https://doi.org/10.31925/farmacia.2024.6.8 **ORIGINAL ARTICLE**

ANTIHYPERTENSIVE EFFECTS OF *HIBISCUS SABDARIFFA* AND *ZINGIBER OFFICINALE* VAR. *RUBRUM* EXTRACTS IN A DEOXYCORTICOSTERONE ACETATE-SALT INDUCED HYPERTENSION RAT MODEL

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Abstract

This study evaluated the antihypertensive activity of the combination of *Hibiscus sabdariffa* Linn (roselle) extract and *Zingiber officinale* var. *Rubrum* (red ginger) extract on deoxycorticosterone-acetate-salt (DOCA-salt) induced hypertension in rats. Thirty male SD rats were randomly divided into six groups (n = 5 *per* group). Hypertension was induced by administering 30 mg/kg b.w. DOCA and 2% NaCl saline for three weeks. The treatment consisted of a normotensive group, a negative control (hypertension without treatment), a positive control (4.5 mg/200 g b.w. captopril orally) and three groups receiving the roselle-red ginger extract combination (50:3.5 mg; 100:7 mg; and 200:14 mg)/300 g b.w. for two weeks. RAAS biomarkers and an inflammatory mediator were measured according to assay procedures. The blood pressure of rats treated with the roselle-red ginger combination extract decreased significantly ($p < 0.05$) compared to the negative control, with values approximating those of the normotensive group. Plasma renin level, serum ACE activity, plasma Ang-II level and IL-17A level also significantly declined in the combination extract group compared to the negative control group. The roselle-red ginger combination extract has antihypertensive activity due to its effects on blood pressure, RAAS biomarkers and inflammatory mediators in the hypertension model rats.

Rezumat

Acest studiu a evaluat activitatea antihipertensivă a combinației de extracte de floare de roselle și ghimbir roșu la șobolani cu hipertensiune arterială indusă de sarea DOCA. Treizeci de șobolani masculi SD au fost împărțiți aleatoriu în șase grupuri (n = 5 *per* grup). Inducerea hipertensiunii arteriale a fost realizată prin administrarea a 30 mg/kg DOCA și soluție 2% NaCl timp de trei săptămâni. Tratamentul grupului normotensiv, control negativ (hipertensiune arterială fără tratament), control pozitiv (4,5 mg/200 grame corp (gc) captopril pe cale orală) și 3 grupuri de combinație de extract de roselle-ghimbir roșu (50:3,5 mg; 100:7 mg; și 200:14 mg)/300 gc a fost efectuat timp de două săptămâni. Biomarkerii sistemului renină-angiotensină-aldosteron și mediatorii inflamatori au fost evaluați conform procedurilor de testare. Tensiunea arterială la șobolanii tratați cu extract combinat de roselle-ghimbir roșu a scăzut semnificativ ($p < 0,05$) în comparație cu grupul control negativ. În plus, nivelul reninei plasmatice, activitatea enzimei de conversie a angiotensinei serice, nivelul angiotensinei II în plasmă și nivelul IL-17A au prezentat, de asemenea, o scădere semnificativă în grupul cu extract combinat în comparație cu grupul martor negativ.

Keywords: antihypertensive, roselle, red ginger, *in vivo*

Introduction

Hypertension is a significant risk factor for the inception of cardiovascular disease, which is still a global health issue due to its high prevalence rate [1]. The patho-physiological causes of hypertension are complex, thus the treatment of this condition may involve multiple approaches. In general, the pharmacological mechanisms of hypertension therapy are reducing peripheral pressure (vasodilators), reducing body fluid volume (diuretics) and inhibiting angiotensin-converting enzyme (ACE-I) activity. Angiotensin enzymes play an essential role in the renin-angiotensin-aldosterone system, the central pathway of blood pressure control and the main target of antihypertensive drugs. An increase in ACE activity will increase the concentration of angiotensin II, causing hypertension [2, 3]. Besides the renin-angiotensin-

