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Original article

Histopathological Blood-Brain Barrier, and Brain Cellular Profile in Preeclampsia Rat Model Treated with Andaliman (*Zanthoxylum acanthopodium* DC.) Seed Extract

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Abstract

Preeclampsia is a pregnancy complication characterized by hypertension and proteinuria after mid-gestation, which can impair the integrity of the blood–brain barrier (BBB) and lead to brain tissue damage. Andaliman (*Zanthoxylum acanthopodium* DC.) contains flavonoids with antioxidant and anti-inflammatory properties that may provide neuroprotection against such pathological changes. This study aims to analyze the histopathology of the BBB and the cellular profile of brain tissue in a preeclampsia rat model treated with different doses of andaliman seed extract. Pregnant rats were allocated into four groups: healthy controls, preeclampsia controls, and preeclampsia models treated with andaliman seed extract at 100 mg/kg body weight (BW) or 200 mg/kg BW. Preeclampsia was induced through high-salt exposure, and brain tissues were processed and evaluated histologically. The preeclampsia control group exhibited severe BBB disruption, including endothelial swelling, perivascular edema, and neuronal disorganization. Treatment with 100 mg/kg BW of andaliman extract resulted in moderate improvement, reducing edema and partially restoring endothelial and neuronal morphology. Conversely, 200 mg/kg BW produced more substantial recovery, characterized by well-preserved vascular boundaries, minimal perivascular spaces, and organized neuronal layers. These findings suggest that andaliman seed extract exerts a dose-dependent neuroprotective effect by maintaining BBB integrity and brain cell architecture in preeclampsia. The 200 mg/kg BW dose demonstrated the most effective outcome, indicating the potential of andaliman as a natural therapeutic agent for hypertensive disorders during pregnancy.

Keywords: *Zanthoxylum acanthopodium* DC., blood–brain barrier, histopathology, preeclampsia, *Rattus norvegicus*

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Introduction

Preeclampsia is a major cause of maternal mortality worldwide, contributing to approximately 24% of maternal deaths in Indonesia (Fitriani & Heni, 2021). This hypertensive disorder develops after 20 weeks of gestation and is commonly associated with systemic organ dysfunction, including impaired cerebral circulation. The blood-brain barrier (BBB) is essential for maintaining central nervous system homeostasis; however, in preeclampsia, excessive production of antiangiogenic factors such as soluble fms-like tyrosine kinase-1 (sFlt-1) antagonizes vascular endothelial growth factor (VEGF), leading to endothelial dysfunction and BBB disruption. This process is further exacerbated by increased proinflammatory cytokines and oxidative stress, resulting in elevated vascular permeability, cerebral edema, seizures, and hypertensive encephalopathy (Younes & Ryan, 2019; Bisson *et al*., 2023).

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Blood–Brain Barrier disruption in preeclampsia also impairs cerebral blood flow autoregulation, causing hyperperfusion or hypoperfusion and subsequent vasogenic edema or ischemic injury. These cerebrovascular disturbances manifest as neurological symptoms, including headache, visual impairment, and seizures, and may progress to posterior reversible encephalopathy syndrome (PRES), a condition characterized by reversible cerebral edema and transient neurological dysfunction, predominantly affecting the occipital and parietal regions (Escudero et al., 2023).

Preeclampsia-induced BBB dysfunction not only affects maternal cerebral health but also has potential long-term consequences for the fetus. Placental hypoxia and impaired uteroplacental perfusion may result in fetal brain hypoxia, altering neuronal development and increasing the risk of neurocognitive and neurological disorders in offspring. Structural changes, including reduced white matter volume and impaired neuronal connectivity, have been reported in children born to preeclamptic mothers, who also exhibit a higher prevalence of developmental delays and seizure disorders. These findings underscore the importance of understanding BBB pathology in preeclampsia to inform preventive and therapeutic strategies aimed at mitigating neurological complications in both mother and child (Younes & Ryan, 2019).

