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Original Article

Modulation of serum transient receptor potential ankyrin 1 (TRPA1) levels by *Phyllanthus emblica* fruit extract in doxorubicin-treated rats

Wenny Wisely[1], Urip Harahap[1], Dadang I. Husori[1], Nur A. Juwita[1]*, Said H. Alfajar[2] and Nabila Apriliani[3]

[1]Department of Pharmacy, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia; [2]Department of Pharmacy, Faculty of Pharmacy, Institut Kesehatan Helvetia, Medan, Indonesia; [3]Department of Pharmacy, Faculty of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Sehat, Medan, Indonesia

*Correspondence: nurairajuwita@usu.ac.id

Abstract

Doxorubicin is widely used as an anticancer agent, but its clinical use is limited by cardiovascular toxicity associated with oxidative stress and inflammatory responses. Transient receptor potential ankyrin 1 (TRPA1) has been implicated in cardiovascular regulation and may contribute to doxorubicin-related cardiovascular alterations. Balakka fruit (*Phyllanthus emblica*) contains bioactive compounds with antioxidant potential; however, its effect on TRPA1 expression in doxorubicin-induced cardiovascular injury remains unclear. The aim of this study was to evaluate the effect of ethanol extract of balakka fruit (EEBF) on TRPA1 levels in doxorubicin-induced experimental animals. Balakka fruit extract was prepared using 70% ethanol maceration, followed by simplicia and extract characterization and phytochemical screening. An in vivo experiment was conducted using six groups: normal control, doxorubicin control, doxorubicin plus quercetin, and doxorubicin plus EEBF at doses of 100, 300, and 500 mg/kg BW. TRPA1 levels were measured using enzyme-linked immunosorbent assay. Phytochemical screening showed that balakka fruit simplicia and extract contained alkaloids, flavonoids, glycosides, saponins, and tannins. Doxorubicin administration increased TRPA1 levels compared with the normal group, while EEBF reduced TRPA1 levels in a dose-related pattern. The EEBF 500 mg/kg BW dose showed the strongest reduction in TRPA1 levels compared with the doxorubicin control group. These findings suggest that EEBF may modulate TRPA1 expression in doxorubicin-induced cardiovascular injury, potentially through its antioxidant-related bioactive compounds. Further studies are needed to evaluate its active compounds, antioxidant and inflammatory pathways, toxicity, and direct cardiovascular effects.

Keywords: Cardiovascular disease, doxorubicin, antioxidant, balakka fruit, TRPA1

Introduction

The heart is a vital organ in the body that functions to pump nutrients throughout the body via the blood vessels; however, mortality caused by cardiovascular diseases exceeds 17 million annually and continues to increase, posing a serious threat to human health [1]. Cardiovascular disease is the leading cause of death in most countries around the world [1]. In Indonesia, cardiovascular disease accounts for 651,481 deaths annually [2]. One form of cardiovascular disease is hypertension, a cardiovascular condition characterized by consistently elevated blood

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pressure, which occurs when arterioles exert excessive pressure on vessel walls due to blood flow, forcing the heart to work harder to maintain pressure [3]. The number of hypertension patients increases annually, with the global population affected expected to exceed 1.5 billion by 2025 [4]. Each year, hypertension and its complications are projected to cause 9.4 million deaths, and globally, 54% of adults are diagnosed with this condition [5]. Elevated blood pressure increases the risk of myocardial infarction, heart failure, stroke, and kidney disorders. In Indonesia, the prevalence of hypertension has risen from 25.8% in 2013 to 34.1% in 2018 [6].

Hypertension arises from a complex interaction between environmental, pathophysiological, genetic, lifestyle factors, and other medical conditions [7]. The underlying mechanisms include age-related changes in kidney function, salt-sensitive hypertension, disruption of the renin-angiotensin-aldosterone system (RAAS), arterial stiffening, and nervous system dysfunction [8]. The RAAS is essential for blood pressure regulation, primarily through the effect of angiotensin II on vascular tone [9]. Additionally, the transient receptor potential ankyrin (TRPA1) channel helps regulate blood pressure in the cardiovascular system by acting as a cardiovascular activator and modulator in hypertension [10]. This channel can also modulate vascular smooth muscle and thereby influence hypertension [10]. Pharmacotherapy for hypertension should be initiated without delay. In individuals with low to moderate cardiovascular risk, lifestyle modifications may be considered as an initial approach, and in such cases, the immediate commencement of medication is not always required. However, under typical circumstances, combination therapy involving a renin–angiotensin system (RAS) inhibitor is recommended to be started as part of the treatment regimen [11].

A chemotherapeutic agent that is frequently used to treat cancer is doxorubicin. However, the side effect resulting from doxorubicin induction is cardiotoxicity, which leads to cardiac remodeling [12]. Doxorubicin induction affects the body's pathophysiology and ultimately causes cardiomyopathy [13]. These outcomes collectively contribute to the decline of heart structure and function. Doxorubicin induction also increases the production of reactive oxygen species (ROS), which affects the cardiovascular system [12].

