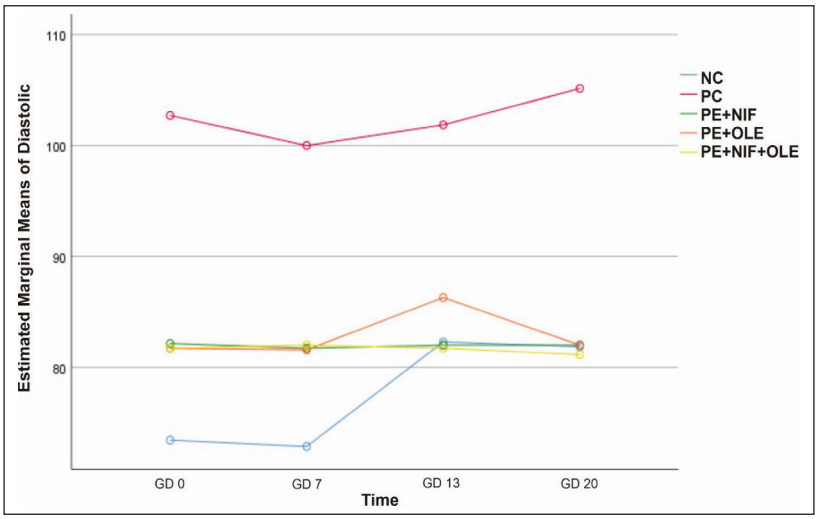

Figure 6. Graph illustrating the difference in diastolic blood pressure among treatment groups.

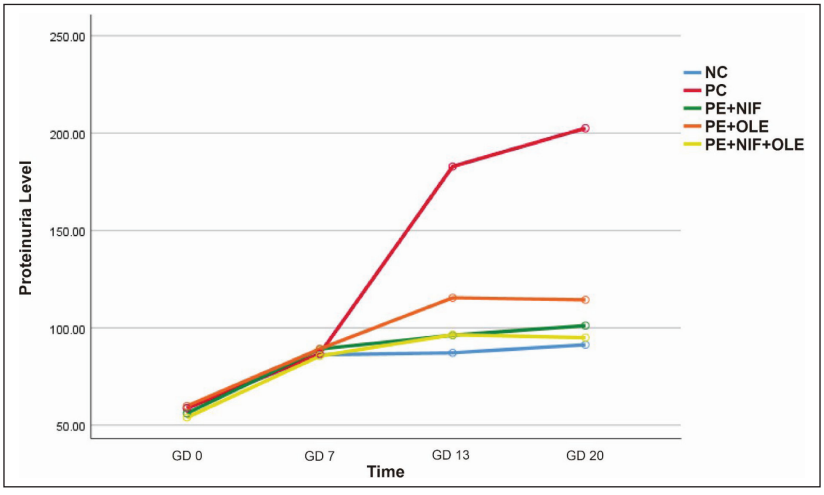

Figure 7. Graph illustrating the Difference in Proteinuria Level Among Treatment Groups.

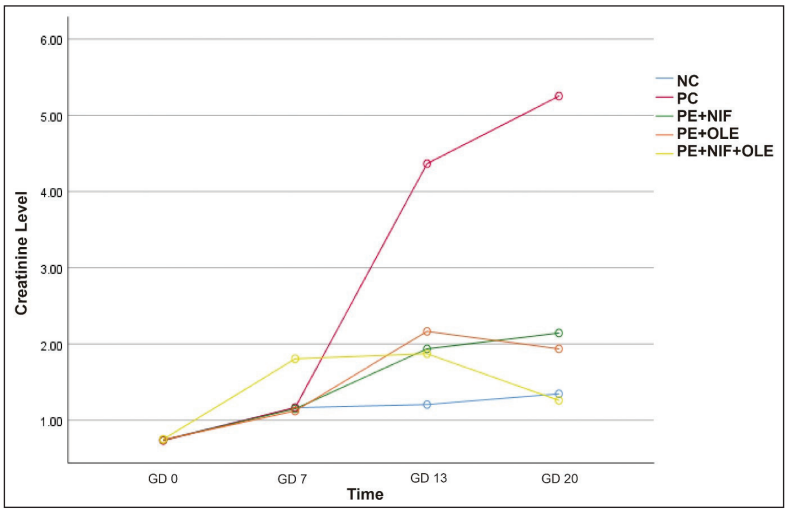

Figure 8. Graph illustrating the Difference in Creatinine Level Among Treatment Groups.

