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Original Article

FasL and cytochrome c-induced apoptosis in cardiac tissue of hypertensive rats treated with *Rhodomyrtus tomentosa*

[Apoptosis inducida por FasL y citocromo c en tejido cardíaco de ratas hipertensas tratadas con *Rhodomyrtus tomentosa*]

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Abstract

Context: Hypertensive heart disease is a leading cause of cardiovascular mortality worldwide. Cardiomyocyte apoptosis, mediated through intrinsic (cytochrome c) and extrinsic (Fas ligand, FasL) pathways, plays a critical role in the progression of hypertensive cardiac injury. *Rhodomyrtus tomentosa,* a medicinal plant rich in flavonoids and polyphenols, exhibits antioxidant and anti-inflammatory properties and may offer cardioprotective benefits.

Aims: To evaluate the effects of *R. tomentosa* on FasL- and cytochrome c-mediated apoptosis in cardiac tissue of hypertensive rats.

Methods: Hypertension was induced in male Wistar rats using L-NAME (40 mg/kg BW/day) for 28 days. Rats were divided into six groups: J− (normal control), J+ (L-NAME only), J0 (enalapril 2 mg/kg BW), and J1–J3 (L-NAME + *R. tomentosa* at 100, 200, and 300 mg/kg BW/day, respectively). Heart tissues were collected for histopathological and immunohistochemical analysis of FasL, cytochrome c, and caspase-3 expression. Serum levels of MDA, NGAL, and SOD were also measured.

Results: *R. tomentosa* significantly reduced myocardial damage, apoptosis marker expression, and oxidative stress in a dose-dependent manner ($p<0.01$). The 300 mg/kg BW dose (J3) showed effects comparable to enalapril, including marked reductions in FasL, cytochrome c, and caspase-3 levels.

Conclusions: *R. tomentosa* exerts cardioprotective effects by modulating both intrinsic and extrinsic apoptotic pathways and attenuating oxidative damage in hypertensive heart tissue. These findings support its potential as a natural adjunctive therapy for hypertensive cardiac injury.

Keywords: caspase 3; cytochrome c; FasL; heart; medicinal plant.

Resumen

Contexto: La cardiopatía hipertensiva es una de las principales causas de mortalidad cardiovascular en todo el mundo. La apoptosis de los cardiomiocitos, mediada por vías intrínsecas (citocromo c) y extrínsecas (ligando Fas, FasL), desempeña un papel fundamental en la progresión de la lesión cardíaca hipertensiva. *Rhodomyrtus tomentosa*, una planta medicinal rica en flavonoides y polifenoles, presenta propiedades antioxidantes y antiinflamatorias y puede ofrecer beneficios cardioprotectores.

Objetivos: Evaluar los efectos de *R. tomentosa* sobre la apoptosis mediada por FasL y citocromo c en el tejido cardíaco de ratas hipertensas.

Métodos: Se indujo hipertensión en ratas Wistar macho utilizando L-NAME (40 mg/kg de peso corporal/día) durante 28 días. Las ratas se dividieron en seis grupos: J− (control normal), J+ (solo L-NAME), J0 (enalapril 2 mg/kg de peso corporal) y J1-J3 (L-NAME + *R. tomentosa* a 100, 200 y 300 mg/kg de peso corporal/día, respectivamente). Se recogieron tejidos cardíacos para el análisis histopatológico e inmunohistoquímico de la expresión de FasL, citocromo c y caspasa-3. También se midieron los niveles séricos de MDA, NGAL y SOD.

Resultados: *R. tomentosa* redujo significativamente el daño miocárdico, la expresión de marcadores de apoptosis y el estrés oxidativo de forma dependiente de la dosis ($p<0,01$). La dosis de 300 mg/kg de peso corporal (J3) mostró efectos comparables a los del enalapril, incluyendo reducciones notables en los niveles de FasL, citocromo c y caspasa-3.

Conclusiones: *R. tomentosa* ejerce efectos cardioprotectores al modular las vías apoptóticas intrínsecas y extrínsecas y atenuar el daño oxidativo en el tejido cardíaco hipertenso. Estos hallazgos respaldan su potencial como terapia adyuvante natural para la lesión cardíaca hipertensiva.

Palabras Clave: caspasa 3; citocromo c; corazón; FasL; planta medicinal.

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INTRODUCTION

Hypertension is a major global health concern and a well-established risk factor for cardiovascular disease, often leading to complications such as myocardial remodeling and cardiac dysfunction (Masenga and Kirabo, 2023). One of the fundamental mechanisms contributing to hypertensive heart damage is apoptosis, a tightly regulated form of programmed cell death. Cardiac apoptosis can be initiated via two primary pathways: the extrinsic pathway, mediated by Fas ligand (FasL), and the intrinsic mitochondrial pathway involving cytochrome c (Krautwald et al., 2010). FasL initiates apoptosis by binding to its receptor (Fas), subsequently activating Fas-associated death domain (FADD) and triggering downstream caspase-8, which culminates in caspase-3 activation (Mustafa et al., 2024). In parallel, mitochondrial stress in hypertensive conditions causes the release of cytochrome c into the cytoplasm, where it interacts with apoptotic protease activating factor-1 (Apaf-1) and ATP, leading to caspase-9 and caspase-3 activation. Excessive activation of these pathways contributes to cardiomyocyte loss and left ventricular remodeling, thereby worsening cardiac function in sustained hypertension. Apoptosis is a primary mechanism involved in the pathophysiology of cardiac injury resulting from hypertension (Kim and Kang, 2010). Apoptosis in cardiac tissue can be initiated by multiple molecular mechanisms, including the extrinsic pathway regulated by Fas ligand (FasL) and the intrinsic system reliant on the release of cytochrome c from mitochondria (Krautwald et al., 2010). The heightened activity of these two pathways might expedite cardiac tissue damage; thus, mitigating excessive apoptosis is a crucial tactic in hypertension treatment (Masenga and Kirabo, 2023).

Hypertension increases mechanical load and oxidative stress in the myocardium, particularly in the left ventricle, leading to hypertrophy and decreased ventricular compliance (Nadar and Lip, 2021). These hemodynamic and biochemical alterations are further compounded by neurohormonal dysregulation, including activation of the sympathetic nervous system and the renin-angiotensin-aldosterone system (RAAS), which elevate levels of angiotensin II and oxidative stress (Ameer, 2022; Oparil et al., 2018). Mitochondrial dysfunction, oxidative injury, and altered redox balance under such conditions collectively drive the apoptotic processes in cardiomyocytes. In chronic hypertension, the baroreceptor response is compromised, resulting in elevated sympathetic tone and the secretion of hormones such as angiotensin II, which constricts blood vessels and enhances sodium and fluid retention (Ameer, 2022). Consequently, blood volume escalates, the cardiac effort intensifies, and blood pressure regulation becomes more challenging. In the hypertensive heart, oxidative stress and elevated mechanical stress can induce apoptosis via the mitochondrial pathway facilitated by cytochrome c (Eirin et al., 2014). In hypertensive conditions, heightened mechanical and oxidative stress can expedite the activation of the FasL/Fas pathway, leading to cardiac remodeling and cardiovascular dysfunction as a result of excessive cardiomyocyte loss (Mustafa et al., 2024).

Given the central role of oxidative stress and apoptosis in hypertensive cardiac injury, targeting these pathways offers a promising therapeutic approach. *Rhodomyrtus tomentosa* (Aiton) Hassk., commonly referred to as *haramonting*, possesses the capability to function as a natural antioxidant that may inhibit apoptosis induced by oxidative stress (Vo and Ngo, 2019). The concentration of phenolic and flavonoid components in *R. tomentosa* extract is crucial for neutralizing free radicals and mitigating lipid peroxidation that can harm cell membranes (Situmorang et al., 2022a). *R. tomentosa* preserves cellular integrity and inhibits the activation of the intrinsic apoptotic pathway by reducing the generation of reactive oxygen species (ROS) and enhancing the activity of antioxidant enzymes, including superoxide dismutase (SOD) and catalase (Situmorang et al., 2023). Consequently, this plant possesses the potential to be formulated as a natural medicinal drug to safeguard cells from harm caused by exposure to hazardous substances or pathological situations that induce oxidative stress (Rumahorbo et al., 2023; Situmorang et al., 2022b). Antioxidants from this plant are crucial in inhibiting apoptosis in hypertensive situations by mitigating oxidative stress and inflammation that lead to cellular damage (Vo and Ngo, 2019). Antioxidant chemicals, including flavonoids, polyphenols, and alkaloids present in numerous herbal plants, can neutralize free radicals, enhance the activity of endogenous antioxidant enzymes, and suppress the production of pro-apoptotic proteins such as Bax and caspase-3 (Situmorang et al., 2025). Previous studies have highlighted the ability of *R. tomentosa* to attenuate oxidative stress and suppress apoptosis in various pathological conditions. However, its role in modulating cardiac apoptosis in hypertension, particularly via FasL and cytochrome c signaling pathways, remains underexplored. This study aims to evaluate the effect of *R. tomentosa* ethanol extract on FasL- and cytochrome c-mediated apoptosis in the cardiac tissue of hypertensive rats. The findings are expected to elucidate the cardioprotective mechanisms of *R. tomentosa* and

support its potential application as a natural therapeutic agent in hypertensive heart disease.

