Full Length Research Article

Andaliman fruit (*Zanthoxylum acanthopodium* DC.) Improves Physiological Condition in Preeclamptic Rat through Angiotensin II Receptor Type 1 Inhibition

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ABSTRACT

Background: Preeclampsia (PE) is a severe pregnancy complication involving the AT1R overactivation. Andaliman fruit showed potential for managing preeclampsia, but their effects on the renin-angiotensin system are not well-known. This study aimed to virtually analyze angiotensin II type 1 receptor (AT1R) inhibition by Andaliman Fruit (*Zanthoxylum acanthopodium* DC.) bioactive compounds and evaluated the effect through an in vivo approach.

Methods: The AT1R protein structure was modeled from the AT1R gene, and computational analysis was performed to identify potential molecules to inhibit AT1R. Wistar rats (n=25) were divided into five groups: normal pregnancy, PE pregnancy, PE + candesartan 5 mg/ kg, PE + Andaliman 100 mg/ kg BW, and PE + Andaliman 200 mg/ kg BW. Blood pressure and proteinuria levels were measured on days 5, 13, and 21 of pregnancy. Serum and kidney Angiotensin- Converting Enzyme (ACE) levels were measured by ELISA, and kidney TNF-alpha levels by dot blotting.

Results: Kaempferol was indicated as the most potential compound to inhibit AT1R. In the late gestation period, Andaliman treatment significantly improved the blood pressure (124.5 mmHg) compared to the PE group (174.1 mmHg) ($P < 0.01$). Proteinuria level showed improvement in a dose- dependent manner. Serum and kidney ACE levels were downregulated significantly ($P < 0.05$) at the highest dose of Andaliman (17.6 and 19.9 ng/mL), as well as the kidney TNF-alpha expression (12,503,447 INT/mm^2).

Conclusion: The study result found bioactive compounds from Andaliman possibly inhibited AT1R to return the blood pressure to normal level, improved proteinuria condition, and lowered the ACE and TNF-alpha protein levels.

INTRODUCTION

Preeclampsia is one of the most life-threatening complications in pregnancy, characterized by the presence of hypertension and proteinuria after 20 weeks of gestation. The World Health Organization in 2022 estimated preeclampsia incidence has reached 10% of pregnancies worldwide [1]. Annually, preeclampsia is responsible for around 70,000 maternal and 500,000 fetal mortalities [2]. The pathogenesis of preeclampsia arises from insufficient invasion of the cytotrophoblast. This will lead to the failure of spiral artery remodeling, creating a narrow spiral artery that cannot supply adequate blood and oxygen. This hypoxia condition stimulated the production of pro-oxidative, pro-inflammatory, and anti-angiogenic factors from the placenta, which caused further dysregulation in the maternal body [3].

Due to its complex etiology, currently no effective curative method is available for preeclampsia [4]. On the other hand, natural resources from plants offer a wide range of bioactive compounds with promising multi targets potential for preeclampsia management [5]. Andaliman (*Zanthoxylum acanthopodium* DC.), a native plant from North Sumatra, Indonesia, has been actively studied for its anti-preeclamptic activity. Research has demonstrated that the Andaliman administration can ameliorate multiple symptoms of preeclampsia. Situmorang et al. [6] was reported that Andaliman nano herbal administration in salt-induced preeclamptic rat model can reduce blood pressure, proteinuria levels, and improve fetal weight. Additionally, Andaliman extract has been shown to suppress the elevation of proinflammatory cytokines in LPS-induced preeclamptic rats [7]. Attenuation of organ damage has also been reported in the kidney and liver [8,9]. However, no studies have reported yet on the effects of Andaliman on the renin-angiotensin system (RAS).

The RAS is a primary cascade responsible for regulating blood pressure, consisting of several peptide cleavages through enzymatic reactions. The first substrate, angiotensinogen, is cleaved by renin to produce angiotensin I (Ang I). The Ang I is later processed by an Angiotensin-Converting Enzyme (ACE) to achieve its active form the angiotensin II (Ang II) [10]. The angiotensin II receptor type 1 (AT1R) is the main receptor that links this system to its pathological role. Overactivation of AT1R may lead to excessive vasoconstriction, water and sodium retention, and stimulating the expression of pro-inflammatory gene such as TNF-a [11]. Notably, AT1R activation also give positive feedback mechanism to upregulate the upstream component of RAS [12]. In preeclamptic conditions, studies have confirmed expression alteration on this pathway and the presence of autoantibodies enhancing the activation of AT1R. Therefore, AT1R becomes one of the important components in preeclampsia underlying the hypertension and proteinuria progression [12,13].

Numerous research has revealed the anti-hypertensive mechanism of most medicinal plants was related to its ability to suppress the RAS. The bioactive compounds such as flavonoids and saponins may work by directly inhibiting the interaction between Ang II and AT1R or occupied ACE active site to prevent the formation of Ang II. Furthermore, some plant also showed modulation in the expression level [14-16]. Thus, we suspected Andaliman may interfere with the RAS cascade to improve the physiological condition in preeclampsia. The ACE inhibition has been evaluated in previous computational study [17]. In this study, we aimed to predict AT1R inhibition virtually, while evaluating the effect of Andaliman methanol extract administration on the blood pressure, proteinuria level, ACE expression, and TNF-α expression, which representing the result of AT1R inhibition in the preeclamptic rat model. Considering the crucial role of the intrarenal RAS in regulating body fluid homeostasis and proteinuria [12], this study primarily focuses on the kidney RAS.