aldosterone system (RAAS), recent studies have shown an association between the inflammatory process as part of the complex immune response and hyper-tension. Thus, intervention against inflammation can be a therapeutic target to improve high blood pressure [4, 5]. Treatment with therapeutic targets of more than one mechanism pathway can be done by consuming herbal medicines. One of the medicinal plants commonly used and proven in pre-clinical and clinical studies to reduce high blood pressure is roselle (*Hibiscus sabdariffa* Linn.) [6, 7]. The marker compounds of roselle extract are anthocyanins. Anthocyanins were found to have various mechanisms as antihypertensives, including vasodilation and inhibition of angiotensin-converting enzyme [8]. In addition to roselle, red ginger (*Zingiber officinale* var. *Rubrum*), a medicinal plant native to Indonesia, has antihypertensive potential by modulating the inflammatory pathway. The compounds responsible for the pharmacological activity of red ginger with high levels are gingerol in the range of 23 - 25% and shogaol by 18 - 25% [9]. An *in vivo* study showed that 6-gingerol administration in multiple sclerosis (MS) animal models can suppress Th17 cell differentiation by inhibiting dendritic cell activation, where Th17 cells that produce IL-17 are the primary effector cells in autoimmune and inflammatory processes [10].

Using roselle in a single form has been widely studied as an antihypertensive. At the same time, the administration of red ginger is expected to support the treatment of hypertension through intervention against inflammation. Pre-clinical studies are necessary to determine the pharmacodynamic effects and provide scientific proof of the efficacy of these combination extracts as herbal medicine [11]. Selecting an appropriate murine model for hypertension research that accurately reflects human hypertension is mandatory [12]. One of the experimental animal models for hypertension outlined in the guidelines from the Food and Drug Administration of the Republic of Indonesia (BPOM RI) pertaining to pre-clinical pharmacodynamic evaluation of herbal remedies is the induction of the deoxy-corticosterone acetate (DOCA), which is typically accompanied by the administration of NaCl solution [13]. The mechanism of hypertension in DOCA-salt-induced animal models is mainly by upregulation of Ang II receptors, increased vasopressin and increased oxidative stress and endothelin [14]. DOCA stimulation can also cause dendritic cells to polarise T cells towards Th17 cells [15]. Thus, mineralocorticoid receptor activation alters the Th17/T-lymphocyte regulatory/ IL-17 pathway in mineralocorticoid-dependent hyper-tension as part of the inflammatory mechanisms contributing to fibrosis and cardiac and renal damage [16]. No study evaluates the antihypertensive effect of a combination of roselle and red ginger extract as a candidate for herbal medicine. Therefore, this research tested the antihypertensive activity of the combination extracts of roselle calyces and red ginger in experimental animal models of DOCA salt-induced hypertension.

Materials and Methods

Materials

The materials used included deoxycorticosterone acetate-salt (DOCA-salt) (Sigma, Pcode 1001376001), captopril (Sigma Aldrich), sodium chloride (Merck), CMC (Daichii), ketamine (Combiphar), distilled water. The combination extracts were roselle (*Hibiscus sabdariffa* Linn.) calyces dry extract and red ginger (*Zingiber officinale* var. *Rubrum*) rhizome dry extract produced by PT. Phytochemindo Reksa, with codes number 2H01E10 and 2R01E15, have been standardised according to procedures established by the Food and Drug Administration of the Republic of Indonesia (BPOM RI).

The dose of rosella-red ginger combination extract for hypertension was determined based on previous studies of the single use of rosella as an antihyper-tensive and red ginger, which has anti-inflammatory activity. The native extract content in the sample (dry extract produced on an industrial scale) of rosella and red ginger is 10% and 15%, respectively. Hence, the dose determination needs to be calculated from this content with the dose of the extract in the literature. The dose of dried rosella extract in the study used was comparable to 100 mg/300 g b.w. of rats, while the dose of red ginger extract was 7 mg/300 g b.w. of rats. This dose was used in the study as the middle dose. The other two doses were multiples of ½ and 2 times the middle dose.

Experimental animals

Male *Sprague-Dawley* (SD) white rats (*Rattus norvegicus*) aged 2 - 3 months with a body weight of 180 - 250 g were obtained from the Biopharma Study Centre of Bogor Agricultural University (IPB; Bogor, Indonesia). The rats were maintained in a well-ventilated animal room under temperature control (25 ± 5°C) and a 12-hour light-dark cycle. Rats were also provided sufficient food and water on an *ad libitum* and uniform basis. All experimental animals were approved by the Ethics Committee for Animal Research of the National Research and Innovation Agency.

Suspensions preparation methods

Captopril

The dose of 4.5 mg/200 g b.w. captopril was achieved by suspending a certain amount of captopril powder in 1 mL of 0.5% CMC solution for each rat. In the process of *per*-oral captopril administration, 200 g b.w. rats received 1 mL of captopril suspension. Therefore, a suspension was made, each 1 mL containing 4.5 mg captopril.