MATERIAL AND METHODS

Reagents

The immunohistochemistry reagents utilized in this study included Invitrogen FasL-CD178 Polyclonal Antibody with Cat #PA5-84227, Invitrogen Cytochrome C Recombinant Rabbit Monoclonal Antibody (ARC1153) with Cat #MA5-35607 100 µL, and Invitrogen Caspase 3 Monoclonal Antibody (74T2) with Cat #700182 from Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA. Furthermore, a phosphate-buffered saline (PBS) solution was employed, consisting of a 50% glycerol and 1% bovine albumin (BSA) buffer (Catalogue Number BS-0812R). L-NAME (Nω-Nitro-L-Arginine Methyl Ester) with catalog number: HB1352 from Hello Bio Limited, Avonmouth, Bristol, UK. Invitrogen Malondialdehyde Competitive ELISA Kit (Catalog # EEL160) from Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA. Rat Neutrophil Gelatinase Associated Lipocalin ELISA kit (NGAL- Catalog number: ELK10659), Rat Superoxide Dismutases ELISA Kit (SOD-Catalog number: ELK8178) from ELK BioTechnology company, Denver, USA.

Vegetal material

R. tomentosa was collected from the Lintong Nihut, Humbang Hasundutan Regency, North Sumatra, Indonesia. The collection site is located at an elevation of 1,000 to 1,500 meters above sea level, with precise GPS coordinates at 2°10′0″N latitude and 98°54′30″E longitude. The area is characterized by peatland ecosystems, with Lintong Nihuta accounting for approximately 479 hectares or 16.03% of the total peatland area within the regency. The plant specimens were authenticated and deposited at the Herbarium Laboratory under voucher number 921/MEDA/2024.

Preparation of *R. tomentosa* leaves

Leaves of *R. tomentosa* were harvested, washed thoroughly under running water to remove surface contaminants, and air-dried in a shaded, well-ventilated area until the moisture content was reduced. The dried leaves were ground into a fine powder using a blender to increase the extraction surface area. A total of 10 kg of powdered material was macerated with 96% ethanol at a ratio of 1:10 (w/v) for 24 to 72 hours with occasional stirring to facilitate optimal extraction of bioactive constituents. The macerate was filtered using a muslin cloth to separate the extract from plant residues. The resulting filtrate was subjected to solvent removal under reduced pressure using a rotary evaporator (EV400VAC, Labtech, Saka, Indonesia) at 40–50°C. The concentrated extract was further dried by freeze-drying to obtain a stable dry extract. The final extract was stored in airtight, light-protected containers at low temperatures to preserve the integrity and stability of its bioactive compounds

Animal handling

A total of 36 male Wistar rats were obtained from the Animal House of the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara (USU). Prior to experimentation, all animals were maintained under controlled environmental conditions, including a 12-hour light/dark cycle, ambient temperature of 22–24°C, and relative humidity of 50–60% during 14 days. Rats were provided *ad libitum* access to standard commercial feed and water. All animal procedures were approved by the Animal Ethics Committee of Universitas Sumatera Utara (Approval No. 0390/KEPH-FMIPA/2024) and conducted in accordance with institutional and national guidelines for the care and use of laboratory animals. Randomization was performed using a random number table to allocate rats into experimental groups. Low-stress handling techniques were employed throughout the study to minimize discomfort, including gentle restraint and calm interaction during treatment and measurement procedures. Animals were monitored daily for signs of stress, pain, or adverse effects related to hypertension induction or treatment. Health status and physical condition were documented to ensure compliance with animal welfare standards.

Animal model of hypertension

Hypertension was induced by intraperitoneal injection of Nω-nitro-L-arginine methyl ester (L-NAME) at a dose of 40 mg/kg BW/day for 28 consecutive days. Systolic and diastolic blood pressure were measured weekly using a non-invasive tail-cuff plethysmography system (Model 229, IITC Life Science Inc., Woodland Hills, CA, USA) to confirm successful induction of hypertension.

Study design

The study comprised 36 rats categorized into 6 groups, with each group containing 6 rats. Group J- represents the Untreated group, while Group J+ consists of rats that received only L-NAME injections. Group J0 consists of rats that received L-NAME injections and were administered enalapril at a dosage of 2 mg/kg body weight per day, serving as a comparator group. J1: Rats with L-NAME injections + 100 mg/kg body weight of *Rhodomyrtus tomentosa*. J2: Rats with L-NAME injections + 200 mg/kg body weight of *R.*

tomentosa. J3: Rats with L-NAME injections + 300 mg/kg body weight of *R. tomentosa*. Blood pressure was frequently assessed utilizing tail-cuff plethysmography (IITC Life Science Inc, Victory Blvd Woodland Hills, United States). The herbal treatment was conducted over a period of 14 days. Rat surgery was conducted under ketamine anesthesia for the excision of the heart and the extraction of blood from the aorta. Upon anesthetization, the rat was placed ventrally on the operating table, with the hind legs secured for support. The abdominal region was disinfected, and a little incision was performed to get access to the bodily cavities. The aorta was subsequently found and meticulously incised using a sterile blade, followed by blood extraction using a syringe for sample purposes. The heart was meticulously exposed and incised for organ extraction. Upon completion of the procedure, the rat was euthanized via an overdose of anesthesia, and all utilized instruments and materials were employed in a sterile manner to avert infection. The slide preparation was conducted with the paraffin technique, followed by hematoxylin and eosin staining, as well as immunohistochemical staining.

Performing tissue preparations using hematoxylin eosin staining

Following excision, rat hearts were immediately rinsed with physiological saline and fixed in 10% neutral-buffered formalin to preserve tissue morphology. Fixed tissues were dehydrated through a graded alcohol series, cleared in xylene, and embedded in paraffin wax according to standard histological procedures (Situmorang and Ilyas, 2018a). Once the paraffin had solidified, the blocks were sectioned at a thickness of 4–5 µm using a rotary microtome. The tissue sections were mounted on clean glass slides and subsequently deparaffinized with xylene and rehydrated through decreasing concentrations of ethanol. Staining was performed using hematoxylin to visualize nuclear structures and eosin to counterstain the cytoplasm and extracellular components. After staining, sections were dehydrated, cleared in xylene, and mounted using Canada balsam as a permanent medium. The slides were then allowed to dry and were examined under a light microscope to assess histopathological alterations (Irianti et al., 2020).

Immunohistochemistry of FasL, cytochrome c and caspase 3

Tissue samples preserved in 10% neutral-buffered formalin were embedded in paraffin and sectioned at a thickness of approximately 4–5 µm. Sections were mounted onto poly-L-lysine-coated glass slides to enhance tissue adherence and subsequently dried on a hot plate at 50–60°C for 1–2 hours. Deparaffinization and rehydration were conducted through sequential immersion in xylene and graded alcohols, followed by rinsing in distilled water and phosphate-buffered saline (PBS) (Simanullang et al., 2022a). Antigen retrieval was performed using citrate buffer (pH 6.0) in a water bath at 95–100°C for 20 minutes to unmask the epitopes. Endogenous peroxidase activity was quenched by incubation in 3% hydrogen peroxide for 10 minutes at room temperature. Nonspecific binding was blocked using 1% bovine serum albumin (BSA) in PBS for 30 minutes. The sections were then incubated overnight at 4°C with the respective primary antibodies: anti-Fas ligand (FasL) (PA5-84227), anti-cytochrome c (MA5-35607), and anti-caspase-3 (700182), all from Invitrogen. After washing in PBS, sections were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 30–45 minutes at room temperature. Color development was achieved using 3,3′-diaminobenzidine (DAB) as the chromogen, followed by counterstaining with hematoxylin. Sections were dehydrated in graded ethanol, cleared in xylene, mounted using permanent media, and evaluated under a light microscope (Simanullang et al., 2022b). IHC Controls: Negative controls were prepared by omitting the primary antibody and replacing it with PBS or antibody diluent to confirm the specificity of secondary antibody binding and DAB staining. Positive controls consisted of rat tissue sections known to express FasL, cytochrome c, and caspase-3 (e.g., liver and spleen tissues), which were processed in parallel under identical conditions to validate the staining protocol and antibody performance. Quantitative analysis of immunohistochemical (IHC) staining for FasL, cytochrome c, and caspase-3 was conducted using ImageJ software (NIH, USA). For each sample, five randomly selected high-power fields (HPF, 400× magnification) were captured per slide using a light microscope equipped with a digital camera. Images were converted to 8-bit grayscale and thresholded to detect positively stained areas (DAB chromogen). The percentage of positive staining area relative to total tissue area was calculated using the "Color Deconvolution" and "Analyze Particles" plugins in ImageJ. Additionally, IHC expression was semi-quantitatively evaluated using a histological immunoreactivity scoring system (IRS) based on staining intensity and the percentage of positive cells. The final IRS score was obtained by multiplying the staining intensity (0 = none, 1 = weak, 2 = moderate, 3 = strong). All scoring was performed by two independent blinded observers to reduce subjective bias.