METHODS

The DNA isolation was performed based on [18]. 0.1 gram of kidney was ground using cold mortar in DNA lysis buffer (10 mM Tris-Cl, 100 mM NaCl, 25 mM EDTA, 0.5% SDS, 0.1 mg/mL Proteinase K). The homogenate was incubated for 3 hours and centrifuged at 13,000 rpm for 4°C 10 minutes. The supernatant was centrifuged by 1x volume PCI 13,000 rpm 25°C for 10 minutes. The upper aqueous phase was then collected and mixed with 2.5 volume absolute ethanol and incubated at -20°C for 1 hour, then centrifugated at 13,000 rpm 4°C for 10 minutes. The pellet was washed with 70% ethanol and centrifuged at 13,000 rpm 4°C for 10 minutes. The pellet was dried overnight and resuspended by Tris-EDTA pH 7.6.

The primer was designed to amplify partial sequence (701 bp) from the third exon of angiotensin II receptor type 1a gene (forward 5’-TCTCAATCTCGCCTTGGCTG-3’ and reverse 5’-

ACAGAGGGTTCAGGCAGTTG-3'), based on Rat Angiotensin Receptor gene (Gen bank ID: M86912.1) as reference sequence. The PCR program consists of hot start 95°C for 5 minutes (1 cycle), denaturation 95°C for 30 seconds, annealing 57°C for 45 seconds, extension 72°C for 45 seconds (35 cycles), and post- extension 72°C for 7 min. The amplification results were confirmed by 1.5% agarose gel electrophoresis. The sequencing was performed using ABIPrims 3730xl DNA Sequencer (Koeln, Germany) and analyzed by Bioedit software [18,19]. The sequence of AT1R gene was translated to amino acid sequence using ExPasy translate tools (https:// web.expasy.org/ translate/). The amino acid sequence was uploaded to the Alpha Fold database (https:// alphafold.ebi.ac.uk) to search the 3D structure. The protein model with the highest homology was selected [20].

Bioactive compound screening for bioavailability and bioactivity

The phytochemical content of Andaliman fruit was collected from previous studies with the total of 51 compounds [21– 23]. The canonical smile and 3D structure of the Andaliman fruit bioactive compounds were selected from the PubChem database (https:// pubchem.ncbi.nlm.nih.gov/). The bioavailability of each compound was evaluated based on Lipinski's Rule of Five (molecular weight ≤ 500, hydrogen bond acceptors ≤ 10, hydrogen bond donors ≤ 5, and MLogP ≤ 5) and gastrointestinal absorption (GIA) rate using SwissADME (http:// www.SwissADME.ch/). The potential of the selected compound to exert the desired bioactivity related to AT1R inhibition (vasodilator, TNF expression inhibitor, and kidney function stimulant) was evaluated using PASS Online. The compounds that meet the criteria then continued for molecular docking analysis [24].

Molecular docking analysis

The ligands (Ang II, candesartan, and bioactive compounds) energy was minimized using open babel tools in PyRx. The ligand was docked to AT1R protein by blind docking method using Autodock Vina in PyRx. Each ligand pose with the highest affinity was selected and merged with AT1R protein in PyMol. The binding affinity threshold was set to -7 Kcal/ mol which indicated strong binding. The interaction was visualized in Biovia Discovery Studio [24,25].

In Vivo Experiment

Preparation of Andaliman Fruit Extract

Andaliman fruits were dried at 40°C and ground. Subsequently, the powder was macerated by absolute methanol (1:10) for 24 hours. The macerate then evaporated at 40°C., and the concentration was adjusted by distilled water [26,27].

Experimental Procedure

Acclimatization was carried out for 7 days before the experiment, then the rats were mated in 2:1 ratio between female and male rats. The presence of a vaginal plug counted as day 0 of pregnancy. The pregnant rats (n = 25) were randomized into five groups, Non- PE (normal pregnancy), PE (10 mL 18% NaCl/ kg body weight), PEcan5 (PE + Candesartan 5 mg/ kg body weight) , PEand100 (PE + Andaliman 100 mg/ kg body weight), and PEand200 (PE + Andaliman 200 mg/kg body weight) [6,28]. The NaCl administration was done by oral gavage from day 5 to 13 with the basis of starting of implantation on day 5, continued by the treatment with candesartan or Andaliman extract from day 14 to 21. The mean arterial pressure was measured by the tail-cuff method, while the proteinuria level was measured by dipstick, on the day 5, 13, and 21 [6]. The measurement on Day 5 reflects the rats' condition before preeclampsia induction, the Day 13 measurement ensures the rats have exhibited the main preeclampsia symptoms (high blood pressure and proteinuria), and the Day 21 measurement analyses the effect of the treatment on these symptoms. The rats terminated on day 21, and then the serum and organs were isolated and stored at -20°C until further use. The protocol was approved by the Brawijaya University Animal Care and Use of Ethics Committee with protocol number 107-KEP-UB-2024 dated 21-06-2024.

Angiotensin-converting enzyme (ACE) concentration measurement

The level of serum and kidney ACE were measured by Rat Angiotensin Converting Enzyme Kit (Bioassay Technology Laboratory) according to the manufacturing procedure.