Discussion

To the best of our knowledge, this is the first study to investigate the potential therapeutic effects of olive leaf extract (OLE) in clinical features (i.e., systolic and diastolic blood pressure) and several biomarkers, such as Neutrophil Gelatinase-Associated Lipocalin (NGAL), Malondialdehyde (MDA), proteinuria and Serum Creatinine (SCr) levels outcomes of rats with pre-eclampsia-like symptoms. This study showed that the combination of nifedipine and OLE significantly improved the results of several biomarkers compared to single treatments.

The progressive deterioration of key physiological parameters over the course of preeclampsia pathogenesis was evident in our study. The steady rise in systolic and diastolic blood pressure underscores the severity of hypertension associated with this condition (Angeli et al. 2011; Stuart et al. 2018). Elevated proteinuria levels are also indicative of glomerular endothelial dysfunction and impaired renal hemodynamics in pre-eclampsia (Garovic 2000). Furthermore, the marked increase in creatinine over time highlights the risk of renal injury and dysfunction (Faiz 2020; MacDonald et al. 2022).

The improved blood pressure regulation could be attributed to the antihypertensive action of nifedipine as a calcium channel blocker coupled with the vasodilatory effects of olive leaf phenolic compounds (Abu-Amara and Meselhy 2016). Reduced proteinuria indicates improved glomerular permeability, which may be mediated by lowered oxidative stress and reduced endothelial activation (Kermanshah et al. 2020). The reno-protective effects, as evidenced by declining creatinine, could arise from reduced renal apoptosis and improved renal hemodynamics (Lafayette et al. 1998). However, the exact molecular mechanisms underlying these physiological effects remain unclear.

OLE also exhibited significant antihypertensive effects, lowering both systolic and diastolic blood pressure in PE rats model. This reduction in blood pressure was consistent with previous studies in Italy highlighting the vasodilatory properties of olive phenolics, particularly oleuropein, which enhance nitric oxide (NO) bioavailability and endothelial function (Ahamad et al. 2019). By preserving nitric oxide signaling disrupted in PE and attenuating oxidative stress-induced vascular damage, OLE likely contributes to improved vascular health and blood pressure regulation. Furthermore, olive leaf extract supplementation markedly reduced proteinuria levels. Proteinuria stems from glomerular endothelial damage and podocyte dysfunction, and is a hallmark of PE predictive of adverse maternal and fetal outcomes (Garovic 2000). The nephroprotective effects of olive leaf extract may be mediated by enhanced endothelial integrity and mitigated oxidative stress and inflammation, suggesting potential to alleviate renal injury in PE (Abu-Amara and Meselhy 2016).

Additionally, biomarker analysis provided insights into molecular mechanisms. We observed that the combination of olive leaf extract (OLE) with nifedipine led to a more significant decrease in soluble Fms-like tyrosine kinase-1 (sFlt-1) levels compared to either treatment alone. This combination may have a synergistic effect in improving placental perfusion and maternal endothelial function. Nifedipine, a calcium channel blocker, is known for its antihypertensive effects, which can help alleviate hypertension in preeclampsia. Additionally, OLE's ability to modulate dysregulation of these angiogenic factors contributes to endothelial dysfunction in PE (Levine et al. 2004; Agrawal et al. 2019). By restoring this balance between pro- and anti-angiogenic factors, olive leaf extract may improve placental perfusion and maternal endothelial function, promoting better pregnancy outcomes. The observed decrease in soluble Fms-like tyrosine kinase-1 (sFlt-1) and increase in placental growth factor (PlGF) provides insights into the molecular mechanisms of olive leaf extract. sFlt-1 is an anti-angiogenic factor produced by the placenta that sequesters and inhibits the pro-angiogenic PlGF, leading to widespread maternal endothelial dysfunction in preeclampsia (Levine et al. 2004). Oleuropein (OLE) and hydroxytyrosol, bioactive phenolics in

olive leaf extract, have been shown to suppress sFlt-1 production and secretion by placental tissues and endothelial cells (Abu-Amara and Meselhy 2016). This may occur by downregulating hypoxia-inducible transcription factors and preventing sFlt-1 mRNA expression and protein accumulation induced by placental oxidative stress (Kermanshah et al. 2020). Additionally, olive leaf extract may directly stimulate PlGF secretion by activating the Akt/eNOS pathway and enhancing VEGF-mediated PlGF expression (Han et al. 2005). By suppressing placental sFlt-1 overproduction and stimulating endothelial PlGF secretion, olive leaf extract can restore balance between these factors. This combination therapy may improve placental vascular development, systemic endothelial quiescence, and vascular tone regulation, thereby alleviating hypertension and proteinuria to improve pregnancy outcomes in preeclampsia.