Statistical analysis of data

Data were presented as mean ± standard deviation (SD). Before conducting statistical comparisons, the

normality of data distribution was assessed with the Shapiro–Wilk test, and the homogeneity of variance was evaluated using Levene's test. Statistical analysis for datasets exhibiting normal distribution and homogeneous variances, such as blood pressure, MDA, SOD, and NGAL levels, was performed using one-way analysis of variance (ANOVA). This was succeeded by Duncan's multiple range test for post hoc analysis to identify pairwise differences among groups. Histopathological and immunohistochemical data (e.g., FasL, cytochrome c, caspase-3 expression; necrosis, edema, congestion) were analyzed using the Kruskal–Wallis test, as they were non-parametric. Subsequently, the Mann–Whitney U test was employed for post hoc pairwise comparisons. p-value less than 0.05 was deemed statistically significant. The significance levels were indicated as follows: $p<0.05$ (*), $p<0.01$ (**), and non-significant (ns, $p>0.05$). Statistical analyses were conducted utilizing SPSS software version 25.0 (IBM Corp., Armonk, NY, USA).

RESULTS

The impact of *R. tomentosa* on blood pressure in L-NAME-injected rats

Fig. 1 demonstrates the impact of *R. tomentosa* administration on systolic and diastolic blood pressure in rats with L-NAME-induced hypertension. In comparison to the untreated control group (J−), L-NAME injection resulted in a significant increase in both systolic and diastolic blood pressure in the J+ group ($p<0.05$; $p<0.01$, respectively), thereby confirming the successful induction of hypertension. The administration of enalapril (J0) and *R. tomentosa* at a dosage of 300 mg/kg BW/day (J3) resulted in a significant reduction in systolic pressure when compared to the J+ group ($p<0.05$). In contrast, the lower doses (J1 and J2) did not produce statistically significant changes ($p>0.05$). A comparable trend was noted in diastolic pressure, with only the J0 and J3 groups showing a significant reduction relative to the hypertensive group ($p<0.05$). Although there were numerical reductions, the J1 and J2 groups did not show a statistically significant difference from J+ ($p>0.05$). The findings suggest that high-dose *R. tomentosa* (J3) has an antihypertensive effect similar to that of enalapril in both systolic and diastolic measurements, whereas lower doses do not achieve significant blood pressure normalization in hypertensive conditions.

Impact of *R. tomentosa* on malondialdehyde (MDA), superoxide dismutase (SOD), and neutrophil gelatinase-associated lipocalin (NGAL) in L-NAME-injected rats

This study evaluated the biochemical profiles of serum oxidative stress and inflammatory markers, specifically MDA, SOD, and NGAL, to determine the effects of *R. tomentosa* on L-NAME-induced hypertensive rats (Fig. 2a–c).

MDA levels, which serve as a marker for lipid peroxidation, were significantly increased in the J+ group relative to the untreated control (J−) ($p<0.05$), suggesting the presence of oxidative stress following L-NAME administration. The administration of enalapril (J0) and *R. tomentosa* at all doses (J1–J3) resulted in a significant reduction of MDA levels compared to J+ ($p<0.05$). No significant differences were found among the J0, J1, J2, and J3 groups ($p>0.05$), indicating similar antioxidant effects across the treatments.

SOD activity was significantly lower in the J+ group compared to the J− group ($p<0.05$), indicating oxidative dysregulation. Treatment with enalapril (J0) or *R. tomentosa* (J1–J3) resulted in a significant increase in SOD levels compared to J+ ($p<0.05$), with J0 and J3 demonstrating a marginally greater restoration of antioxidant capacity. No significant differences were found between the J1–J3 groups ($p>0.05$).

NGAL levels, recognized as a sensitive biomarker for inflammation and tissue injury, were significantly higher in the J+ group compared to the J− group ($p<0.01$), suggesting inflammatory stress induced by L-NAME. Enalapril (J0) and *R. tomentosa* at the highest dose (J3) significantly decreased NGAL expression relative to J+ ($p<0.01$ and $p<0.05$, respectively). NGAL levels in the J1 and J2 groups were lower than in the J+ group, yet did not achieve statistical significance ($p>0.05$), suggesting a limited anti-inflammatory effect at reduced doses. The findings indicate that *R. tomentosa*, especially at elevated doses, demonstrates antioxidant and anti-inflammatory properties similar to enalapril in L-NAME-induced hypertensive rats, as shown by decreases in MDA and NGAL levels and the restoration of SOD activity.

Histological changes in hypertensive rat heart tissue after administration of *R. tomentosa*

The extent of cardiac tissue injury was assessed by quantifying necrotic cells, edematous areas, and vascular congestion in heart sections of L-NAME-induced hypertensive rats (Fig. 3a–c). The administration of L-NAME resulted in a significant increase in the number of necrotic cells in the J+ group relative to the untreated control (J−) ($p<0.01$).

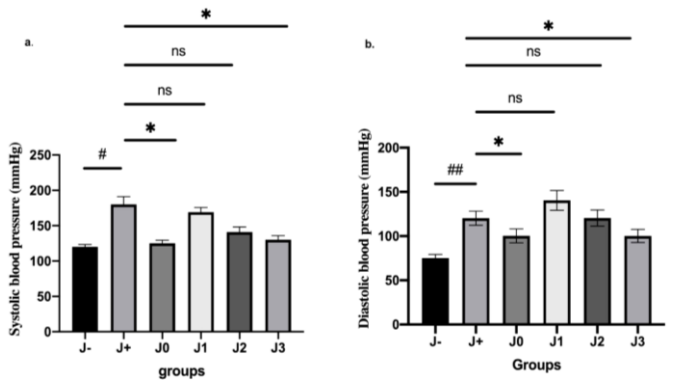

Figure 1. Alterations in systolic **(a)** and diastolic **(b)** blood pressure in L-NAME-treated rats following the injection of *R. tomentosa.*

J-: Untreated group, J+: The rats received only L-NAME injections. J0: The rats received L-NAME injections + enalapril at a dosage of 2 mg/kg BW/day, J1: Rats with L-NAME injections + 100 mg/kg BW/day of *R. tomentosa*. J2: Rats with L-NAME injections + 200 mg/kg BW/day of *R. tomentosa*. J3: Rats with L-NAME injections + 300 mg/kg BW/day of *R. tomentosa.* Values are expressed as mean ± SD (n = 36), Statistical analysis was performed using one-way ANOVA followed by Duncan's post-hoc test (#p<0.05 vs. J-, ##p<0.01 vs. J-, *p<0.05 vs. J+, nsp>0.05).

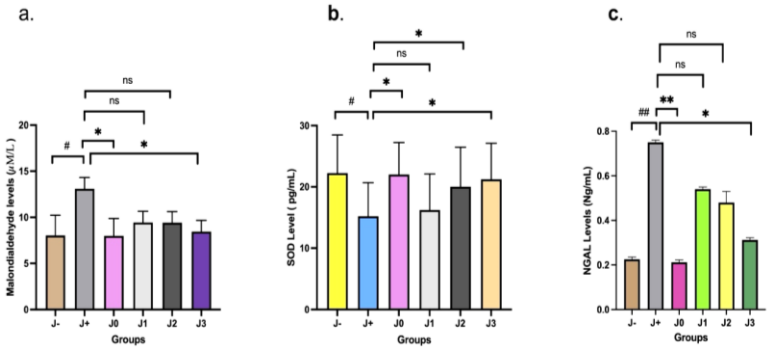

Figure 2. Impact of *R. tomentosa* administration on several blood analysis parameters in L-NAME injected rats.

(a) Malondialdehyde (MDA), **(b)** Superoxide Dismutase (SOD), **(c)** Neutrophil Gelatinase-Associated Lipocalin (NGAL). J-: Untreated group, J+: The rats received only L-NAME injections. J0: The rats received L-NAME injections + enalapril at a dosage of 2 mg/kg BW/day, J1: Rats with L-NAME injections + 100 mg/kg BW/day of *R. tomentosa*. J2: Rats with L-NAME injections + 200 mg/kg BW/day of *R. tomentosa*. J3: Rats with L-NAME injections + 300 mg/kg BW/day of *R. tomentosa*. Values are expressed as mean ± SD (n = 36), Statistical analysis was performed using one-way ANOVA followed by Duncan's post-hoc test (#p<0.05 vs. J-, ##p<0.01 vs. J-, *p<0.05 vs. J+, **p<0.01 vs. J+, nsp>0.05).

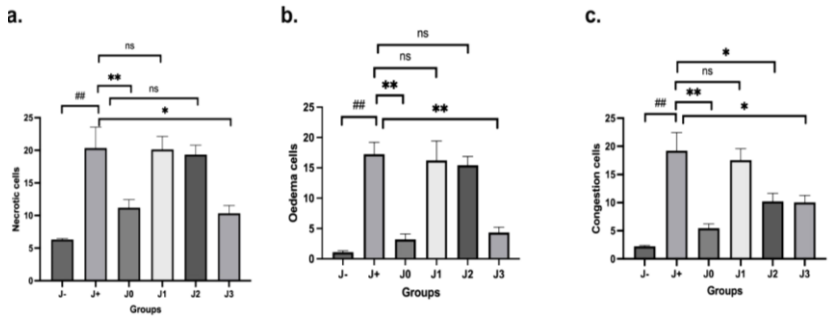

Figure 3. Analysis of the amount of heart tissue damage in hypertensive rats after administration of *R. tomentosa*.