Kidney TNF-α measurement

Approximately 0.1 gram of the kidney was ground using cold mortar in protein lysis buffer (1 mM PMSF in DMSO, 50 mM KH_2PO_4 pH 7.4, 0.5% Nonidet P-40). The homogenate was centrifuged at 10,000 rpm 4°C for 20 minutes, and the supernatant was stored [18]. The TNF-α concentration in the kidney was measured by dot blotting. Protein concentration was adjusted by Tris-Cl pH 6.8. The protein was incubated on PVDF membrane in the dot blot apparatus for 30 minutes, then the membrane was blocked by PBS-skim milk 5% for 1 hour. The primary antibody was using TNF alpha rabbit polyclonal antibody 1:1500 (BIOSSUSA) and goat anti-rabbit IgG alkaline phosphatase-conjugated 1:2500 (Invitrogen) for the secondary antibody. The intensity of the dot was visualized by GelDoc and measured using ImageJ [19,29].

Data analysis

The data analysis was performed using IBM SPSS Statistics. Data normality was assessed by the Shapiro-Wilk test. A t-test was used to assess whether differences between treatment groups were statistically significant.

RESULTS

Angiotensin II Receptor Type 1a Gene Sequencing and Protein Modelling

The exon 3 region from the AT1R gene from the kidneys was amplified and sequenced (Figure 1). The agarose visualization confirmed the amplicon size around 700 bp for all treatment groups (Figure 1B). No genetic difference was found among the treatment groups. However, three single nucleotide polymorphisms (SNP) were found in the treatment groups against the control gene sequence (Figure 1A), which consisted of a transversion mutation (T28 -> A28), and two transition mutations (T271->C271 and T380->C380). The translated protein sequence (Figure 1C) showed 100% similarity with the P25095 AT1Ra model in AlphaFold. This 3D structure then used for the molecular docking analysis.

Bioavailability Screening and Bioactivity Prediction

The bioavailability screening showed that 39 compounds met the parameters, with only 22 compounds predicted to exert biological activity related to the AT1R inhibition and subjected to molecular docking. in silico screening for bioavailability and bioactivity of the compounds revealed five most potential candidates, including kaempferol, quercetin, resveratrol, farnesyl acetate, and (e,e)-farnesyl acetone. Based on the screening results, these compounds were shown to fulfill Lipinski's rule of 5 and have high GIA rate (Table 1). Bioactivity prediction showed these compounds potentially exert bioactivity related to AT1R inhibition with Pa > 0.3 (Figure 2).

Molecular Docking

Figure 3 and Table 2 show the interaction of the AT1R receptor with the native substrate Ang II, control drug, and selected bioactive compounds. From the total of 22 compounds, only 5 compounds meet the binding affinity threshold (-7 Kcal/mol). The native substrate Ang II formed 23 interactions with AT1R, consisting of salt bridge, electrostatic interactions, hydrogen bonds, and hydrophobic bonds with -10.8 Kcal/ mol binding affinity. All the ligands were found the occupied the same site with the Ang II. The control ligand candesartan has the closest affinity to Ang II (-10.1 Kcal/ mol). The bioactive compounds with the highest affinity to bound to AT1R were kaempferol (-8.6 Kcal/ mol), followed by quercetin (-8.4 Kcal/ mol), resveratrol (-7.8 Kcal/ mol), farnesyl acetate (-7.4 Kcal/ mol), and (e,e)- farnesyl acetone (-7.4 Kcal/ mol). The candesartan binds to 6 target residues of Ang II, while the bioactive compounds interact with 3 to 4 target residues. Several residues which not the target of Ang II were also found to interact with candesartan and bioactive compounds, namely TYR32, PRO285, and ILE288.

Physiological Parameters

The mean arterial pressure and proteinuria level on days 5, 13, and 21 of pregnancy are shown in Figure 4. Throughout the pregnancy, the control group was not experienced either blood pressure elevation or proteinuria. Preeclampsia induction managed to increase the blood pressure on the 4 treatment groups after day 13, ranging from 160 to 171 mmHg accompanied by proteinuria (<30 mg/dL - <300 mg/dL). At the end of pregnancy, the blood pressure of the untreated preeclampsia (PE) group remained high (174 mmHg) with severe proteinuria condition. Administration of candesartan and both dose of Andaliman methanolic extract significantly reduced blood pressure ($P < 0.01$) against the PE group, respectively at 122, 119, and 124.5 mmHg. The improvement of proteinuria by the 200 mg Andaliman dose group was slightly higher than the group using candesartan, while the 100 mg Andaliman did not show promising improvement.

ACE Concentration

Figure 5 shows the ELISA measurement of kidney and serum ACE. Preeclampsia inductions were observed to upregulate ACE expression in the kidney and serum (27.4 and 26.9 ng/mL). Candesartan treatment showed non-significant reduction in tissue ACE (23.8 ng/mL), whereas serum ACE expression was significantly reduced (19.4 ng/mL) ($P < 0.05$). In contrast, lower doses of Andaliman decreased tissue ACE expression significantly (18.8 ng/mL), whereas serum ACE was not significantly different (23.1 ng/mL). In the higher dose, Andaliman treatment significantly reduced ACE expression in both the kidney and serum (17.6 and 19.9 ng/mL).

TNF-α Expression

TNF-α expression level in the kidney is showed in Figure 6. The preeclampsia group showed much higher TNF-α expression in the kidney (19,800,316 INT/mm^2) than the normal pregnancy (11,291,787 INT/mm^2). The 2 doses of Andaliman were observed to reduce TNF-α expression by highly significant ($P < 0.01$), respectively 12,872,178 and 12,503,447 INT/mm^2 for the 100 mg and 200 mg doses. Overall, the candesartan treatment reduced TNF-α expression, but the result is varied among the individual and not considered as significant (15,187,390 INT/mm^2).