OLE treatment showed reduced caspase-3 expression, indicating suppressed apoptosis, which is implicated in improper placentation and trophoblast turnover in preeclampsia (Allaire et al. 2000). The bioactive phenolics in OLE, such as oleuropein and hydroxytyrosol can inhibit apoptosis by modulating Bcl-2 proteins, preventing cytochrome c release, and inhibiting caspase activation (Bulotta et al. 2013). OLE also lowered malondialdehyde (MDA) levels, indicating reduced lipid peroxidation resulting from placental oxidative damage in preeclampsia (Kermanshah et al. 2020). Oleuropein can attenuate MDA production by enhancing antioxidant enzyme activities, decreasing NADPH oxidase activity, and scavenging reactive oxygen species (ROS) (Abu-Amara and Meselhy 2016). Additionally, OLE reduced neutrophil gelatinase-associated lipocalin (NGAL) levels, a biomarker of renal tubular injury. This reno-protective effect may occur via suppressed renal macrophage infiltration and inflammation by downregulating NF-kB activity (Han et al. 2005). The antioxidant effects of olive leaf extract likely also protect against renal oxidative damage that can cause NGAL upregulation. Overall, the reduction in apoptosis, oxidative stress, and renal injury markers highlights the multifaceted therapeutic potential of olive leaf extract in preeclampsia through modulation of pathways including inflammation, oxidative stress, and apoptosis. The combination therapy of nifedipine and OLE led to a further decrease in average caspase-3 levels compared to OLE treatment alone, suggesting a synergistic effect in suppressing apoptosis. Nifedipine, known for its calcium channel blocking properties, may complement OLE's anti-apoptotic effects by improving placental perfusion and reducing oxidative stress, thereby further reducing apoptosis. This combination therapy plays a crucial role in improving placental health and pregnancy outcomes in PE by targeting multiple pathways, including inflammation, oxidative stress, and apoptosis.

The synergistic effects observed with combination therapy of nifedipine and olive leaf extract suggest that olive leaf extract may complement standard antihypertensive drugs like nifedipine to enhance efficacy while minimizing side effects. Nifedipine is a calcium channel blocker that lowers blood pressure by relaxing vascular smooth muscle and dilating peripheral arteries and arterioles (Xu et al. 2021). Oleuropein and hydroxytyrosol in olive leaf extract have been shown to induce acute endothelium-independent vasodilation in animal models of hypertension, possibly by interfering with calcium influx into vascular smooth muscle (Abu-Amara and Meselhy 2016). This complementary vasodilatory mechanism may underlie the enhanced antihypertensive action of the combination therapy. Furthermore, long-term intake of oleuropein-enriched olive leaf extract has been found to reduce blood pressure in genetic hypertension by improving vascular function and attenuating inflammation and oxidative stress, effects associated with angiotensin converting enzyme (ACE) inhibition (Singh et al. 2008). The combination of these long-term vascular benefits with nifedipine's acute vasodilatory effects likely contributes to the synergistic blood pressure lowering efficacy of the combination therapy. The multi-targeted actions of olive leaf bioactive on pathways like oxidative stress, ACE activity, and vascular function highlight the value of integrating this herbal therapy with conventional drugs like nifedipine for a more effective management of hypertension in preeclampsia.

While this study provides valuable insights into the potential therapeutic effects of olive leaf extract (OLE) and nifedipine in a rat model of preeclampsia, several limitations should be acknowledged. The study focused on a limited set of physiological and molecular parameters, and further research is needed to explore the broader effects of OLE and nifedipine on maternal and fetal outcomes. The mechanisms underlying the observed effects were not fully elucidated, and future studies should aim to clarify the molecular pathways involved. Additionally, the long-term safety and efficacy of OLE and nifedipine in pregnant individuals remain to be established. Finally, the study did not assess the potential interactions between OLE and nifedipine with other medications commonly used in pregnancy, which could be important for clinical application.

Conclusion

This study highlights the potential of olive leaf extract (OLE) as a nutraceutical adjunct in the management of preeclampsia, both alone and in combination with nifedipine. The results demonstrate that the combination therapy of OLE and nifedipine exhibits synergistic effects, suggesting that OLE may complement conventional antihypertensive drugs to enhance efficacy while minimizing side effects. However, further research is needed to elucidate the molecular mechanisms involved and to evaluate the clinical safety and efficacy of OLE in human trials, paving the way for the development of novel treatment strategies.

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Competing interests

The author has declared that no competing interests exist.

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