(a) Necrosis cells, **(b)** Edema cells, **(c)** Congestion cells. J-: Untreated group, J+: Rats received only L-NAME injections. J0: Rats received L-NAME injections + enalapril at a dosage of 2 mg/kg BW/day, J1: Rats with L-NAME injections + 100 mg/kg BW/day of *R. tomentosa*. J2: Rats with L-NAME injections + 200 mg/kg BW/day of *R. tomentosa*. J3: Rats with L-NAME injections + 300 mg/kg BW/day of *R. tomentosa.* Values are expressed as mean ± SD (n = 36), Statistical analysis was performed using Kruskal-Wallis followed by Mann-Whitney's post-hoc test (##p<0.01 vs. J-, *p<0.05 vs. J+, **p<0.01 vs. J+,nsp>0.05).

Treatment with enalapril (J0) and the maximum dosage of *R. tomentosa* (J3) resulted in a significant reduction in necrotic cell counts compared to the J+ group (p<0.01 and p<0.05, respectively). The J1 and J2 groups exhibited no significant reductions (p>0.05), suggesting a limited protective effect at lower doses. A comparable trend was noted for edema, with the J+ group exhibiting a significant rise in the count of edematous cells relative to the J− group (p<0.01). Significant reductions in edema were noted in the J0 and J3 groups (p<0.01 vs. J+), while the J1 and J2 groups did not demonstrate significant improvements (p>0.05). The data indicate that only high-dose *R. tomentosa* and enalapril effectively reduce hypertensive-induced vascular leakage and fluid accumulation. Congestion scores were significantly higher in the J+ group compared to the J− group (p<0.01). The J0 and J3 groups exhibited a significant reduction in congestion relative to the J+ group (p<0.01 and *p<0.05, respectively). The J2 group exhibited a downward trend; however, statistical significance was not achieved (p>0.05). J1 did not significantly reduce congestion (p>0.05). The histological assessments indicate that the highest dose of *R. tomentosa* (J3) provides protection similar to enalapril in reducing necrosis, edema, and vascular congestion in hypertensive heart tissue, whereas lower doses exhibit reduced efficacy (Fig. 4).

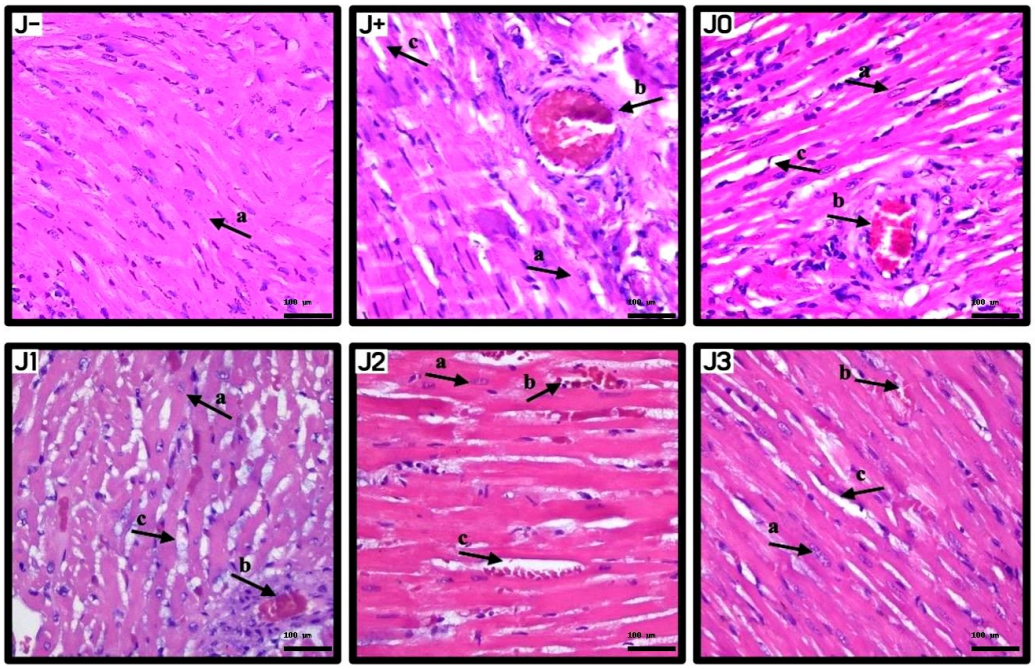

Figure 4. Histology of the heart of rats given *R. tomentosa* after L-NAME induction with HE staining.

(a) Necrosis, **(b)** Congestion, **(c)** Edema. J-: Untreated group, J+: Rats received only L-NAME injections. J0: Rats received L-NAME injections + enalapril at a dosage of 2 mg/kg BW/day, J1: Rats with L-NAME injections + 100 mg/kg BW/day of *R. tomentosa*. J2: Rats with L-NAME injections + 200 mg/kg BW/day of *R. tomentosa*. J3: Rats with L-NAME injections + 300 mg/kg BW/day of *R. tomentosa*. Magnification 400×.

FasL expression in hypertensive heart tissues following administration of *R. tomentosa*

The expression of FasL protein in cardiac tissue was analyzed through immunohistochemistry (IHC), with quantitative results illustrated in the accompanying bar graph (Fig. 5). In the untreated control group (J−), FasL expression was minimal, evidenced by the absence of brown staining and low quantification values. After L-NAME induction (J+), there was a significant increase in FasL expression ($p<0.01$ vs. J−), demonstrated by intense cytoplasmic brown staining in myocardial cells and the highest mean expression value across all groups. Treatment with enalapril (J0) or *R. tomentosa* at dosages of 100 mg/kg BW/day (J1) and 200 mg/kg BW/day (J2) led to slight decreases in FasL expression; however, these alterations were not statistically significant relative to the hypertensive group (J+) ($p>0.05$). The highest dose of *R. tomentosa* (300 mg/kg BW/day, J3) significantly decreased FasL expression compared to the J+ group ($p<0.05$), although it did not return to basal levels. This indicates a dose-dependent inhibition of FasL-mediated apoptosis. Histological analysis revealed that elevated FasL expression was associated with extensive and pronounced brown immunostaining within the cytoplasm of cardiomyocytes, especially in the J+ and J1 groups. In these groups, myocardial fibers exhibited increased fragmentation, accompanied by structural disarray and signs of inflammatory infiltration. Conversely, hearts from the J3 group exhibited diminished FasL staining, improved tissue architecture, and a lower presence of inflammatory cells. The morphological patterns observed substantiate the quantitative differences and underscore the potential role of *R. tomentosa* in modulating FasL-mediated apoptotic signaling in hypertensive cardiac injury.

Cytochrome c expression in hypertensive heart tissues following administration of *R. tomentosa*

Immunohistochemical staining demonstrated a significant increase in cytochrome c expression in the cardiac tissue of rats treated with L-NAME (J+ group) relative to the untreated control (J−), characterized by pronounced brown cytoplasmic staining across the myocardial fibers ($p<0.01$). The elevated expression indicates mitochondrial-mediated apoptotic activity induced by hypertensive stress. Quantitative analysis indicated a significant elevation in cytochrome c levels in the J+ group, with a mean expression surpassing threefold compared to the control (Fig. 6). Treatment with enalapril (J0) and *R. tomentosa* at all doses (J1–J3) led to partial reductions in cytochrome c expression compared to the hypertensive group (J+). Nonetheless, these reductions failed to achieve statistical significance ($p>0.05$), suggesting that neither standard antihypertensive treatment nor moderate doses of *R. tomentosa* significantly inhibited cytochrome c release. Only the highest dose of *R. tomentosa* (J3) demonstrated a decreasing trend that neared statistical significance ($p<0.05$ vs J+), while expression levels continued to exceed baseline (J−).

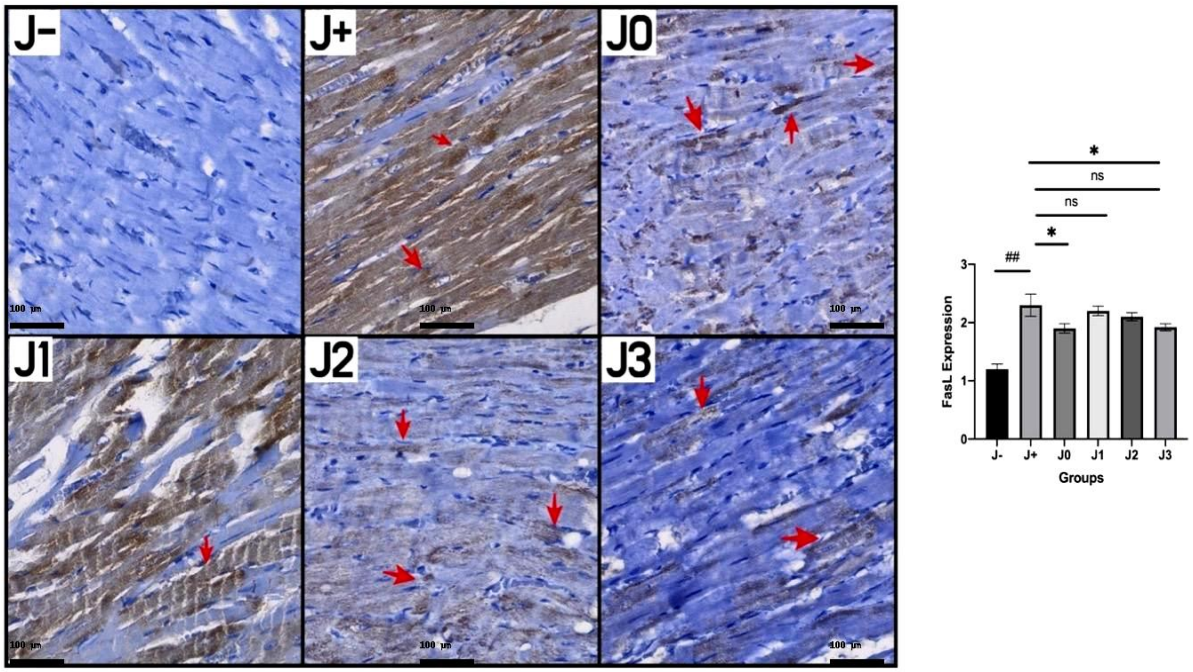

Figure 5. Immunohistochemical analysis of Fas ligand (FasL) expression in cardiac tissue of hypertensive rats following *R. tomentosa* treatment.