Andaliman (*Zanthoxylum acanthopodium* DC.) is a traditional spice from North Sumatra, Indonesia, that has

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attracted scientific interest because of its pharmacological potential. Andaliman seeds contain bioactive compounds, including flavonoids, alkaloids, and essential oils, which exhibit antioxidant and antimicrobial activities. Ethanol extracts of andaliman seeds demonstrate antioxidant activity with an IC_{50} value of 239.06 ppm, categorized as moderate based on the DPPH radical scavenging assay (Effendi, 2020). Despite its moderate potency, the flavonoid content, particularly quercetin-like compounds, plays a critical role in mitigating oxidative stress by neutralizing reactive oxygen species (ROS) and stabilizing cellular membranes. Furthermore, in vivo studies suggest that the antioxidant effect may be potentiated by synergistic interactions among bioactive constituents, contributing to cumulative biological effects beyond what is indicated by in vitro IC_{50} values alone (Ompusunggu & Irawati, 2021; Adrian et al., 2023). Phytochemical analyses have identified abscisic acid as one of the major active constituents, while essential oil components contribute to antibacterial activity against several pathogenic bacteria (Effendi, 2020; Reinoviar et al., 2019; Ompusunggu & Irawati, 2021).

This research was conducted to evaluate the histopathological alterations of the blood–brain barrier and the cellular organization of brain tissue in a preeclampsia rat model, and to compare the effects of andaliman (*Zanthoxylum acanthopodium* DC.) seed extract administered at doses of 100 mg/kg and 200 mg/kg body weight. The investigation highlights the limited characterization of cerebral pathology in preeclampsia and emphasizes the importance of preserving the blood-brain barrier integrity to prevent neurological complications. By analyzing dose-dependent responses, the research aims to determine the optimal neuroprotective concentration of andaliman extract. This study provides novel insight into the neuropathological manifestations of preeclampsia and highlights andaliman as a potential natural therapeutic candidate for hypertensive disorders during pregnancy.

Methods

Sample Determination

All animal procedures in this study were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Universitas Brawijaya under ethical clearance number 107-KEP-UB-2024. Thirty female rats aged ≥10 weeks (170–200 g) were mated with males at a ratio of 1:2 for four consecutive nights. Pregnancy was confirmed by the presence of a vaginal plug and supported by physiological and behavioral indicators, including nipple enlargement, weight gain, increased food intake, nesting behavior, abdominal enlargement, and hair loss around the nipples. The day following confirmation was defined as gestational day (GD) 0. Only pregnant rats with normal baseline blood pressure and no proteinuria were included.

Pregnant rats were randomly allocated into four experimental groups. One group served as a normal pregnancy control and did not receive any treatment or hypertension induction. The positive preeclampsia group consisted of pregnant rats induced with hypertension through oral administration of an 18% NaCl solution. Two additional treatment groups comprised hypertensive pregnant rats that received andaliman (Zanthoxylum acanthopodium DC.) seed extract at doses of 100 mg/kg body weight and 200 mg/kg body weight, respectively.

Prior to intervention, all animals were reassessed to ensure compliance with the inclusion criteria, including confirmed pregnancy, body weight ranging from 170 to 200 g, good general health condition, absence of visible injury, normotensive baseline status (systolic blood pressure < 140 mmHg and diastolic blood pressure < 90 mmHg), and absence of proteinuria. Hypertension induction was performed in the positive preeclampsia group and both treatment groups from gestational day 7 to day 13. Induction was achieved through daily oral administration of an 18% NaCl solution at a dose of 10 mL/kg body weight using an oral gavage technique, following the protocol established by Mao et al. (2015). The normal pregnancy control group did not receive NaCl induction or any treatment.

Systolic and diastolic blood pressure as well as proteinuria were measured on GD7, GD14, and GD21. Blood pressure was assessed using a non-invasive tail-cuff method with a CODA™ mouse and rat tail-cuff system at the Animal Disease Diagnosis Laboratory, Faculty of Veterinary Medicine, Universitas Brawijaya. Proteinuria was evaluated by placing rats individually in metabolic cages for 1–2 h or until approximately 2 mL of urine was collected, followed by dipstick analysis (Accubiotech 10 P). On GD22, all animals were euthanized under intramuscular ketamine anesthesia. Brain tissues were carefully harvested, rinsed three times with 1× phosphate-buffered saline (PBS), and preserved for subsequent histopathological analysis.