Figures

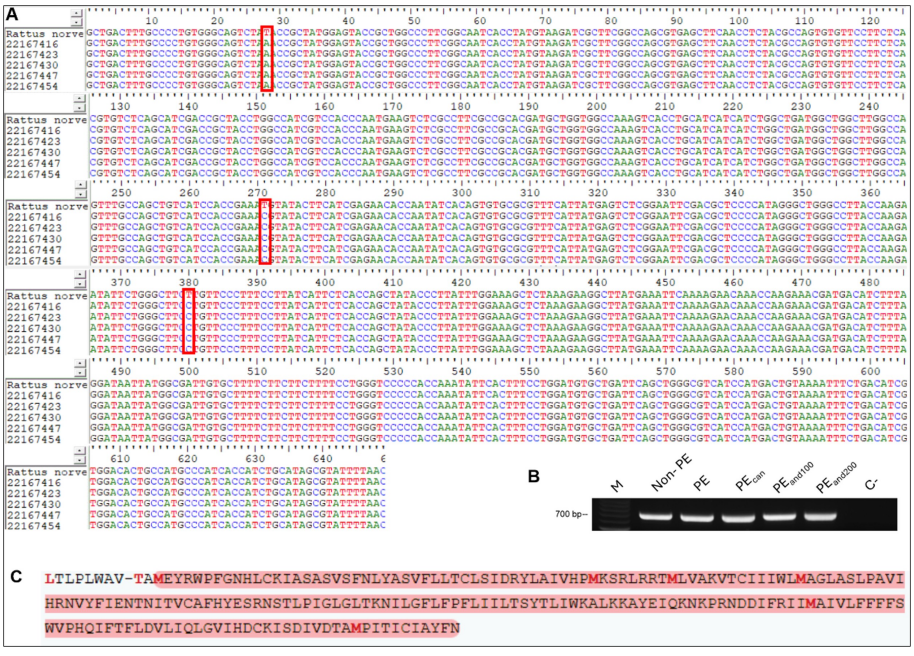

Figure 1: Gene and protein analysis of angiotensin II receptor type 1a (AT1R) across all experimental groups: A) nucleotide sequence of the AT1R gene; B) kidney AT1R amplicons visualized on a 1.5% agarose gel; C) translated protein sequence generated using ExPASy. The red box indicates the single nucleotide polymorphism (SNP) observed relative to the control sequence. M: marker; C−: negative control.

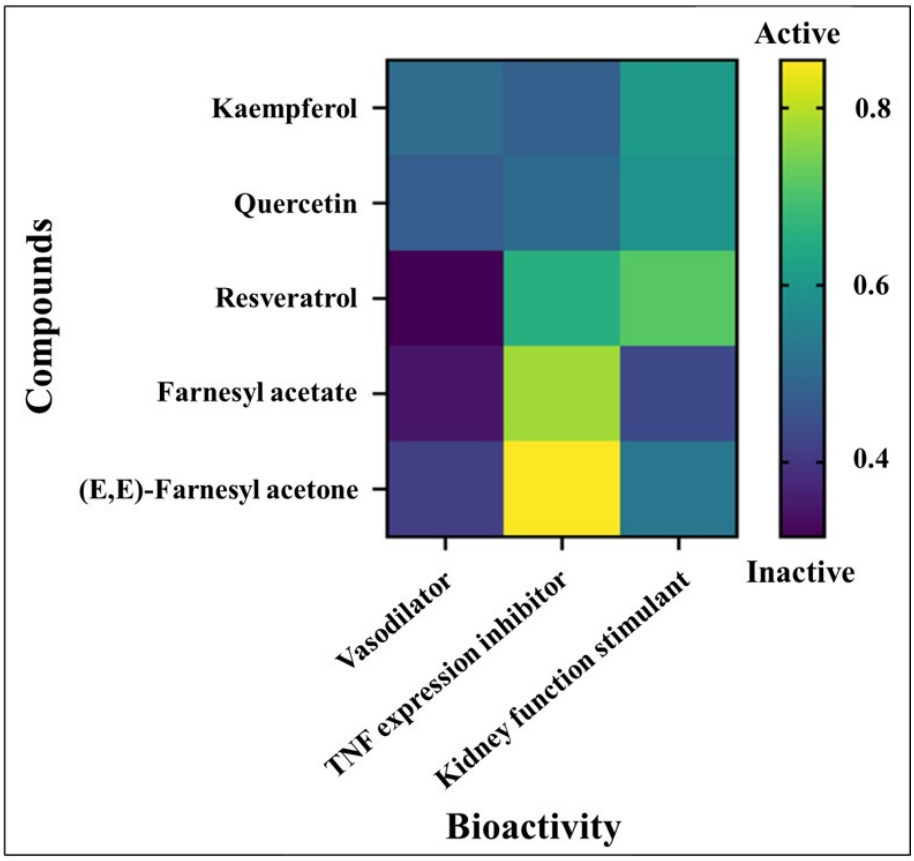

Figure 2: Bioactivity prediction result using PASS Online web server. The 5 potential compounds exhibited active probability > 0.3 for the 3 bioactivities related to AT1R inhibition.