J−: untreated control; J+: L-NAME only; J0: L-NAME + enalapril 2 mg/kg BW; J1: L-NAME + *R. tomentosa* 100 mg/kg BW; J2: 200 mg/kg BW; J3: 300 mg/kg BW. Red arrows indicate positive FasL expression (brown staining) in cardiomyocytes, observed under 400× magnification. FasL expression was significantly increased in the J+ group compared to J− ($^{\#\#}p<0.01$), while treatment with enalapril (J0) and high-dose *R. tomentosa* (J3) markedly reduced FasL expression ($^{*}p<0.05$ vs. J+), suggesting an anti-apoptotic effect. Statistical analysis used Kruskal–Wallis followed by Mann–Whitney post hoc test. Data are presented as mean ± SD (n = 6 per group). $^{ns}p>0.05$

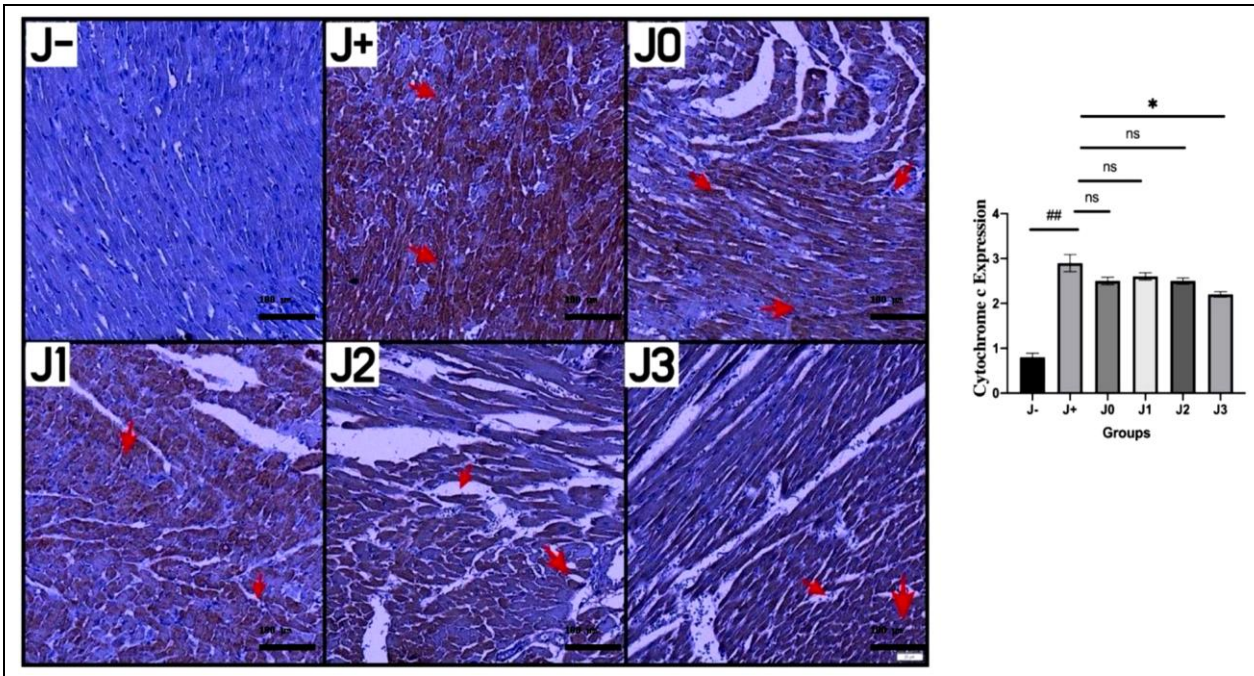

Figure 6. Expression of cytochrome c in cardiac tissue of L-NAME-induced hypertensive rats treated with *R. tomento*sa.

J−: untreated control; J+: L-NAME only; J0: L-NAME + enalapril 2 mg/kg BW; J1: L-NAME + *R. tomentosa* 100 mg/kg BW; J2: 200 mg/kg BW; J3: 300 mg/kg BW. Immunohistochemical staining shows mitochondrial cytochrome c localization (brown) at 400× magnification. Cytochrome c was highly expressed in the J+ group ($^{\#\#}p<0.01$ vs. J−), indicating mitochondrial-mediated apoptosis. Treatment with J0 and J3 significantly decreased cytochrome c expression compared to J+ ($^{*}p<0.05$), suggesting inhibition of apoptotic signaling. No significant change was observed in J1 and J2 ($^{ns}p>0.05$). Statistical analysis used Kruskal–Wallis followed by Mann–Whitney post hoc test. Data are presented as mean ± SD (n = 6 per group).

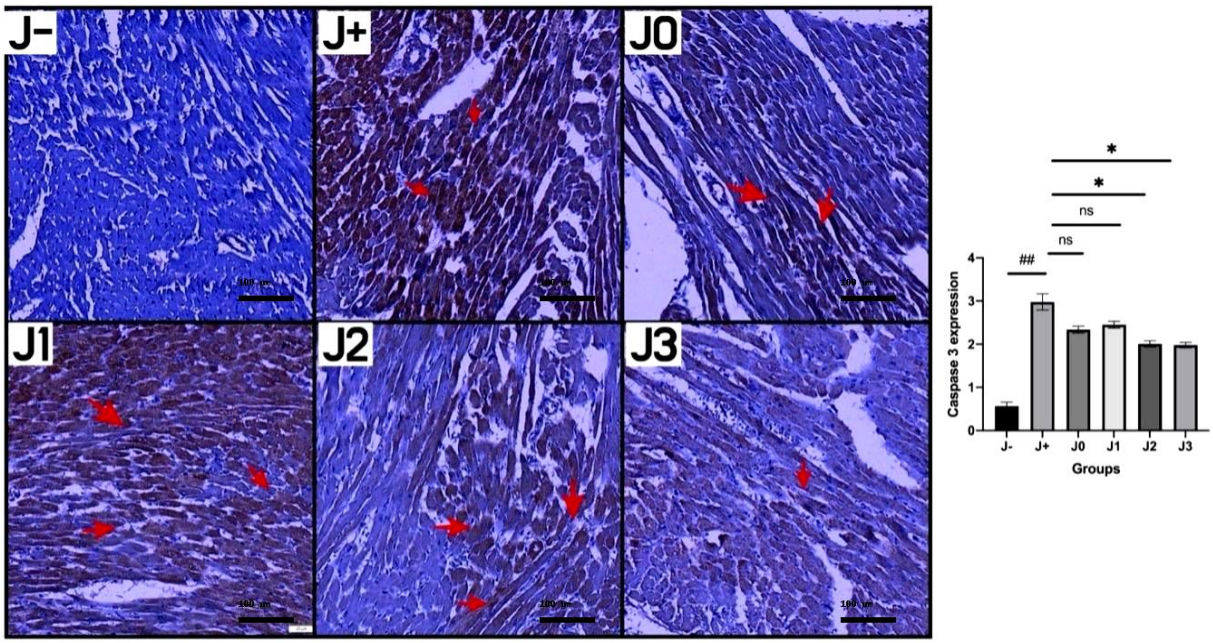

Figure 7. Expression of caspase 3 in hypertensive heart histology.

J-: Untreated group, J+: The rats received only L-NAME injections. J0: Rats received L-NAME injections + enalapril at a dosage of 2 mg/kg BW/day, J1: Rats with L-NAME injections + 100 mg/kg BW/day of *R. tomentosa*. J2: Rats with L-NAME injections + 200 mg/kg BW/day of *R. tomentosa*. J3: Rats with L-NAME injections + 300 mg/kg BW/day of *R. tomentosa*. Red arrows indicated positive caspase 3 expressions, magnification 400×. The J+ group displayed significantly elevated caspase-3 expression compared to J− ($^{\#\#}p<0.01$), indicating active apoptosis. Enalapril (J0) and high-dose *R. tomentosa* (J3) significantly reduced caspase-3 levels ($^{*}p<0.05$ vs J+), while J1 and J2 did not differ significantly (ns, $p>0.05$). These findings highlight the apoptosis-inhibiting effect of higher *R. tomentosa* doses. Statistical analysis was performed using Kruskal-Wallis followed by Mann-Whitney's post-hoc test ($^{\#\#}p<0.01$ vs J-, $^{*}p<0.05$ vs J+, $^{ns}p>0.05$).

Histological analysis revealed pronounced cytochrome c staining (red arrows) in the cytoplasm of myocardial cells within the J+ group, indicating mitochondrial leakage and the activation of apoptosis. Groups J1 and J2 displayed significant brown staining, whereas the J3 group demonstrated diminished staining intensity and a relatively intact cardiac structure, suggesting a possible reduction in mitochondrial apoptosis. The findings indicate that high-dose *R. tomentosa* may reduce cytochrome c-mediated apoptotic signaling in hypertensive cardiac injury, although it does not achieve complete normalization.