Combination of extracts

The dried extracts of roselle flower and red ginger were weighed according to the administration dose and then suspended in 0.5% CMC solution. When

the preparation was administered orally, 200 g b.w. rats received 1 mL of extract suspension. Therefore, a suspension was made in which each 1 mL contained several extracts according to the dose to be given.

Induction of hypertension

Thirty rats were utilised, of which twenty-five were induced with hypertension using a mineralocorticoid induction model. Hypertension induction is achieved through the administration of 30 mg/kg b.w. DOCA and 2% NaCl salt. DOCA administration was conducted once a week by dissolving DOCA powder in 0.5 mL of corn oil and then injecting subcutaneously in the cervical spine of the rat for 3 weeks or 21 days until the rat's blood pressure stabilised at > 140/90 mmHg [13, 17]. Simultaneously, a 2% NaCl solution served as the drinking water for rats, provided orally during the induction phase before medication administration.

Drug treatments

There were six treatment groups, each group consisting of 5 rats. Group I was not subjected to DOCA-salt induction and received CMC-Na orally as a normotensive control (N). Group II developed hypertension and was given CMC-Na orally as a negative control (HTN). Group III (hypertensive rats) was administered captopril at a dose of 4.5 mg/kg b.w. orally as a positive control (CAP). Group IV (hypertensive rats) were given a combination of roselle-red ginger extract with a small dose combination (50 mg/300 g b.w. roselle extract and 3.5 mg/300 g b.w. red ginger extract) orally as a combination dose group 1 (D1). Group V (hypertensive rats) were given the middle combination dose (100 mg/300 g b.w. of roselle extract and 7 mg/300 g b.w. of red ginger extract) orally as a combination dose group 2 (D2). Group VI (hypertensive rats) were given the high combination dose (200 mg/300 g b.w. of roselle extract and 14 mg/300 g b.w. of red ginger extract) orally as a combination dose group 3 (D3). Suspensions of extracts, captopril and CMC were prepared daily. All treatments were performed every day for two weeks.

Measurement of blood pressure

The blood pressure (including systolic, diastolic and mean arterial pressure) was measured using the CODA non-invasive tail-cuff blood pressure system (Kent-Scientific, Torrington, CT) every week before starting induction and during treatment following the method described by Hem *et al*. [18]. The blood pressure of the rats measured before starting the induction was declared as the baseline blood pressure. Before measurement, the rats were calmed first by being put into a chamber or animal holder at 30 - 37°C. Then, the occlusion cuff was attached to the rat's tail, followed by a VPR cuff as a pulse detector. The cuff automatically inflates and presses the rat's tail when the device is in running condition. Then, the blood flow pulse is detected and the blood pressure parameters appear on the monitor screen.

Determination of biomarkers of RAAS level and inflammatory mediator level

The analysed RAAS biomarkers were renin level, angiotensin-converting enzyme (ACE) activity, and angiotensin II levels. At the same time, interleukin-17A (IL-17A) levels as inflammatory mediators were analysed. The plasma renin level, activity of serum ACE, plasma ang-II levels and plasma IL-17A level were measured after the second week of treatment. Plasma renin was measured using Rat Renin Elisa Kit Sigma Aldrich USA (catalogue number: MAK 157) and plasma angiotensin II using Angiotensin II EIA Kit Sigma Aldrich USA (catalogue number: RAB0010). ACE activity was measured with serum samples using the ACE1 Colorimetric Activity Assay Kit Sigma Aldrich Germany (catalogue number: MAK419). Inflammatory mediator IL-17A was measured using an ELISA kit from Elabscience China with rat blood plasma samples after sacrifice.

Statistical analysis

Each data point is presented as the mean ± standard deviation. The statistical analysis was conducted using the SPSS version 23.0 software (IBM, Armonk, NY) and GraphPad Prism 9 software. Multiple group means were compared using a one-way analysis of variance (ANOVA) test or the Kruskal-Wallis test. Then, T-Test, Tukey HSD post-hoc, or Mann-Whitney tests were used to determine significant differences between treatment groups. If the p-value was less than 0.05, it was considered statistically significant for all tests.

Results and Discussion

Blood pressure after induction of hypertension in rats

Figure 1 illustrates the progression of blood pressure changes during the induction phase. It can be observed that systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) values in the DOCA-salt-treated group of rats exhibited a statistically significant increase ($p < 0.05$) after three weeks of induction (Figures 1B - 1F). The normotensive group's systolic and diastolic blood pressures, which comprised rats not administered DOCA-salt, demonstrated no statistically significant difference during the induction period. By the third week, the blood pressure of the rats that had been induced was ≥ 140/90 mmHg with an average increase of ± 36 mmHg in blood pressure's systolic and diastolic values. In contrast, the rats in the normotensive control group did not exhibit a significant increase in blood pressure. These findings indicate that DOCA-salt is a reliable method for inducing hypertension. Rats exhibiting an elevation in blood pressure are designated hypertensive and subsequently allocated to the treatment groups.