Paraffin Block Preparation

The preparation of paraffin blocks began with thawing the brain tissue at 4°C, followed by washing with phosphate-buffered saline (PBS) until it was clean. The tissues were then fixed in 4% paraformaldehyde (PFA) for 24 hours (Zhanmu et al., 2020). Fixed tissues were coronally sliced into 0.3–0.5 cm sections and dehydrated through a graded series of alcohol concentrations (30%, 50%, 60%, 70%, 80%, 90%, 96%, and absolute ethanol I) for 30 minutes each, followed by immersion in absolute ethanol II overnight. Clearing was performed using xylol–alcohol mixtures in ratios of 1:3, 2:2, and 3:1 for 1 hour each, followed by two immersions in pure xylol for 1 hour each. Tissue infiltration was performed using xylol–paraffin mixtures (3:1, 1:1, and 1:3) for 1 hour each. Afterward, the samples were embedded in molten paraffin at 55°C for 4 hours and then cooled overnight.

The paraffin blocks were sectioned at a thickness of 4 µm using a microtome. Histological ribbons were floated on warm water (37°C), mounted on poly-L-lysine–coated slides, and adhered on a hot plate at 42°C for 24 hours. Deparaffinization was conducted by immersing slides in xylol I, II, and III for 5 minutes each. Rehydration was performed through a descending alcohol series, starting from absolute ethanol I and II, followed by 96%, 80%,

70%, 60%, 50%, 40%, and 30% alcohols for 3 minutes each, and then rinsed under running water for 15 minutes. The sections were stained with hematoxylin for 5 minutes, washed under running water for 10 minutes, and counterstained with eosin for 3 minutes after preliminary dehydration in 30%, 50%, 60%, and 70% alcohols. Final dehydration was conducted through 70%, 80%, 90%, 95%, and 100% alcohols for 3 minutes each, followed by clearing in xylol. Slides were mounted with Entellan and covered with cover glass. The preparations were observed using an Olympus BX53 light microscope (Rika & Fatchiyah, 2017).

Histopathological and Cellular Profile Analysis

Histopathological examination was performed to evaluate blood–brain barrier (BBB) integrity and the brain cellular profile in preeclampsia rat models by assessing morphological changes in capillary endothelial cells, perivascular astrocytes, vessel walls, and perivascular spaces. Tissue sections from the posterior cerebral cortex were observed using an Olympus BX53 light microscope at 1000× magnification. BBB alterations were assessed semi-quantitatively using a scoring system: score 0 (normal morphology, endothelial cells intact and continuous), score 1 (mild changes, slight endothelial irregularity or focal discontinuity, mild displacement or thinning of pericyte coverage), score 2 (moderate changes, evident endothelial cell swelling with partial detachment, partial detachment or loss of pericyte coverage), and score 3 (severe changes, severe endothelial damage with discontinuity, widespread loss of pericytes along vessel wall), based on endothelial continuity, vessel shape, tight junction integrity, pericyte arrangement, astrocyte endfeet attachment, and perivascular edema. Observations were conducted in five randomly selected fields, and mean scores were calculated for each group (Fatchiyah et al., 2021).

Histopathological assessment of the brain cellular profile was conducted to evaluate neuronal morphological changes indicative of nuclear degeneration in the posterior region of the cerebral cortex. Neuronal damage was identified based on characteristic nuclear abnormalities, including pyknosis (defined by nuclear shrinkage and chromatin condensation), karyorrhexis (showed by fragmentation of the nucleus), and karyolysis (indicated by the dissolution of nuclear material). Neurons exhibiting one or more of these degenerative features were classified as damaged, whereas neurons with round nuclei and evenly distributed chromatin were considered intact. The percentage of damaged neurons was calculated relative to the total neuronal count in each field, and the data were expressed as mean ± standard deviation (SD) to represent the degree of neuronal injury across the different treatment groups.

Statistical Analysis

All data were analyzed statistically using the Shapiro–Wilk test for normality and Levene's test for homogeneity. Data that met the assumptions of normality and homogeneity were further analyzed using a one-way analysis of variance (one-way ANOVA), followed by Duncan's post hoc test to determine significant differences among groups. Statistical significance was set at $p < 0.05$. Data analysis was conducted using SPSS and GraphPad Prism version 9.0.0 (Fatchiyah et al., 2021; Reyhanditya et al., 2022).

Results

Histopathological Observation of the Blood–Brain Barrier (BBB)

Histopathological examination was performed to evaluate morphological alterations in the blood–brain barrier (BBB). The representative histological images and the comparison of morphological scores are presented in Figure 1.