Caspase 3 expression in hypertensive heart tissues following administration of *R. tomentosa*

The quantitative and histological analysis of caspase-3 expression revealed a significant increase in L-NAME-induced hypertensive rats (J+ group) compared to the untreated control (J−) (Fig. 7). This was evidenced by a notable elevation in brown cytoplasmic staining and a corresponding rise in expression level ($p<0.01$). This suggests increased activation of the executioner phase of apoptosis due to hypertensive stress. In the J0 group (enalapril-treated), caspase-3 expression was elevated, albeit slightly lower than in the J+ group; however, this difference was not statistically significant ($p>0.05$). Comparable nonsignificant reductions were noted in the J1 and J2 groups administered 100 and 200 mg/kg BW/day of *R. tomentosa,* respectively. Only the highest dose group (J3) demonstrated a statistically significant reduction in caspase-3 expression relative to the J+ group ($p<0.05$), although levels remained above baseline (J−). Histological analysis revealed intense cytoplasmic brown staining, indicative of active caspase-3 expression, primarily in the J+ and J1 groups (red arrows), suggesting extensive apoptotic signaling. The J3 group demonstrated significantly diminished staining intensity and a more organized myocardial structure. These findings indicate that *R. tomentosa*, especially at elevated doses, reduces apoptosis through the modulation of caspase-3 expression, thus maintaining cardiac tissue structure in hypertensive conditions.

DISCUSSION

L-NAME injection elevates systolic and diastolic blood by inhibiting nitric oxide synthase, leading to a significant reduction in nitric oxide (NO) production (Shin et al., 2014). This reduction results in blood vessel contraction (vasoconstriction) and an increase in peripheral vascular resistance. Systolic blood pressure rises as the heart exerts greater force to overcome heightened resistance. Furthermore, the absence of NO inhibition diminishes the vasodilatory effect on the blood vessel wall during diastole, resulting in an elevation of diastolic blood pressure (Flint et al.,

2019). Decreased NO levels disturb the equilibrium between vasodilation and vasoconstriction, resulting in a persistently elevated vascular tone. This condition may induce experimental hypertension in research models (Tejero et al., 2019). Administering the maximum dosage of herbs may mitigate hypertension induced by L-NAME administration. *R. tomentosa* exhibits antioxidant properties that mitigate oxidative stress, safeguard blood vessels from damage, and support healthy endothelial function (Situmorang et al., 2024). *R. tomentosa* enhances the body's natural production of nitric oxide by neutralizing free radicals that impede its synthesis (Situmorang et al., 2024). The current study confirms that administration of *R. tomentosa* particularly at the highest dose, alleviates this hypertensive state.

L-NAME injection in rats influences the body's antioxidant levels, particularly resulting in a reduction of SOD levels (Bar and Kara, 2024). Decreased levels of NO lead to an increased formation of free radicals, which can diminish the activity of the SOD enzyme, responsible for converting free radicals into a more stable form. The reduction in SOD levels signifies heightened oxidative stress within the body, potentially leading to cellular damage. On the other hand, increased NGAL levels in rats injected with L-NAME may indicate a response to oxidative stress and tissue damage. The elevation of NGAL may correlate with the body's response to injury induced by oxidative stress, which arises from reduced SOD activity (Ismi et al., 2023). NGAL is increasingly recognized as a sensitive biomarker of oxidative stress-induced inflammation and cellular damage. Our findings mirror recent data where *Moringa oleifera* supplementation restored SOD activity and reduced NGAL levels in preclinical cardiovascular models (Romejiko et al., 2023). Treatment with *R. tomentosa* significantly improved these biomarkers, suggesting a strong antioxidant capacity. Unlike earlier studies that cited older sources such as Ismi et al. (2023), recent evidence emphasizes that modulation of NGAL and SOD levels is a hallmark of effective cardioprotective phytotherapy (Zheng et al., 2023). Lower levels of SOD can result in the accumulation of free radicals, subsequently inducing inflammation and prompting an increase in NGAL as an adaptive response to cellular damage (Younus, 2018). *R. tomentosa* possesses antioxidants that significantly enhance SOD levels while reducing MDA and NGAL levels, which serve as markers of oxidative stress and cellular damage (Situmorang et al., 2024). SOD is an enzyme that protects cells from free radical damage by converting superoxide radicals into less harmful hydrogen peroxide. Antioxidants enhance SOD activity, thereby preventing free radical accumulation that can result in lipid peroxidation and elevated MDA levels, which are indicators of cell membrane damage (Ilyas et al., 2019). Furthermore, antioxidants may reduce NGAL levels, a biomarker for inflammation and kidney injury, by mitigating inflammation and oxidative stress (Zheng et al., 2023). Effective utilization of antioxidants enhances cellular protection against oxidative stress, thereby reducing organ damage and improving overall health.

Histological analysis further revealed that L-NAME-induced rats exhibited marked myocardial damage, including necrosis, edema, and vascular congestion. Oxidative stress arises from an imbalance between free radicals and the body's capacity to neutralize them, leading to damage in heart cells, blood vessels, and heart muscle (Münzel et al., 2017). The damage increases capillary permeability, leading to fluid accumulation in the extracellular space and causing edema. L-NAME interferes with blood circulation, triggers congestion, and reduces oxygen supply to heart tissue, potentially leading to heart cell necrosis due to insufficient oxygen and nutrients (Vasques-Nóvoa et al., 2022). The antioxidants present in *R. tomentosa* may offer protection against damage induced by L-NAME. Antioxidants neutralize free radicals, which reduce oxidative damage to cardiac cells and blood vessels (Idris et al., 2022). Minimizing oxidative damage can reduce edema and congestion resulting from vascular injury. High-dose *R. tomentosa* attenuated these pathological features, likely through inhibition of lipid peroxidation and enhancement of microvascular integrity. Recent meta-analytical data support that flavonoid-rich herbs, including *R. tomentosa*, improve cardiac tissue integrity by stabilizing endothelial permeability and modulating inflammatory cascades (Situmorang et al., 2024). Furthermore, antioxidants may inhibit necrosis by preserving cellular integrity and enhancing microcirculation disorders (Manurung et al., 2021). Consequently, antioxidants derived from natural herbs such as *R. tomentosa* play a crucial role in preserving optimal cardiac structure and function in the context of oxidative stress (Vo and Ngo, 2019).

FasL is a key pro-apoptotic ligand upregulated under hypertensive oxidative stress. Its excessive activation can lead to unnecessary cardiomyocyte loss and progressive cardiac dysfunction (Zhazykbayeva et al., 2020). Oxidative stress in the heart, as induced by L-NAME injection in hypertension, can lead to increased FasL levels, which may initiate apoptosis in compromised cardiac cells, thereby mitigating the buildup of non-functional cells (Zhazykbayeva et al., 2020). Excessive activation of FasL can lead to additional damage to heart tissue; therefore, appropriate regulation of FasL is crucial to mitigate severe tissue injury (Młynarska et al., 2024a). Conversely, antioxi-

dants in plants are essential for mitigating the effects of oxidative stress on the heart. Oxidative stress arises from an imbalance between the generation of ROS and the capacity of the body's antioxidant system to counteract them (Muscolo et al., 2024). In the context of cardiac physiology, elevated ROS can impair cellular structures and exacerbate cardiovascular disease. Antioxidants, whether endogenous or exogenous, serve to neutralize ROS, inhibit the oxidation of lipids and proteins, and safeguard cardiac DNA from damage (Sharifi-Rad et al., 2020). Antioxidants contribute to the maintenance of heart function, facilitate the repair of existing damage, and lower the risk of heart disease associated with oxidative stress (Młynarska et al., 2024b). Our study confirms that *R. tomentosa* at high dose downregulates FasL expression, suggesting a protective role in preserving cardiomyocyte viability, which reduces FasL and p53 activation in myocardial tissue through PRDX2 modulation (Situmorang et al., 2024). These findings indicate that apoptosis-regulatory properties of traditional herbs warrant further exploration, particularly in hypertensive contexts where such pathways are underexplored.