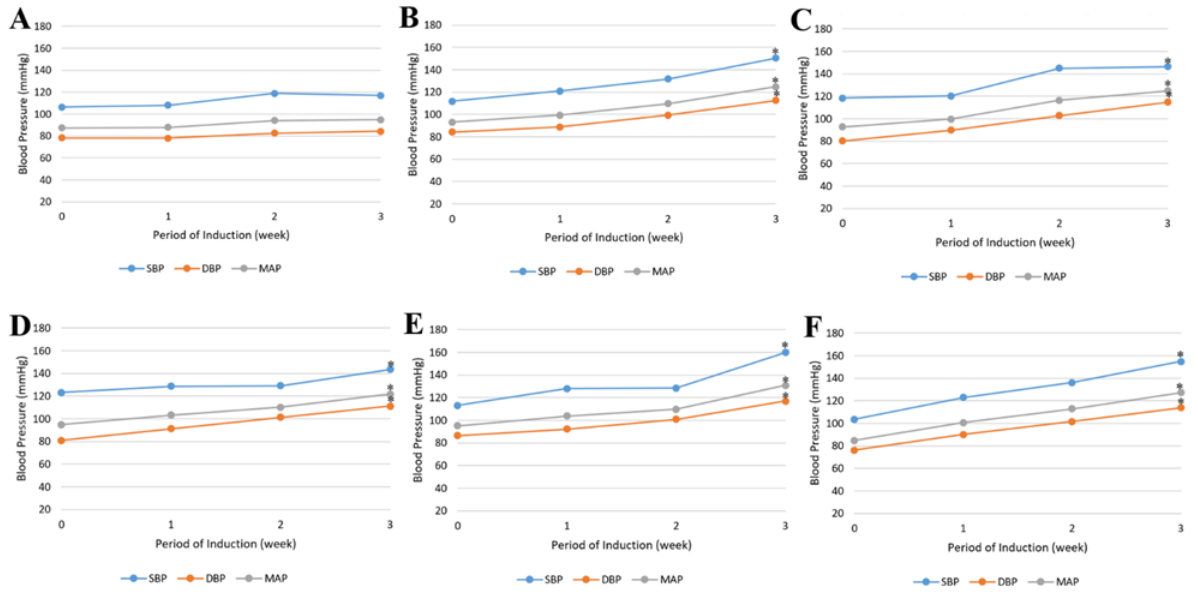

Figure 1.

Progress of Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP) and Mean Arterial Pressure (MAP) values during induction of hypertension in each group (n = 5 rats *per* group): A: Normotensive group; B: Negative control group; C: Positive control group; D: Dosage 1 combination extracts group; E: Dosage 2 combination extracts group; F: Dosage 3 combination extracts group

*p < 0.05 compared to the baseline (before induction)

Effects of combination of roselle and red ginger extracts on blood pressure in DOCA-salt model rats

After a three-week DOCA-salt induction period, in which the blood pressure of the rats was monitored until it reached a threshold of ≥ 140/90 mmHg, the treatment was initiated for the antihypertensive activity test of roselle and red ginger extracts that were assessed until two-weeks. The results of blood pressure values of rats in each group after two weeks of treatment are presented in Figure 2. The systolic blood pressure of rats (Figure 2A) from the normotensive group (N), negative control (HTN), positive control (CAP), combination dose 1 (D1), combination dose 2 (D2) and combination dose 3 (D3) demonstrated the following mean values: 114 ± 6.37; 149 ± 8.41; 107 ± 7.13; 124 ± 6.90; 114 ± 3.38; 124 ± 3.63 mmHg, respectively. The diastolic blood pressure values of rats (Figure 2B) after treatment in groups N, HTN, CAP, D1, D2 and D3 were 80 ± 4,15; 111 ± 4.73; 77 ± 6.65; 88 ± 1.94; 78 ± 3.93; 82 ± 1.36 mmHg, respectively. In parallel, the mean arterial pressure (Figure 2C) exhibited values in each group as follows: 91 ± 4.63; 123 ± 5.55; 86 ± 6.40; 99 ± 3.22; 90 ± 3.26; and 96 ± 1.76 mmHg, respectively.