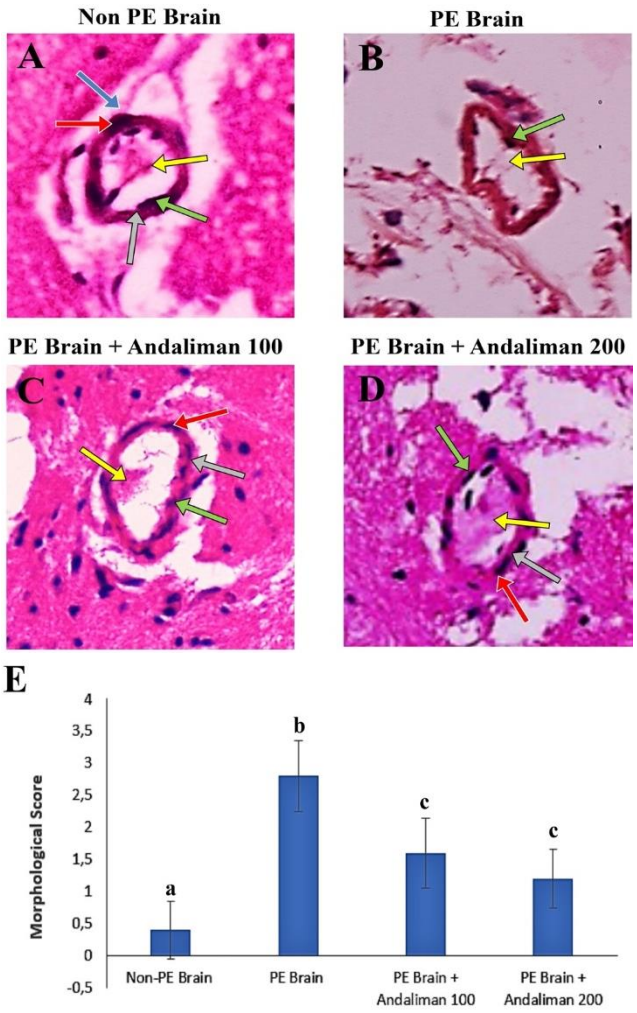

Fig. 1. Histopathological profile and morphological scoring of the blood–brain barrier (BBB) in PE rats treated with andaliman extract. (A) Non-PE brain: non-preeclamptic brain. (B) PE brain: preeclamptic brain. (C) PE + Andaliman100: preeclamptic brain treated with Andaliman 100 mg/kg BW. (D) PE + Andaliman 200 = preeclamptic brain treated with Andaliman 200 mg/kg BW. (E) Morphological scoring of the blood–brain barrier. Blue arrow: astrocyte endfeet, green arrow: endothelial cells, red arrow: pericytes, grey arrow: tight junctions, yellow arrow: the luminal side of the vessel. Hematoxylin–Eosin staining, 1000× magnification.

Qualitative histopathological analysis of the blood-brain barrier (BBB) using Hematoxylin-Eosin staining (Figure 1A) demonstrated varied morphological features across the treatment groups. The Non-PE brain control

group exhibited cerebral blood vessels that were round and regular in shape, characterized by intact and tightly arranged layers of endothelial cells, pericytes, tight junctions, and astrocyte endfeet. This morphology contrasted sharply with that of the PE brain group, where the blood vessels became highly irregular and exhibited severe disorganization. The endothelial cells appeared swollen and discontinuous, accompanied by significant widening of the perivascular space. Administration of Andaliman extract at doses of 100 and 200 mg/kg BW resulted in morphological improvement. The PE brain + Andaliman 100 group showed partial restoration; the blood vessel shape appeared more regular compared to the PE brain group, with endothelial cells becoming denser and exhibiting reduced discontinuity. The most extensive improvement was observed in the PE brain + Andaliman 200 group, where the blood vessels appeared highly regular and closely resembled a perfect circular shape, and the endothelial cell condition showed dense, organized layers with minimal widening of the perivascular space.

These findings were further supported by the quantitative analysis of BBB morphological scores presented in Figure 1B. The Non-PE brain group exhibited the lowest mean score (0.4 ± 0.55), indicating minimal structural disruption, whereas the PE brain group showed the highest score (2.8 ± 0.45), reflecting substantial BBB damage. Treatment with Andaliman extract resulted in a progressive reduction in BBB injury in a dose-dependent manner. The PE brain + Andaliman 100 group demonstrated a reduced score of 1.6 ± 0.55, while the most significant improvement was observed in the PE brain + Andaliman 200 group, in which the score decreased to 1.2 ± 0.45. These results provided clear evidence of a dose-dependent protective effect of Andaliman extract on BBB morphology.