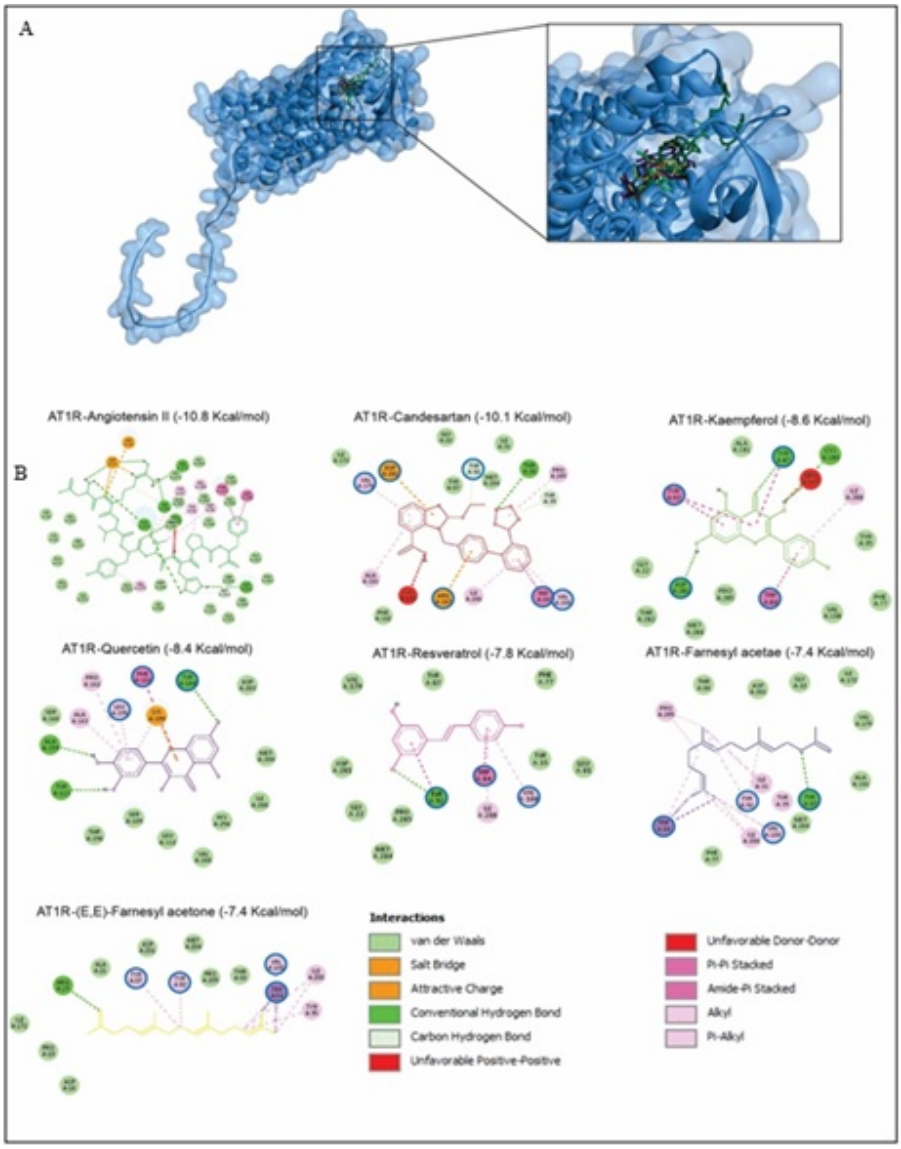

Figure 3: Molecular interaction of angiotensin II receptor type 1a with the native substrate (angiotensin II), candesartan, and Andaliman fruit bioactive compounds. A) 3D structure of the receptor-ligand complex B) 2D diagram of ligand interactions. The blue circle marked target residue that is similar with the native substrate.

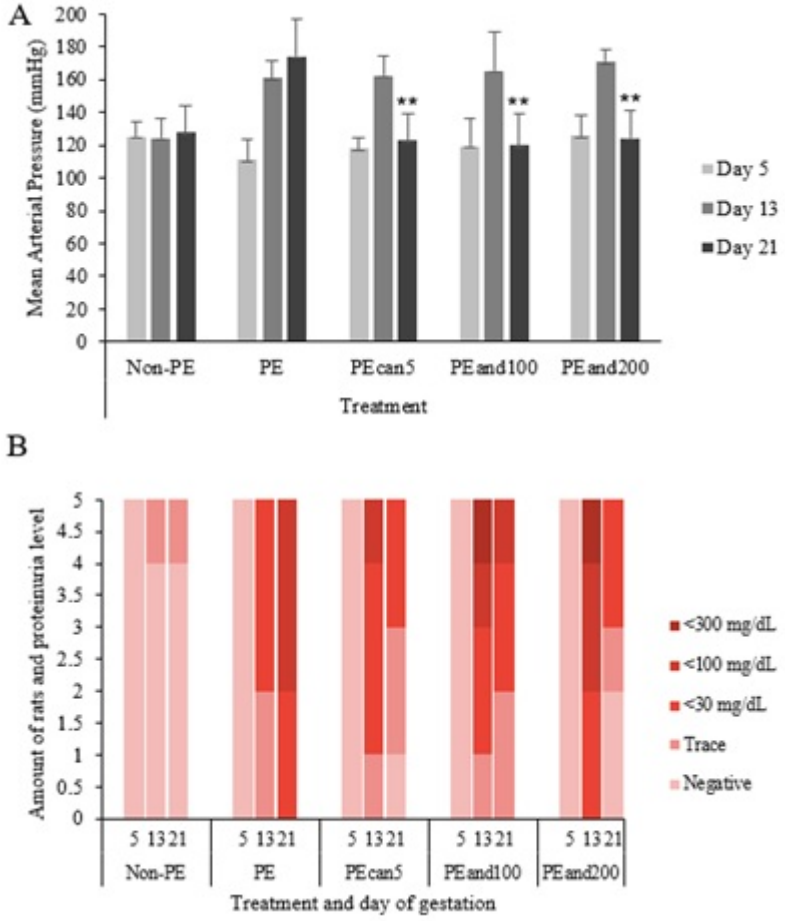

*Figure 4: Physiological Data on day 5, 13, and 21 among the treatment groups A) Blood Pressure B) Proteinuria. ** P < 0.01 versus the PE group.*