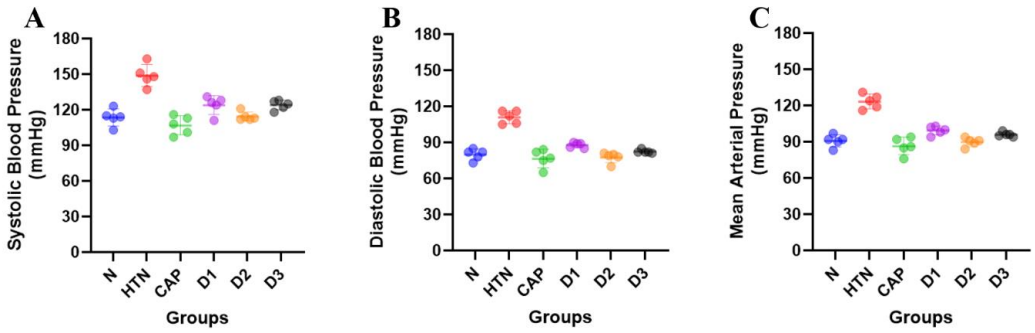

Figure 2.

After two weeks of treatment, the blood pressure values of rats in each group (n = 5 rats *per* group): A: Systolic blood pressure values of rats in each group; B: Diastolic blood pressure values of rats in each group; C: Mean arterial pressure values of rats in each group

In Figure 3, which shows the comparison of rat blood pressure before and after treatment, it can be seen that the systolic and diastolic blood pressure of rats in the positive control group (CAP), combination dose 1 (D1), combination dose 2 (D2) and combination dose 3 (D3) decreased significantly ($p < 0.05$) compared to the negative control group (HTN) and the values were not significantly different ($p > 0.05$) with the normo-

tensive group. There was a statistically significant difference ($p < 0.05$) between the systolic blood pressure of the combination dose group 1 (D1) and combination dose 3 (D3) compared to the positive control (CAP), as illustrated in Figure 3A. Significant differences were also observed between the diastolic blood pressure of the combination dose group 1 (D1) and the positive control (CAP) presented in Figure 3B. The combination of extracts dose 2 (D2) yielded blood pressure values that were not significantly distinct from the positive control group (rats administered captopril at 4.5 mg/ 200 g b.w.). Figure 3C illustrates the percentage of reduction in blood pressure before and after treatment. The highest reduction in systolic blood pressure was observed in the combination dose 2 (D2), which was ± 45 mmHg (27.6%). This group also exhibited the highest diastolic blood pressure reduction ± 39 mmHg (33.2%).

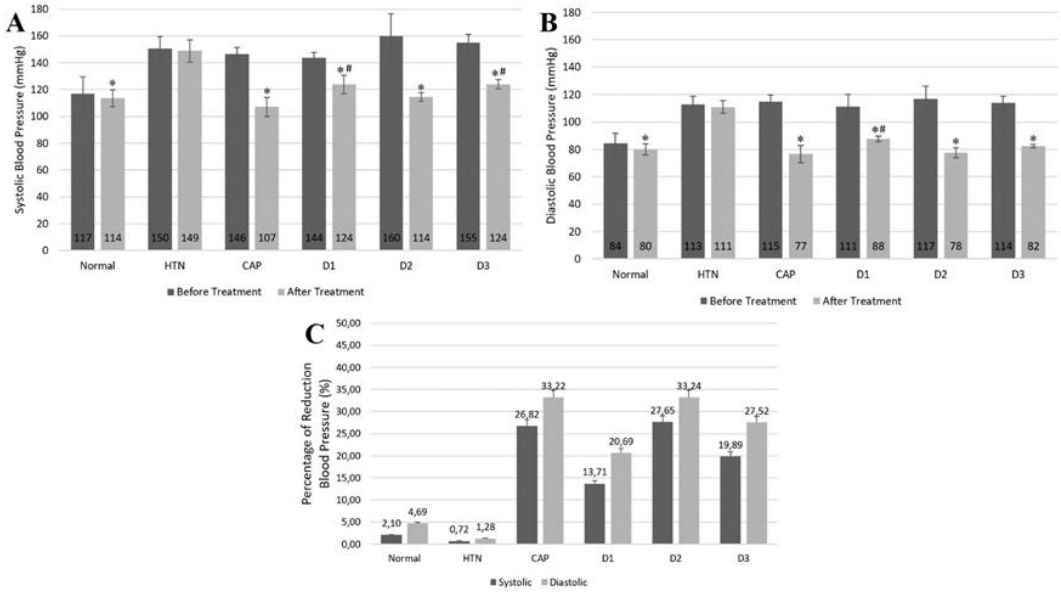

Figure 3.