Cellular Profile of Brain Tissue

Histopathological examination was conducted to evaluate neuronal morphological alterations in brain tissue among the different treatment groups. Representative histological images and a comparison of nuclear degeneration profiles are presented in Figure 2.

Histopathological observation (Figure 2A) revealed that the Non-PE brain group exhibited normal neuronal morphology, characterized by round nuclei, evenly distributed chromatin, and no signs of nuclear degeneration, indicating preserved neuronal structure and function. The PE brain group exhibited pronounced cellular alterations.

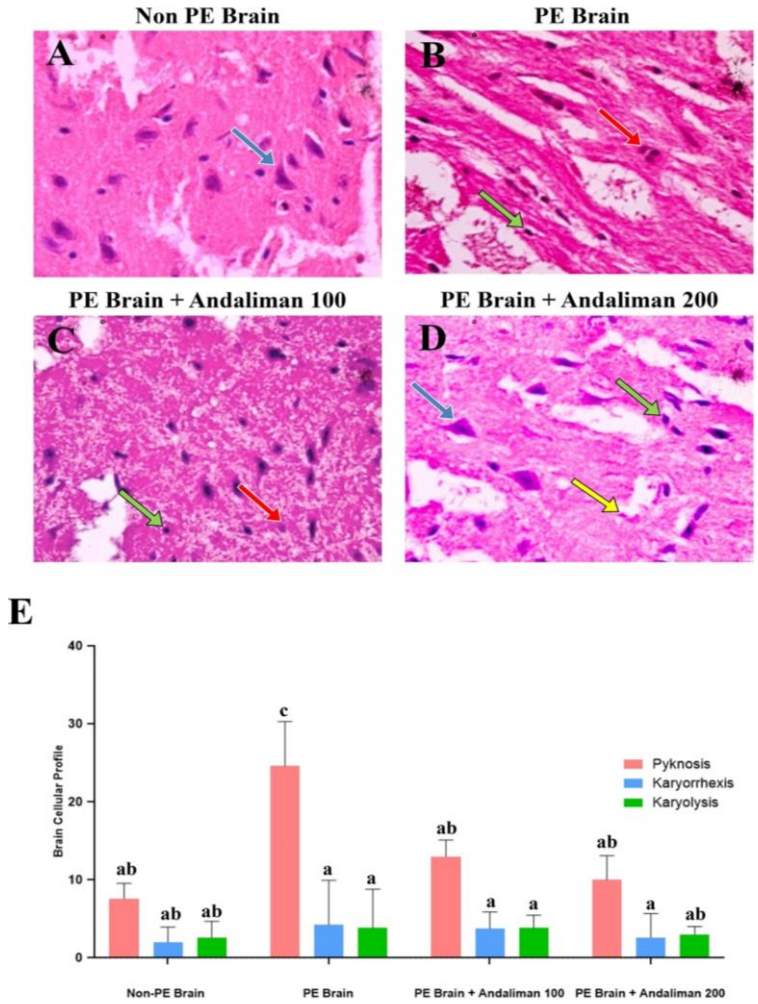

Fig. 2. Histopathological and cellular profiles of brain tissue in PE rats treated with andaliman extract. (A) Non-PE brain: non-preeclamptic brain. (B) PE brain: preeclamptic brain. (C) PE + Andaliman100: preeclamptic brain treated with Andaliman 100 mg/kg BW. (D) PE + Andaliman 200 = preeclamptic brain treated with Andaliman 200 mg/kg BW. (E) Quantification of nuclear degeneration in brain tissue. Blue arrow: normal cell, green arrow: pyknosis, red arrow: karyorrhexis, yellow arrow: karyolysis. Hematoxylin–Eosin staining, 1000× magnification.

Numerous neurons showed pyknosis (nucleus shrinkage and chromatin condensation, green arrows), karyorrhexis (nuclear fragmentation, red arrows), and karyolysis (nuclear dissolution, yellow arrows). These degenerative features reflect severe neuronal necrosis, likely caused by oxidative stress and inflammation associated with preeclampsia. The tissue also appeared more disorganized, with less distinct cellular boundaries and widespread vacuolation. Treatment with Andaliman 100 mg/kg BW markedly reduced neuronal damage compared to the PE group. Although some degenerating nuclei were still observed, most neurons exhibited normal morphology and clearer nuclear integrity. The PE + Andaliman 200 mg/kg BW group demonstrated even greater neuroprotection, with only a few pyknotic or fragmented nuclei remaining, suggesting a strong restorative effect of andaliman in a dose-dependent manner.