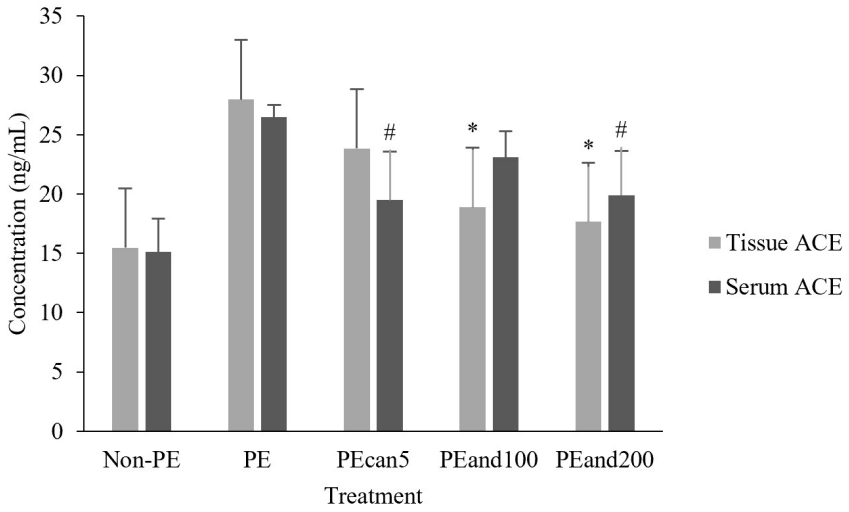

*Figure 5: ACE concentration in serum and kidney measured by ELISA. */# P < 0.05 versus the PE group.*

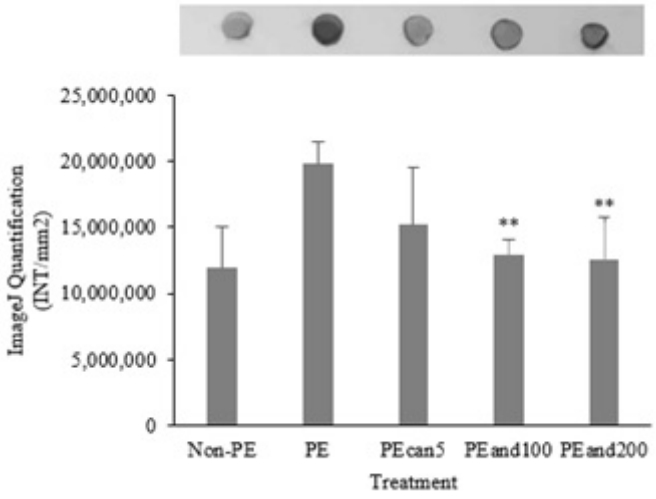

*Figure 6: Kidney TNF-α expression measured by dot-blotting. ** P < 0.01 versus the PE group.*

Tables

CID	Compounds	Lipinski rule of 5					GIA	Reference
		MW	H-bond acceptors	H-bond donors	Consensus Log P	Total Violation		
5280863	Kaempferol	286.24	6	4	1.58	0	High	[22]
5280343	Quercetin	302.24	7	5	1.23	0	High	[22]
6603962	Resveratrol	228.24	2	3	2.48	0	High	[22]
638500	farnesyl acetate	264.4	2	0	4.73	0	High	[21]
1711945	(e, e)-farnesyl acetone	262.43	1	0	5.04	1	High	[23]

Table 1: Bioavailability prediction result using SwissADME web server. The 5 potential compounds showed no more than 1 Lipinski rule violation and high GIA rate, which indicate good oral bioavailability. MW: molecular weight, GIA: gastrointestinal absorption.

Ligand (Affinity)	Types of interaction	Residues
Ang II (-10.8 Kcal/mol)	Salt bridge	ASP17
	Electrostatic interaction	ASP16, ASP17
	Hydrogen bond	ASP17, ASP17, ARG167, TYR184, TYR184, ASP263, ILE266, ILE266, ILE271, ASP281
	Hydrophobic bond	TRP84, TYR87, TYR87, TYR92, VAL108, VAL179, PHE182, LEU195, LEU195, GLY196
Candesartan (-10.1 Kcal/mol)	Salt bridge	ARG167
	Electrostatic interaction	ASP281
	Hydrogen bond	TYR35, THR88, TYR92
	Hydrophobic bonds	TYR35, TRP48, TYR92, TRP84, VAL108, VAL179, ALA181, PRO285, PRO285 and ILE288
Kaempferol (-8.6 Kcal/mol)	Hydrogen bond	TYR87, CYS180, ASP281
	Hydrophobic bond	TRP84, TRP84, TYR87, TYR92, TYR92, ILE288
Quercetin (-8.4 Kcal/mol)	Electrostatic interaction	LYS199
	Hydrogen bond	TYR113, ALA159, TYR184
	Hydrophobic bonds	PRO162, ALA163, PHE182, LEU195, LYS199, LYS199
Resveratrol (-7.8 Kcal/mol)	Hydrogen bond	TYR92
	Hydrophobic bonds	TRP84, TRP84, TYR92, VAL108, ILE288
Farnesyl acetate (-7.4 kcal/mol)	Hydrogen bond	TYR87
	Hydrophobic bonds	ILE31, TYR35, TRP84, TRP84, TRP84, TRP84, TYR92, TYR92, TYR92, TYR92, VAL108, VAL108, PRO285, PRO285, ILE288
(e,e)-farnesyl acetone (-7.4 Kcal/mol)	Hydrogen bond	ARG24
	Hydrophobic bonds	TYR35, TRP84, TRP84, TRP84, TRP84, TRP84, TRP84, TYR87, TYR92, TYR92, VAL108, MET284, PRO285, ILE208, ILE208

Table 2: Interactions summary between the ligands and AT1R. Residues in bold indicate shared targets of Ang II and the inhibitor.