Comparison of blood pressure values of rats before and after treatment in each group (n = 5 rats/group):
A: Systolic blood pressure values of rats in each group; B: Diastolic blood pressure values of rats in each group;
C: Percentage of reduction blood pressure values of rats in each group
*$p < 0.05$ compared to the HTN group; #$p < 0.05$ compared to the CAP group

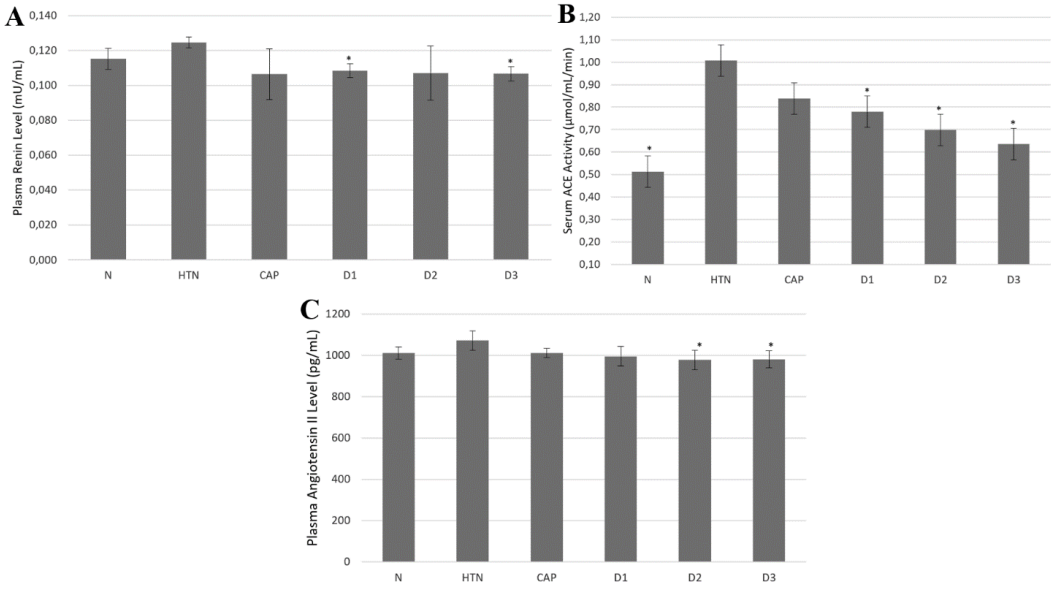

Figure 4.

RAAS biomarkers level after two weeks of treatment in each group:
A: plasma renin level; B: serum ACE activity; C: plasma angiotensin II level; D: plasma IL-17A level
*$p < 0.05$ compared to the HTN group

Effects of combination of roselle and red ginger extracts on RAAS biomarkers level

As illustrated in Figure 4A, plasma renin levels of rats subjected to treatment with a combination of roselle extract and ginger showed a significant difference ($p < 0.05$) between the combination dose 1 (D1) and the combination dose 3 (D3) groups compared to the negative control group (HTN). The ACE activity in Figure 4B indicates that the group of rats that were administered the extract combination dose 1 (D1), combination dose (D2) and combination dose 3 (D3) following two weeks of treatment exhibited statistically significant differences ($p < 0.05$) when compared to the negative control group (HTN). The angiotensin II level in the plasma of rats presented in Figure 4C demonstrates the group that received a combination of roselle, and red ginger extract (D2 and D3) exhibited a significant difference ($p < 0.05$) in comparison to the negative control group (HTN).

Effects of combination of roselle and red ginger extracts on inflammatory mediator level

Figure 5 illustrates that the IL-17A level in the plasma of rats treated with the roselle-red ginger combination extract between the group combination dose 2 (D2) and 3 (D3) exhibited a statistically significant difference ($p < 0.05$) in comparison to the negative control group (HTN).