Quantitative analysis (Figure 2E) showed that the PE brain group recorded the highest nuclear degeneration scores, with pyknosis at 24.60 ± 5.68, karyorrhexis at 4.25 ± 5.68, and karyolysis at 3.80 ± 4.97, indicating severe neuronal necrosis. The Non-PE group showed the lowest levels of degeneration (pyknosis 7.60 ± 1.95, karyorrhexis 2.00 ± 1.95, karyolysis 2.60 ± 2.07). Both andaliman-treated groups exhibited significant reductions in all degeneration parameters. Andaliman 100 mg/kg reduced pyknosis to 13.00 ± 2.12, while Andaliman 200 mg/kg further decreased it to 10.00 ± 3.08, with corresponding reductions in karyorrhexis and karyolysis. ANOVA showed significance ($p < 0.05$), confirming a significant difference among the groups ($F = 7.694$, $P = 0.0113$, $R^2 = 0.6310$), indicating that andaliman treatment markedly attenuated neuronal degeneration compared to the PE brain group. Furthermore, Tukey's multiple comparison test revealed significant differences between pyknosis and karyorrhexis ($p = 0.0188$) and between pyknosis and karyolysis ($p = 0.0203$), while no significant difference was found between karyorrhexis and karyolysis ($p = 0.9987$). These results suggest that pyknosis represents the most prominent form of nuclear degeneration, whereas karyorrhexis and karyolysis occur at relatively lower and comparable levels.

Discussion

Neuroprotective Effect of Andaliman Extract on BBB Morphology

Histopathological observation (Figure 1) demonstrated clear morphological differences in the blood–brain barrier (BBB) among experimental groups, reflecting the impact of high-salt–induced preeclampsia and the protective effects of andaliman extract. The Non-PE brain group exhibited normal vascular architecture, characterized by round and regular blood vessels, continuous endothelial lining, compact perivascular organization, and preserved basement membranes. This morphology reflects intact BBB function under physiological conditions and is consistent with the normal organization of the neurovascular unit, in which endothelial cells, pericytes, and astrocytic endfeet collectively maintain cerebral homeostasis (Alahmari, 2021).

In contrast, the PE brain group showed marked BBB disruption, including irregular and distorted vascular walls, endothelial cell swelling and discontinuity, and pronounced widening of the perivascular space. These alterations are closely associated with high salt–induced hypertension, which activates the renin–angiotensin–aldosterone system (RAAS) and increases angiotensin II signaling through AT1 receptors. Excessive AT1R activation promotes vasoconstriction, oxidative stress, and the release of antiangiogenic factors such as soluble fms-like tyrosine kinase 1 (sFlt 1), leading to endothelial dysfunction and increased BBB permeability (Sánchez-Aranguren et al., 2014; Tomimatsu et al., 2019; León et al., 2021). Elevated oxidative stress and inflammatory mediators further damage endothelial junctions and basement membranes, resulting in vascular leakage and neurovascular impairment characteristic of preeclampsia (Escudero et al., 2023; Beckett et al., 2023; Bisson et al., 2023).

Administration of andaliman (*Zanthoxylum acanthopodium* DC.) extract resulted in dose-dependent improvement of BBB morphology. Partial restoration was observed at 100 mg/kg BW, whereas near-normal vascular architecture and minimal perivascular widening were evident at 200 mg/kg BW. Quantitative BBB scoring supported these findings, demonstrating significant attenuation of BBB damage following andaliman treatment. The protective effects are likely mediated by the antioxidant and anti-inflammatory properties of andaliman bioactive compounds, which mitigate oxidative stress and preserve endothelial integrity (León et al., 2021; Escudero et al., 2023).

Quantitative BBB morphological scoring corroborated these observations, showing the lowest scores in the Non-PE group and the highest in the PE brain group. Andaliman treatment significantly reduced BBB damage scores in a dose-dependent manner, with the 200 mg/kg BW dose providing the greatest protective effect (Figure 1E). The neuroprotective properties of andaliman are likely mediated by its rich content of flavonoids, alkaloids, and phenolic compounds, which exhibit antioxidant and anti-inflammatory activities. These compounds can suppress reactive oxygen species (ROS), reduce endothelial inflammation, and improve vascular function, thereby stabilizing endothelial junctions and limiting BBB disruption (Effendi, 2020; Reinoviar et al., 2019; Ompusunggu & Irawati, 2021; Adrian et al., 2023).