DISCUSSION

In silico methods have become a powerful tool in identifying mechanism of action from a compound. The Lipinski rule of 5 and GIA rate parameters were required to ensure the compounds can be absorbed properly and enter the blood stream to give the intended effects [30]. The bioactivity prediction integrated molecular structure, biological, and chemical data with modeling algorithms to predict biological activity [31]. Molecular docking method then evaluated the interactions of the selected compounds with the protein targets. Our computational analysis showed that 5 potential bioactive compounds occupied the same site and shared several similar target residues with the native substrate, indicating the compounds may act as competitive inhibitors [32]. However, they showed lower affinity toward AT1R compared to candesartan. Moreover, the candesartan was bound to six similar target residues of Ang II, while the bioactive compounds were bound to only three to four similar residues. Nonetheless, bioactive compounds might work synergistically in inhibiting a protein target [33].

The candesartan might lower blood pressure and proteinuria by specifically inhibiting the AT1R, preventing the activation of secondary messengers (IP3, DAG, and PLCβ) and several kinase families (Src, Rho, WNK, and SPAK) which are responsible for inducing vascular smooth muscle cell contraction [11]. It also reduced the activation of mitogen-activated protein kinase (MAPK) signaling that contributed to fibrosis and inflammation in renal tissue [34], thus, reducing the kidney damage and proteinuria level. The dose of 5 mg/kg is recommended by several study to be effectively treat RAS related hypertension and several cardiovascular issue [35– 37]. We avoided higher dose as there is probability of miscarriage. The blood pressure- lowering mechanism of Andaliman fruit has not been elucidated yet, but the computational analysis (Figure 3) suggested the possibility of AT1R inhibition. Several researches have also reported kidney improvement under Andaliman treatment, including decreased kidney necrosis in preeclamptic rats [8], decreased urea level, kidney damage score, and increased glomerular diameter in tartrazine-induced rats [38]. These studies suggested that the bioactive flavonoid compounds of Andaliman fruit can donate electrons to free radicals to protect the kidney and its functionality.

Modulation of ACE expression by candesartan and Andaliman methanolic extract is derived from disruption of the "feed forward" mechanism in the RAS. Activation of AT1R by Ang II somehow created positive feedback which mediated the upregulation of RAS components including ACE [12]. The research by Koka et al. [39] showed AT1R activation by Ang II infusion upregulated ACE in the human kidney tubular epithelial cell line, while AT1R blockade using losartan managed to completely prevent this upregulation. Unfortunately, our candesartan treatment to inhibit AT1R didn't show significant decrease in the tissue ACE, which may be affected by the drug dose and in vivo environment. Another factor that may cause candesartan to reduce serum ACE levels more effectively is related to its pharmacokinetic properties. Candesartan has a high plasma protein binding capacity, which may limit its distribution into tissues [40]. In contrast, the compounds kaempferol and quercetin have lower plasma protein binding compared to candesartan, allowing them to more easily moved from the bloodstream to kidney tissues. This may cause the candesartan to primarily inhibit systemic AT1R while the bioactive compounds targeted more AT1R in the tissue.

We observed that the expression of kidney TNF-α (Figure 6) was proportional to the tissue ACE expression (Figure 5), where higher tissue ACE concentration was related to higher TNF- α expression. Elevation of ACE expression can mediate higher conversion of Ang I to Ang II [41]. Then Ang II is recognized by AT1R, and finally, NF-κB is activated to stimulate the expression of the pro- inflammatory cytokine TNF- α [37]. Elevated TNF- α contributes to inflammation and endothelial dysfunction, exacerbating kidney damage [42]. The varied and insignificant reduction of TNF-α expression under candesartan treatment suggest the involvement of other pathways related to TNF- α activation in the preeclamptic rat kidney outside the RAS [43]. However, regarding the kidney damage, the lower kidney TNF- α expression in the 100 mg Andaliman group did not result in better proteinuria condition (Figure 2B). In contrast, although candesartan treatment could not significantly reduce TNF-α expression, it still showed better improvement for proteinuria. Therefore, TNF-α alone was insufficient as a single marker for kidney damage severity.

While our study limiting the AT1R as the target to reduce TNF-α, it is important to acknowledge the potential involvement of other inflammatory pathways. The review by Al-Khayri et al. [44] highlighted several mechanisms of plant derived compounds to reduce TNF-α. Some membrane

receptors such toll-like receptor (TLR), endothelial growth factor receptor (EGFR), and tumor necrosis factor receptor (TNFR) which involved in TNF-α expression are reported to be the targets of anti-inflammatory medicinal plant. Moreover, some research also reported the possibility of bioactive compounds to enter the cell and inhibited the downstream pathway of the receptor including the NF-κB itself to prevent TNF-α expression. Additionally, the compounds may regulate TNF-α expression by modulating oxidative stress via the Nrf2 signaling pathway and inhibiting cyclooxygenase (COX), which reduces prostaglandin levels and subsequently suppresses NF-κB and MAPK activation. The bioactive compounds in Andaliman might interact with these pathways, while candesartan was not.