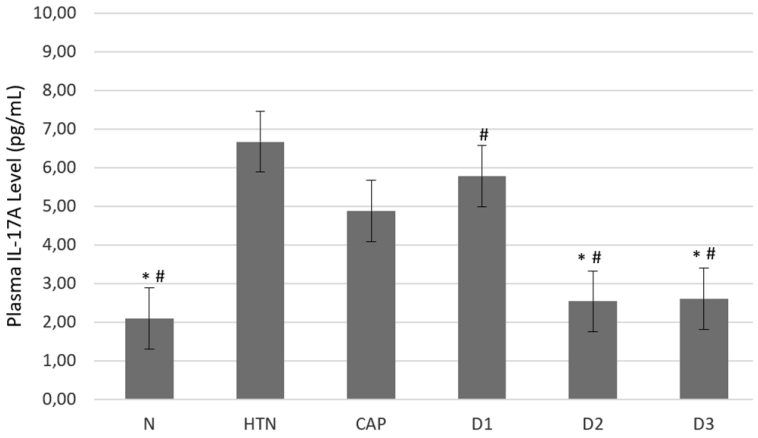

Figure 5.

Interleukin 17A level after two weeks of treatment in each group

*p < 0.05 compared to the HTN group; #p < 0.05 compared to the CAP group

Hypertension is a multifaceted condition with numerous causes and treatment pathways. Recent studies demonstrated a potential link between inflammation and hypertension, indicating that interventions upon the inflammation pathway could offer a promising avenue for managing high blood pressure [4, 5]. Identifying drug candidates derived from natural sources that target this pathway is crucial in searching for effective hypertension therapies. The single-use of roselle has been extensively researched as an antihypertensive [7, 8, 19-22]. At the same time, the administration of red ginger is expected to support the treatment of hypertension through an intervention against inflammation.

In this study, the hypertensive condition of the animal model was observed to occur after three weeks of DOCA-salt administration. Hypertension induced by DOCA produced hypertension associated with the system of renin-angiotensin-aldosterone (RAAS). This is because deoxycorticosterone is a steroid hormone produced by the adrenal glands that has activity as a mineralocorticoid and acts as a precursor to aldosterone [23]. Aldosterone formation can be influenced by renal control mechanisms, which include the renin-angiotensin-aldosterone system. Renin is produced by the kidney that converts angiotensinogen into angiotensin I (Ang I). Angiotensin I is converted by angiotensin-converting enzyme (ACE) into angiotensin II. The physiological effects of Ang II arise from its binding to AT1 receptors, which cause vasoconstriction [24]. DOCA-salt administration also causes oxidative stress due to increased superoxide, a free radical that can damage the physiology of several tissues in the body, including endothelial cells. Endothelial damage can lead to impaired endothelial stability (endothelial dysfunction), which then causes vasoconstriction and hypertension [14].

After a two-week treatment period for each group of rats, the blood pressure results for the rats given a combination of rosella-red ginger extract demonstrated that all three combination dose groups (D1, D2 and D3) exhibited a significant reduction. These findings indicate that combining roselle-red ginger extracts can affect blood pressure. The effect of this combination on lowering blood pressure can be caused by roselle extract containing anthocyanin compounds (cyanidin-3-O-sambubioside and delphinidin-3-O-sambubioside), which work as vasodilators through inhibition of ACE enzyme activity [8, 21, 22]. In addition, the 6-gingerol compound contained in the red ginger extract has an anti-inflammatory effect by inhibiting the synthesis

of inflammatory mediators through inhibition of the cyclooxygenase enzymes COX-1, COX-2 and 5-lipoxygenase (5-LO) [25], thus preventing chronic inflammation which causes endothelial dysfunction. This is consistent with *in silico* research indicating that 6-gingerol has the potential to act as a vasodilator, thereby reducing blood pressure [26].

The results of plasma renin levels in the DOCA-salt-induced rat group as a negative control (HTN) showed higher values than the normotensive group rats. Still, statistically, the increase was not significant. DOCA (deoxycorticosterone-acetate) that enters the body is converted into DOC (deoxycorticosterone), an aldosterone analogue. DOC can bind to and activate mineralocorticoid (MR) receptors like aldosterone, indirectly suppressing aldosterone synthesis [23]. When aldosterone levels are low, the body responds by stimulating renin production as part of RAAS. However, DOCA with a high salt (NaCl) diet can also cause sodium retention, which triggers negative feedback by reducing renin production [27]. Although there was a slight increase in renin level in the DOCA-salt-treated rats in response to low aldosterone, this effect was insignificant due to the dominance of a more potent negative feedback mechanism resulting from increased blood pressure caused by sodium and water retention.