Cellular Protection by Andaliman Extract Based on Histopathological and Nuclear Degeneration Profiles

Histopathological examination revealed marked differences in the cellular profile of brain tissue among the normal (Non-PE brain), preeclamptic (PE brain), and treatment groups receiving *Zanthoxylum acanthopodium* DC. (andaliman) extract at doses of 100 and 200 mg/kg BW. In the Non-PE brain group, neurons exhibited normal morphology characterized by round nuclei, evenly distributed chromatin, and well-defined cell boundaries, indicating healthy and stable brain tissue. In contrast, the PE brain group showed distinct neuronal damage, including *pyknosis* (nuclear shrinkage and chromatin condensation), *karyorrhexis* (nuclear fragmentation), and *karyolysis* (nuclear dissolution), which are characteristic indicators of

necrosis and apoptosis induced by oxidative stress and inflammation (Bisson et al., 2023; Younes & Ryan, 2019). High salt–induced hypertension exacerbates cerebral endothelial dysfunction and compromises BBB integrity, allowing inflammatory mediators and plasma components to infiltrate brain tissue. This process disrupts cerebral homeostasis, induces hypoxia, and promotes neuroinflammation, ultimately accelerating neuronal death (Canjels et al., 2022; Beckett et al., 2023; Wu et al., 2023). The high levels of nuclear degeneration observed in the PE brain group in this study are consistent with these mechanisms.

Administration of andaliman extract at 100 mg/kg BW reduced nuclear degeneration compared with the untreated PE brain group. Most neurons appeared more intact, with chromatin still evenly distributed, although mild *pyknosis* was occasionally observed. A higher dose (200 mg/kg BW) demonstrated a more pronounced protective effect, showing markedly fewer degenerating nuclei and a more organized tissue structure. These findings indicate that andaliman exerts a dose-dependent neuroprotective effect by reducing oxidative and inflammatory injury (Adrian et al., 2023; Ompusunggu & Irawati, 2021).

Quantitative analysis of nuclear degeneration supported the histological observations. The PE brain group exhibited the highest scores for *pyknosis*, *karyorrhexis*, and *karyolysis*, while the Non-PE brain group had the lowest values. Treatment with andaliman extract significantly decreased these parameters, with the most significant reduction observed at 200 mg/kg BW. These findings align with those of Effendi (2020) and Reinoviar et al. (2019), who reported that andaliman fruit extract possesses potent antioxidant activity through DPPH radical scavenging, thereby preventing oxidative damage to cellular membranes and nuclei.

The Role of Andaliman Antioxidants in Suppressing Reactive Oxygen Species (ROS) in the Pathophysiology of Preeclampsia

High salt intake during pregnancy contributes to hypertension through both hemodynamic and molecular mechanisms. Excess sodium promotes water retention, increasing plasma volume and blood pressure, while simultaneously disrupting angiogenic regulation. Excessive salt intake has been associated with reduced vascular endothelial growth factor (VEGF) expression, leading to endothelial dysfunction. This reduction is closely linked to angiotensin II type 1 receptor (AT1R) activation, which stimulates the release of soluble fms-like tyrosine kinase-1 (sFlt-1), thereby decreasing VEGF bioavailability and promoting the early development of preeclampsia-related endothelial injury (Sulistyowati et al., 2015).

High salt consumption also activates the renin–angiotensin–aldosterone system (RAAS). Increased angiotensin II signaling through AT1R induces vasoconstriction, sodium retention, sympathetic activation, and aldosterone release, resulting in sustained hypertension (Ames et al., 2019). During pregnancy, excessive AT1R activation impairs uteroplacental perfusion, leading to placental hypoxia and triggering oxidative stress and antiangiogenic factor release, which are central to preeclampsia pathogenesis (Sánchez-Aranguren et al., 2014; Tomimatsu et al., 2019).