Additionally, cytochrome c and caspase-3, both critical mediators of the intrinsic apoptotic pathway, were upregulated following L-NAME treatment. Increased cytosolic cytochrome c indicates mitochondrial dysfunction, triggering caspase cascades that culminate in cardiomyocyte apoptosis (Ishikawa et al., 2021). This study observed that *R. tomentosa* administration reduced both cytochrome c and caspase-3 expression in cardiac tissue. These findings align with recent research demonstrating that phenolic-rich extracts, such as myricetin from *R. tomentosa*, attenuate apoptosis and inflammation in endothelial cells. Antioxidants in *R. tomentosa* play a crucial role in regulating cytochrome c levels in hypertensive hearts by mitigating oxidative damage associated with elevated blood pressure (Amponsah-Offeh et al., 2023). In hypertension, excessive oxidative stress can impair the structure and function of cardiac cells, particularly by impacting mitochondria, which are crucial for energy metabolism and the regulation of apoptosis (Amponsah-Offeh et al., 2023). Cytochrome c, a component of the apoptotic pathway, can be released from mitochondria following cellular damage. Antioxidants neutralize free radicals that induce oxidative damage, thereby reducing excessive cytochrome c release and preventing excessive apoptosis in cardiac cells (Vo and Ngo, 2019). Oxidative stress in heart cells arises from an imbalance between free radical production and the antioxidant system's capacity to neutralize them (Vo and Ngo, 2019). This condition can lead to the activation of caspase 3, which is involved in the signaling pathway that initiates apoptosis (Situmorang and Ilyas, 2018b). Oxidative stress can damage cellular components, including lipids, proteins, and DNA, leading to the activation of caspase 3 (Hussar, 2022). The activation of caspase 3 results in the degradation of critical cellular structures, leading to damage and death of heart cells, which may contribute to the progression of heart disease, including heart failure (Hussar, 2022). The role of antioxidants is crucial in mitigating the adverse effects of oxidative stress on cardiac cells (Zhazykbayeva et al., 2020). Antioxidants, derived from natural sources like vitamins C and E or flavonoid compounds, as well as those synthesized by the body, play a crucial role in neutralizing free radicals and mitigating oxidative damage to cells (Lobo et al., 2010). The decrease in oxidative stress may impede the activation of caspase 3 and the apoptosis process induced by oxidative stress (Ismi et al., 2023; Situmorang and Ilyas, 2018b). Antioxidants derived from plants like *R. tomentosa* serve to protect heart cells by preserving redox balance, mitigating cellular damage, and lowering the likelihood of additional heart injury caused by oxidative stress (Zhao et al., 2020).

Various phytochemicals found in *R. tomentosa*, including rhodomyrtone, flavonoids (e.g., myricetin, quercetin), and triterpenoids, have been documented to influence apoptosis-related pathways. Rhodomyrtone inhibits mitochondrial-mediated apoptosis by suppressing cytochrome c release and the subsequent activation of caspase-3 and caspase-9 in multiple cancer and endothelial cell models (Kong and Yu, 2021). Furthermore, myricetin has demonstrated the ability to reduce oxidative stress-induced apoptosis by increasing Bcl-2 expression and decreasing Bax and cleaved caspase-3, thus preserving mitochondrial integrity (Na-Phatthalung et al., 2021). The findings elucidate the mechanisms by which *R. tomentosa* phytoconstituents may influence apoptotic signaling, corroborating the observed decreases in FasL, cytochrome c, and caspase-3 in this study. The cardioprotective effects noted in the hypertensive rat model may be partially ascribed to the modulation of caspase-dependent apoptotic pathways by these bioactive compounds.

Although the results are encouraging, it is important to acknowledge several limitations. While earlier research on *R. tomentosa* has detailed its phytochemical composition and indicated involvement in apoptotic pathways (Situmorang et al., 2021), the current study lacks molecular validation via TUNEL assay, Western blotting, or qRT-PCR, which are essential for confirming the inhibition of apoptosis pathways at the protein or gene level. Future research should aim to address these gaps within the same experimental framework to enhance mechanistic un-

derstanding. Furthermore, while previous research has investigated the phytochemical profiling of *R. tomentosa*, this specific extract batch has not undergone such analysis. Future studies should utilize advanced techniques like LC-MS/MS to verify the bioactive constituents, ensuring reproducibility and consistency of compounds. The duration of the intervention was limited, resulting in uncertainty regarding the long-term efficacy, toxicity, and reversibility of the effects. This study is primarily preclinical in nature. While *R. tomentosa* demonstrated efficacy similar to enalapril in certain outcomes, these results should not be generalized to clinical situations without additional validation. Meta-analyses have consistently warned against the extrapolation of conclusions from animal models to human therapy in the absence of rigorous clinical trials.

CONCLUSION

The present study demonstrated that *R. tomentosa* exerted cardioprotective effects in a hypertensive rat model by modulating both intrinsic and extrinsic apoptotic pathways. A significant dose-dependent reduction in the expression of FasL, cytochrome c, and caspase-3 was observed, particularly at the highest dose (300 mg/kg BW), which showed effects comparable to the standard treatment with enalapril. These findings were accompanied by improvements in oxidative stress markers, including decreased MDA and NGAL, and increased SOD activity. The cardioprotective activity of *R. tomentosa* is likely associated with its antioxidant and anti-apoptotic properties, which mitigate myocardial damage induced by hypertensive conditions. While the results are promising, they remain limited to an *in vivo* preclinical model. Therefore, further investigations, particularly involving molecular validation and phytochemical characterization of active compounds, are essential to confirm the underlying mechanisms and to evaluate the potential translational application of *R. tomentosa* as an adjunctive therapy for hypertensive cardiac injury.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

Ameer OZ (2022) Hypertension in chronic kidney disease: What lies behind the scene. Front Pharmacol 13: 949260. https://doi.org/10.3389/fphar.2022.949260

Amponsah-Offeh M, Diaba-Nuhoho P, Speier S, Morawietz H (2023) Oxidative stress, antioxidants and hypertension. Antioxidants 12(2): 281. https://doi.org/10.3390/antiox12020281

Bar S, Kara M (2024) Linalool exerts antioxidant activity in a rat model of diabetes by increasing catalase activity without antihyperglycemic effect. Exp Ther Med 28(3): 359. https://doi.org/10.3892/etm.2024.12648

Eirin A, Lerman A, Lerman LO (2014) Mitochondrial injury and dysfunction in hypertension-induced cardiac damage. Eur Heart J 35(46): 3258–66. https://doi.org/10.1093/eurheartj/ehu436

Flint AC, Conell C, Ren X, Banki NM, Chan SL, Rao VA, Melles RB, Bhatt DL (2019) Effect of systolic and diastolic blood pressure on cardiovascular outcomes. N Engl J Med 381(3): 243-251. https://doi.org/10.1056/NEJMoa1803180

Hussar P (2022) Apoptosis regulators Bcl-2 and caspase-3. Encyclopedia 2(4): 1624–1636. https://doi.org/10.3390/encyclopedia2040111

Idris M, Sukandar ER, Purnomo AS, Martak F, Fatmawati S (2022) Antidiabetic, cytotoxic and antioxidant activities of *Rhodomyrtus tomentosa* leaf extracts. RSC Advances 12(41): 25697–25710. https://doi.org/10.1039/D2RA03944C

Ilyas S, Murdela F, Hutahaean S, Situmorang PC (2019) The effect of haramounting leaf ethanol extract (*Rhodomyrtus tomentosa* (Aiton) Hassk.) on the number of leukocyte type and histology of mice pulmo (*Mus Musculus L.*) exposed to electronic cigarette. Open Access Maced J Med Sci 7(11): 1750-1756. https://doi.org/10.3889/oamjms.2019.467

Irianti E, Ilyas S, Hutahaean S, Rosidah R, Situmorang PC (2020) Placental histological on preeclamptic rats (*Rattus norvegicus*) after administration of nanoherbal haramonting (*Rhodomyrtus tomentosa*). Res J Pharm Tech 13(8): 3879–3882. https://doi.org/10.5958/0974-360X.2020.00686.1

Ishikawa W, Kazama S, Suzuki T, Yamana R, Miyazaki Y, Tanaka K, Usami M, Takizawa T (2021) L-NAME, a nitric oxide synthase inhibitor, increases the protein expression of both executioner and inhibitor of apoptosis in the placental bed of mid-to-late pregnant rats. Congenit Anom (Kyoto) 61(5): 183–187. https://doi.org/10.1111/cga.12420

Ismy J, Syukri M, Emril DR, Sekarwana, N, Ismy J (2023) Superoxide dismutase reduces creatinine and NGAL by restoring oxidative balance during sepsis. Emerg Sci J 6(2): 286–294. https://doi.org/10.28991/ESJ-2022-06-02-06

Kim NH, Kang PM (2010) Apoptosis in cardiovascular diseases: mechanism and clinical implications. Korean Circ J 40(7): 299–305. https://doi.org/10.4070/kcj.2010.40.7.299

Kong Y, Yu Z (2021) The health beneficial properties of *Rhodomyrtus tomentosa* as potential functional food. Food Res Int 140: 109859. https://doi.org/10.1016/j.foodres.2020.109859

Krautwald S, Ziegler E, Rölver L, Linkermann A, Keyser KA, Steen P, Wollert KC, Korf-Klingebiel M, Kunzendorf U (2010) Effective blockage of both the extrinsic and intrinsic pathways of apoptosis in mice by TAT-crmA. J Biol Chem 285(26): 19997-20005. https://doi.org/10.1074/jbc.M110.122127

Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 4(8): 118–126. https://doi.org/10.4103/0973-7847.70902

Manurung RD, Ilyas S, Hutahaean S, Rosidah R, Situmorang PC (2021) Diabetic wound healing in FGF expression by nano

herbal of *Rhodomyrtus tomentosa* L. and *Zanthoxylum acanthopodium* fruits. Pak J Biol Sci 24(3): 401–408. https://doi.org/10.3923/pjbs.2021.401.408

Masenga SK, Kirabo A (2023) Hypertensive heart disease: Risk factors, complications and mechanisms. Front Cardiovasc Med 10: 1205475. https://doi.org/10.3389/fcvm.2023.1205475

Młynarska E, Biskup L, Możdżan M, Grygorcewicz O, Możdżan Z, Semeradt J, Uramowski, M, Rysz J, Franczyk B (2024b) the role of oxidative stress in hypertension: The insight into antihypertensive properties of vitamins A, C and E. Antioxidants 13(7): 848. https://doi.org/10.3390/antiox13070848