The etiology of preeclampsia in humans remains incomplete, probably because the disease involves complex interactions between various systems in the body, including the immune, nervous, cardiovascular, reproductive, and other systems [45]. Consequently, mimicking the human preeclampsia condition in an animal model has become challenging [46]. Several methods have been developed to induce pre-eclampsia in rat model, such administration of N(ω)-nitro-L-arginine methyl ester (L-NAME), DOCA-salt, and NaCl. The L-NAME act as nitric oxide synthase (NOS) inhibitor, reducing the availability of the nitric oxide which important for vasodilatation and maintaining vascular fuction [47]. Meanwhile the DOCA is a synthetic mineralocorticoid that mimics aldosterone to induce water retention. We used the NaCl induction as it may decrease NO level and induce oxidative stress [48], water retention [49], also it is more affordable and reproducible [50].

The early sodium intake manipulation in pregnant rats was performed by Beauséjour et al. [51]. Replacement of drinking water with 1.8% NaCl in late pregnancy causes physiological changes that resemble preeclampsia, especially increased blood pressure and proteinuria. Tyurenkov et al. [50] experimented by administering 1.8% NaCl solution during pregnancy. The study reported edema, endothelial dysfunction, and most importantly, impaired trophoblast invasion of the placenta. NaCl induction has been adopted in several studies evaluating the effect of Andaliman administration on preeclampsia in Wistar rats [6,9,52]. The studies used 6% NaCl at a dose of 3 mL/day/200 g body weight (BW) injected subcutaneously from day 6 to 12. The results showed no significant increase in diastolic blood pressure with this treatment, and re-evaluation of the NaCl dose was suggested. Thus, we decided to use a higher dose of 10 mL of 18% NaCl/kg rat body weight/day via oral gavage as reported by [28]. Unfortunately, no study has evaluated the presence of AT1R autoantibody in salt-induced preeclamptic rats, but the overexpression of the RAS prior to salt induction particularly in the kidney has been confirmed [53].

Currently there is no study describing the exact mechanism of how NaCl induced preeclampsia, we suggested it may relate to the activation of RAS. The downstream signaling of RAS, particularly the NADPH oxidase can generate ROS and cause oxidative stress, while the NF-κB pathway trigger the inflammatory cascade [11]. This can damage the cells lining the vessels and cause endothelial dysfunction, resulted in decreased NO availability which important for placenta angiogenesis, spiral artery remodeling, and trophoblast invasion [2]. After the preeclampsia onset, the symptoms then exacerbate to the wider dysregulation troughout the body.

The alteration of RAS expression in human preeclampsia was summarized by Leal et al. [13]. In circulating RAS there is an abnormal decrease in the expression of renin, Ang II, and aldosterone, while the expression of circulating ACE was inconsistent, as some research reported upregulation, and others reported no difference or decreased expression. On the contrary, components of the local or tissue RAS are consistently upregulated. In our animal model, we found that both serum (circulating) and kidney (local) ACE were upregulated. As the consequence, the number of Ang II might be higher and activating more AT1R, resulted in excessive vasoconstriction. The increased kidney renin release led to water and sodium retention, which resulted in elevated blood pressure. The highly stimulated TNF-α expression also damages renal podocytes and glomerulus, allowing the protein to escape to the urine [12]. Normally, the overexpression of the kidney RAS leads to the suppression of renin release, thereby limiting the systemic activation of RAS. This mechanism is believed to explain the distinct alterations observed between human tissue and systemic RAS in preeclampsia. Since we observed increased ACE expression in systemic RAS, we suggest future research to comprehensively map the different expression pattern of circulating RAS component between preeclampsia in the human and animal model.

Even though RAS inhibitor can improve the physiological condition in the maternal body, the

blockade of RAS over-activation in preeclampsia is paradoxical. Several adverse outcomes for the fetus have been reported [54]. The current theory suspects the RAS antagonist drugs may cross the placenta and inhibit the RAS in the fetus. As a consequence, the baby undergoes renal function disturbance, delayed growth, hypotension, and even miscarriage [55,56]. Fortunately, the research by Situmorang et al. [6] showed Andaliman treatment does not affect fetal number and weight. With its minimum side effect and numerous beneficial biological activities, such antioxidant and anti- inflammatory properties, Andaliman bioactive compounds may be more suitable in inhibiting AT1R overactivation in preeclampsia.

This study result indicates that Andaliman improves physiological outcomes in a salt-retention preeclamptic rat model, potentially through RAS involvement. Computational analysis suggested 5 bioactive compounds from Andaliman fruit, namely kaempferol, quercetin, resveratrol, farnesyl acetate, and (e,e)- farnesyl acetone possibly inhibiting AT1R, which supported by improvement in blood pressure, proteinuria, attenuated ACE overexpression in serum and kidney, and reduced TNF-α expression.

CONFLICT OF INTEREST

The authors declare that there are no competing interests.

AUTHOR CONTRIBUTIONS

Muhammad Shafala Safa and Azmi Noer: conceptualizations, writing- review and editing, laboratory work, data analysis and interpretation. Fatchiyah Fatchiyah: conceptualizations, supervision, resources, writing-review and editing.

Muhammad Shafala Safa and Azmi Noer contributed equally to this study.

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