Oxidative stress, arising from an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, plays a pivotal role in endothelial dysfunction in preeclampsia. Excess sodium intake enhances ROS generation, reducing nitric oxide (NO) bioavailability and further impairing vascular homeostasis. Elevated oxidative stress also increases sFlt-1 and soluble endoglin (sENG) levels while suppressing VEGF and transforming growth

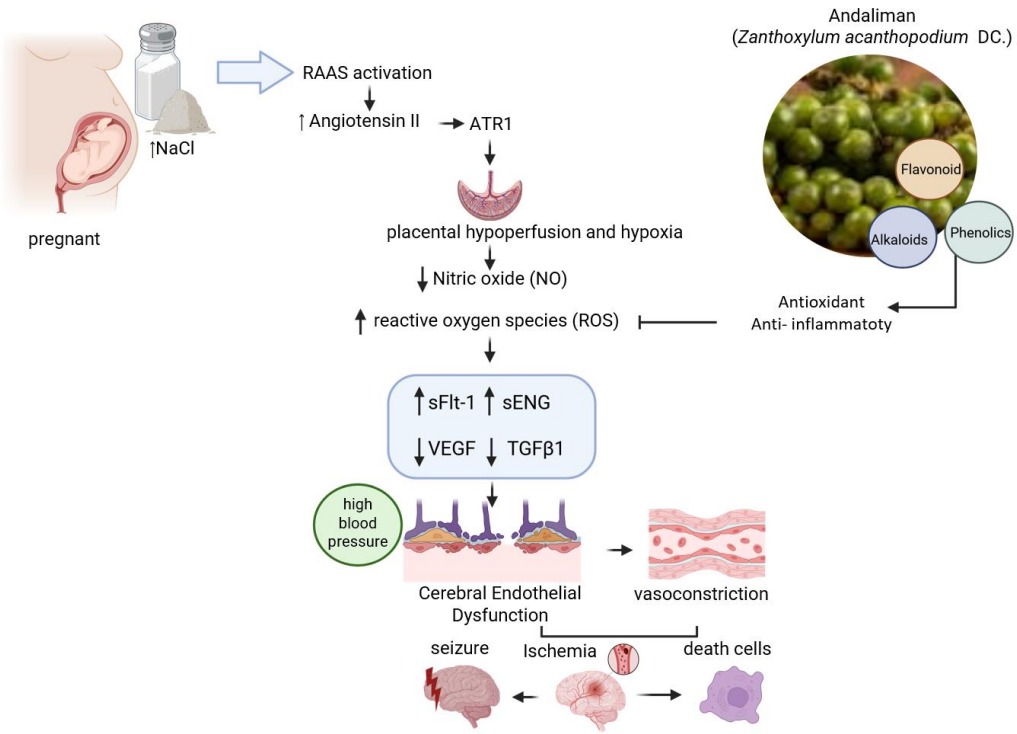

Fig. 3. Proposed mechanism of salt-induced preeclampsia and the protective role of *Zanthoxylum acanthopodium* (andaliman)

factor-β1 (TGF-β1), thereby exacerbating angiogenic imbalance and inflammation (Sánchez-Aranguren et al., 2014).

Cerebral endothelial dysfunction disrupts autoregulation of cerebral blood flow, resulting in vasoconstriction, ischemia, and compensatory vasodilation. In damaged endothelium, this response leads to increased blood–brain barrier permeability and vasogenic edema, contributing to the development of Posterior Reversible Encephalopathy Syndrome (PRES) and its neurological manifestations, including seizures and eclampsia (Narkhed & Karnad, 2021).

Andaliman (Zanthoxylum acanthopodium DC.) extract, rich in flavonoids, alkaloids, and phenolic compounds, exhibits potent antioxidant and anti-inflammatory properties. By suppressing ROS production, preserving NO bioavailability, and restoring angiogenic balance, andaliman may protect cerebral endothelial integrity, reduce brain tissue injury, and mitigate neurological complications associated with preeclampsia (Figure 3; Santoso et al., 2023).

Conclusion

Zanthoxylum acanthopodium DC. (Andaliman) extract significantly improved BBB integrity and neuronal morphology in a preeclampsia model in a dose-dependent manner. The 100 mg/kg dose produced moderate improvements in endothelial structure and neuronal organization, reducing nuclear degeneration but not fully restoring normal histology. In contrast, the 200 mg/kg dose showed a significantly more substantial protective effect ($p < 0.05$, ANOVA), with a more intact BBB, well-arranged neuronal layers, and minimal cellular damage. This enhanced efficacy is likely attributed to the antioxidant and anti-inflammatory activities of Andaliman's bioactive compounds, particularly flavonoids and polyphenols, which mitigate oxidative stress and stabilize neuronal membranes. Therefore, the 200 mg/kg dose is considered the most effective in providing cellular protection against preeclampsia-induced brain injury.

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