Młynarska E, Hajdys J, Czarnik W, Fularski P, Leszto K, Majchrowicz G, Lisińska W, Rysz J, Franczyk B (2024a) The role of antioxidants in the therapy of cardiovascular diseases-A literature review. Nutrients 16(16): 2587. https://doi.org/10.3390/nu16162587

Münzel T, Camici GG, Maack C, Bonetti NR, Fuster V, Kovacic JC (2017) Impact of oxidative stress on the heart and vasculature: Part 2 of a 3-Part Series. J Am Coll Cardiol 70(2): 212–229. https://doi.org/10.1016/j.jacc.2017.05.035

Muscolo A, Mariateresa O, Giulio T, Mariateresa R (2024) Oxidative stress: The role of antioxidant phytochemicals in the prevention and treatment of diseases. Int J Mol Sci 25(6): 3264. https://doi.org/10.3390/ijms25063264

Mustafa M, Ahmad R, Tantry IQ, Ahmad W, Siddiqui S, Alam M, Abbas K, Moinuddin, Hassan MI, Habib S, Islam S (2024) Apoptosis: A comprehensive overview of signaling pathways, morphological changes, and physiological significance and therapeutic implications. Cells 13(22): 1838. https://doi.org/10.3390/cells13221838

Nadar SK, Lip GYH (2021) The heart in hypertension. J Hum Hypertens 35: 383–386. https://doi.org/10.1038/s41371-020-00427-x

Na-Phatthalung P, Teles M, Voravuthikunchai SP, Tort L, Fierro-Castro C (2018) Immunomodulatory effects of *Rhodomyrtus tomentosa* leaf extract and its derivative compound, rhodomyrtone, on head kidney macrophages of rainbow trout (*Oncorhynchus mykiss*). Fish Physiol Biochem 44(2): 543–555. https://doi.org/10.1007/s10695-017-0452-2

Oparil S, Acelajado MC, Bakris GL, Berlowitz DR, Cífková R, Dominiczak AF, Grassi G, Jordan J, Poulter NR, Rodgers A, Whelton PK (2018) Hypertension. Nat Rev Dis Primers 4: 18014. https://doi.org/10.1038/nrdp.2018.14

Romejko K, Markowska M, Niemczyk S (2023) The review of current knowledge on neutrophil gelatinase-associated lipocalin (NGAL). Int J Mol Sci 24(13): 10470. https://doi.org/10.3390/ijms241310470

Rumahorbo CGP, Situmorang PC, Zagoto ERMP, Nisfa L, Hasanah U (2023) Effects of micro-colloidal *Rhodomyrtus tomentosa* on MMP9, GLUT-1, and IL-1β expression in *Rattus norvegicus* cervical cancer. J Pharm Pharmacogn Res 11(3): 537–546. https://doi.org/10.56499/jppres23.1618_11.3.537

Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, Rajkovic J, Tsouh Fokou PV, Azzini E, Peluso I, Prakash Mishra A, Nigam M, El Rayess Y, Beyrouthy ME, Polito L, Iriti M, Martins N, Martorell M, Docea AO, Setzer WN, Calina D, Cho WC, Sharifi-Rad J (2020) Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. Front Physiol 11: 694. https://doi.org/10.3389/fphys.2020.00694

Shin IW, Hah YS, Kim C, Park J, Shin H, Park KE, Ok SH, Lee HK, Chung YK, Shim HS, Lim DH, Sohn JT (2014) Systemic blockage of nitric oxide synthase by L-NAME increases left ventricular systolic pressure, which is not augmented further by Intralipid®. Int J Biol Sci 10(4): 367-376. https://doi.org/10.7150/ijbs.8048

Simanullang RH, Situmorang PC, Herlina M, Noradina, Silalahi B (2022a) Cytochrome c expression by andaliman (*Zanthoxylum acanthopodium*) on cervical cancer histology. Pak J Biol Sci 25(1): 49–55. https://doi.org/10.3923/pjbs.2022.49.55

Simanullang RH, Situmorang PC, Herlina M, Noradina, Silalahi B, Manurung SS (2022b) Histological changes of cervical tumours following *Zanthoxylum acanthopodium* DC treatment, and its impact on cytokine expression. Saudi J Biol Sci 29(4): 2706–2718. https://doi.org/10.1016/j.sjbs.2021.12.065

Situmorang PC, Ilyas S (2018a) Description of testis histology of *Mus Musculus* after giving nano herbal *Rhodomyrtus tomentosa* (Haramonting). Asian J Pharm Clin Res 11(11): 460–463. https://doi.org/10.22159/ajpcr.2018.v11i11.29042

Situmorang PC, Ilyas S (2018b) Review: Germinal cell apoptosis by herbal medicine. Asian J Pharm Clin Res 11(9): 24-31 http://dx.doi.org/10.22159/ajpcr.2018.v11i9.26400

Situmorang PC, Ilyas S, Hutahaean S, Rosidah R (2021) Components and acute toxicity of nanoherbal haramonting (*Rhodomyrtus tomentosa*). J Herbmed Pharmacol. 10(1): 139–148. https://doi.org/10.34172/jhp.2021.15

Situmorang PC, Ilyas S, Siahaan DAS, Restuati M, Sari ER, Chairunisa C, Maliki MF (2022a) Effect of *Rhodomyrtus tomentosa* Hassk. on HIF1α and VEGF expressions on hypertension placental. J Pharm Pharmacogn Res 10(6): 1076–1086. https://doi.org/10.56499/jppres22.1517_10.6.1076

Situmorang PC, Ilyas S, Syahputra RA, Sari RM, Nugraha AP, Ibrahim A, Rumahorbo CGP (2024) Role of *Rhodomyrtus tomentosa* (Aiton) Hassk. in regulating the expression of fibroblast growth factor family in the liver of rat in a breast cancer model. J Pharm Pharmacogn Res 12(4): 800–813. https://doi.org/10.56499/jppres23.1810_12.4.800

Situmorang PC, Simanullang RH, Syahputra RA, Hutahaean MM, Sembiring H, Nisfa, Sari ER (2023) Histological analysis of TGFβ1 and VEGFR expression in cervical carcinoma treated with *Rhodomyrtus tomentosa*. Pharmacia 70(1): 217–223. https://doi.org/10.3897/pharmacia.70.e96811

Situmorang PC, Syahputra RA, Simanullan RH (2022b) EGFL7 and HIF-1a expression on human trophoblast placental by *Rhodomyrtus tomentosa* and *Zanthoxylum acanthopodium* . Pak J Bio Sci 25(2): 123–130. https://doi.org/10.3923/pjbs.2022.123.130

Situmorang PC, Syahputra RA, Siregar ES, Putri MSS, Tallei TE, Setiawan E (2025) Effect of *Rhodomyrtus tomentosa* (Aiton) Hassk. on aortic hyperglycemia via JAK family expression. J Pharm Pharmacogn Res 13(4): 979–990. https://doi.org/10.56499/jppres24.2134_13.4.979

Tejero J, Shiva S, Gladwin MT (2019) Sources of vascular nitric oxide and reactive oxygen species and their regulation. Physiol Rev 99(1): 311–379. https://doi.org/10.1152/physrev.00036.2017

Vasques-Nóvoa F, Angélico-Gonçalves A, Alvarenga JMG, Nobrega J, Cerqueira RJ, Mancio J, Leite-Moreira AF, Roncon-Albuquerque R Jr (2022) Myocardial oedema: pathophysiological basis and implications for the failing heart. ESC Heart Fail 9(2): 958-976. https://doi.org/10.1002/ehf2.13775

Vo TS, Ngo DH (2019) The health beneficial properties of *Rhodomyrtus tomentosa* as potential functional food. Biomolecules 9(2): 76. https://doi.org/10.3390/biom9020076

Younus H (2018) Therapeutic potentials of superoxide dismutase. Int J Health Sci (Qassim) 12(3): 88-93. https://pmc.ncbi.nlm.nih.gov/articles/PMC5969776/

Zhao Z, Wu L, Xie J, Feng Y, Tian J, He X, Li B, Wang L, Wang X, Zhang Y, Wu S, Zheng X (2020) *Rhodomyrtus tomentosa* (Aiton.): A review of phytochemistry, pharmacology and

industrial applications research progress. Food Chem 309: 125715. https://doi.org/10.1016/j.foodchem.2019.125715

Zhazykbayeva S, Pabel S, Mügge A, Sossalla S, Hamdani N (2020) The molecular mechanisms associated with the physiological responses to inflammation and oxidative stress in cardiovascular diseases. Biophys Rev 12(4): 947–968. https://doi.org/10.1007/s12551-020-00742-0

Zheng M, Liu Y, Zhang G, Yang Z, Xu W, Chen Q (2023) The applications and mechanisms of superoxide dismutase in medicine, food, and cosmetics. Antioxidants 12(9): 1675. https://doi.org/10.3390/antiox12091675

AUTHOR CONTRIBUTION:

Contribution	Situmorang PC	Tanjung M	Syahputra RA	Nugraha AP	Lutfia A
Concepts or ideas	x	x			
Design	x	x			
Definition of intellectual content		x	x	x	
Literature search			x		
Experimental studies			x	x	
Data acquisition	x	x			x
Data analysis	x	x			x
Statistical analysis	x				
Manuscript preparation	x		x	x	
Manuscript editing	x				
Manuscript review	x	x	x	x	x

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