The observed decline in serum ACE activity in rats administered a combination of roselle-red ginger extract (D1, D2, D3) may be attributed to the inhibitory effect of the combination of extracts on ACE activity. Roselle extract contains anthocyanin compounds, including cyanidin-3-O-sambubioside, cyanidin-3-glucoside, delphinidin-3-O-sambubioside and delphinidin-3-glucoside which have been demonstrated to inhibit angiotensin-converting enzyme competitively [20, 21]. Anthocyanins inhibit ACE activity by competing with the substrate for the enzyme's active site. Delphinidin-3-glucoside, for instance, binds to ACE, disrupting its normal function. The specific structural features of anthocyanins, including the distribution of hydroxyl groups and the number of monomer units, contribute to their inhibitory activity [29-31]. Therefore, anthocyanins can prevent ACE from converting angiotensin I into angiotensin II, which has a strong vasoconstrictor effect when it binds to AT-1 receptors.

Elevated levels of angiotensin II in the plasma can cause hypertension due to the vasoconstrictive properties of angiotensin II [24]. This study indicates that there was no statistically significant difference between the negative control group (HTN) and the normotensive group, which was consistent with the findings for renin, where the increase was also not significant. However, elevated plasma renin levels in response to low aldosterone levels will still result in elevated angiotensinogen converted to Ang I and subsequently to Ang II by ACE [28]. Meanwhile, angiotensin II levels in the plasma of rats given a combination of roselle-red ginger extract (D1, D2, D3) had lower values than the negative control group (HTN). This may be attributed to the decreased activity of serum ACE, which converts angiotensin I into angiotensin II. Consequently, the levels of angiotensin II in the blood plasma are reduced.

Interleukin-17A is a pro-inflammatory cytokine that plays a role in the inflammatory process. Consequently, increased levels of IL-17A can be a marker of tissue damage. Chronic inflammation and tissue damage can result from excessive DOCA-salt administration. DOCA stimulation can cause dendritic cells to polarise T cells towards Th17 cells. Thus, the activated mineralocorticoid receptor can change the Th17/T-lymphocyte regulatory/IL-17 pathway in mineralocorticoid-dependent hypertension [15]. In contrast, the IL-17A levels in the group of rats administered a combination of roselle-red ginger extract after two weeks of treatment were found to be lower than those observed in the negative control group (HTN), and there were significant differences in the doses of combinations 2 and 3 (D2 and D3). The decrease in IL-17A levels can be caused by the 6-gingerol compound in the red ginger extract, which has anti-inflammatory activity. 6-Gingerol can inhibit the activation of Th17 cells to reduce IL-17A production. This is consistent with a study on multiple sclerosis (MS) animal models, which demonstrated that the administration of 6-gingerol can suppress Th17 cell differentiation by inhibiting dendritic cell activation. Th17 cells produce IL-17, the main effector cells in autoimmune and inflammatory processes [10]. Gingerol and shogaol derivatives in red ginger extract have been shown to reduce IL-17A expression in various experimental models, including experimental autoimmune encephalomyelitis mice, demonstrating their anti-inflammatory effects [32]. They regulate the NF-κB signalling pathway, inhibiting pro-inflammatory cytokines like IL-17A. Additionally, 6-shogaol inhibits the NLRP3 inflammasome, reducing cytokines IL-1β and IL-18, thereby mitigating inflammation. Red ginger constituents also interfere with MAPK and PI3K/Akt/mTOR pathways, reducing pro-inflammatory mediators and cell activation while targeting upstream regulators like AMPK and NRF2, influencing IL-17A production [33-34].

The anti-hypertensive test results indicated that the combination of roselle-red ginger extracts containing anthocyanin and gingerol has antihypertensive activity as it affects systolic and diastolic blood pressure, levels of RAAS biomarkers (renin level, ACE activity and angiotensin-II level) and IL-17A inflammatory mediator in the blood. In this experimental study, the measurement results of all parameters for the three groups administered with the combination of roselle-red ginger extracts (D1, D2 and D3) exhibited a reduction in values, though not necessarily identical. This can occur due to the combined extracts' varying compound levels and mechanisms of action.

Conclusions

The combination of roselle-red ginger extracts has been evaluated for its antihypertensive activity. The antihypertensive activity of the combination extracts was demonstrated by its effect on reducing systolic and diastolic blood pressure, mean arterial pressure, as well as on the levels of renin-angiotensin-aldosterone system biomarkers and the inflammatory mediator IL-17A in the blood of DOCA-salt-induced hypertensive rats. Further, this combination of roselle-red ginger extracts can be formulated as an antihypertensive herbal medicine.

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Conflict of interest

The authors declare no conflict of interest.

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