ROS are caused by increased oxidation originating from oxidative metabolic disorders, which produce large amounts of oxidative substances, ultimately leading to an increased inflammatory response [14]. An increased inflammatory response can interfere with the cardiovascular system. ROS can increase the synthesis of inflammatory cytokines, causing inflammation in vascular cells and thereby impairing their function. Furthermore, ROS can also induce changes in vascular function, including vascular smooth muscle cell hyperplasia and structural remodeling [15].

The balakka plant (*Phyllanthus emblica* L.) belongs to the Euphorbiaceae family and grows abundantly in Southeast Asia, including Indonesia. It has been studied as a natural source for nutrition and medicine. Traditionally, people use the balakka plant to treat various diseases [16]. Its fruit contains many active compounds such as flavonoids, tannins, and ascorbic acid, which possess antibacterial, hepatoprotective, and cardioprotective properties [17]. The abundant content of ascorbic acid and flavonoids in balakka fruit has been reported to exhibit high antioxidant activity of 3.032 µg/mL using the DPPH method, which is categorized as very strong [18,19]. The aim of this was therefore to analyze the effect of the administration of ethanolic extract of balakka fruit (EEBF) at doses of 100, 300, and 500 mg/kg BW, which serves as an antioxidant, on TRPA1 levels in rats treated with doxorubicin.

Methods

Preparation of *Phyllanthus emblica* extracts

Balakka fruit (*P. emblica*) was collected from South Tapanuli, North Sumatra, Indonesia, washed with water, subsequently drained, and weighed. They were dried in a drying cabinet at 40–60°C until fully dried. After drying, the balakka fruit simplicia were pulverised using a mixer. The extract was made by the maceration method, with 500 g of dry simplicia powder and 5 L of 70% ethanol as a solvent for 24 hours, with intermittent stirring at ambient temperature (25°C). This extraction method pertains to a similar published protocol [20].

Characteristics of simplicia and extracts

Simplicia was characterized by determining its water content, water-soluble extractive content, and ethanol-soluble extractive content. Total ash and acid-insoluble ash contents were also determined to assess the inorganic mineral content and the purity of the simplicia. For the extract, only the water content, total ash content, and acid-insoluble ash content were determined [21].

Phytochemical screening of balakka fruit extracts

Phytochemical screening of the balakka fruit simplicia and extract was performed using standard qualitative methods to detect the presence of alkaloids, flavonoids, glycosides, saponins, steroids or triterpenoids, and tannins. The screening was performed to identify the groups of secondary metabolites present in the sample [22].

Preparation of 0.5% sodium carboxymethylcellulose (S-CMC) suspension

A 0.5% S-CMC suspension was prepared by dispersing 0.5 g of S-CMC into a mortar containing 20 mL of heated water. The mixture was covered and left to stand for 30 minutes until a transparent mass was formed, then ground until a smooth gel consistency was obtained. The gel was diluted with a small amount of water, transferred into a 100 mL volumetric flask, and made up to volume with water.

Preparation of quercetin and extract of balakka fruit solutions

Quercetin was administered to the rats at a dose of 25 mg/kg BW. To prepare the solution, 0.5 mg of quercetin was accurately weighed and then dissolved in 50 mL of S-CMC. Three different extracts were prepared at doses of 100 mg/kg BW, 300 mg/kg BW, and 500 mg/kg BW. The 100 mg/kg BW dose was prepared by grinding 100 mg of ethanol extract of balakka fruit (EEBF) and 10 mL of 0.5% S-CMC suspension until homogeneous. The same process was carried out for the 300 mg/kg BW and 500 mg/kg BW doses.

Animal grouping and treatment

The in vivo experiment was performed using male white rats (*Rattus norvegicus*) weighing 180–200 grams from the Pharmacology Laboratory at the Universitas Sumatera Utara, Medan, Indonesia. The rats had been acclimatized in the animal facility of the Faculty of Pharmacy at the Universitas Sumatera Utara for one week prior, in well-ventilated and hygienic facilities.

Thirty rats were allocated into six groups, each containing five rats. The normal group received no treatment. The DOX group served as the negative control, consisting of rats induced with doxorubicin at 25 mg/kg BW for 30 days. The DOX+QR group served as the positive control, receiving the same doxorubicin induction treatment along with quercetin administration at 25 mg/kg BW for 14 days. The other three groups each received doxorubicin induction (25 mg/kg BW, 30 days) and were subsequently given ethanol extract at different doses for 14 days: DOX+100 group (100 mg/kg BW), DOX+300 group (300 mg/kg BW), and DOX+500 group (500 mg/kg BW). The number of experimental animals was determined using the Federer formula [23].

TRPA1 analysis using ELISA

At the end of the experimental period, rat blood was collected in an EDTA tube and centrifuged at 3,000 rpm for 10 minutes. The serum was collected and subsequently analyzed using ELISA to quantify TRPA1 levels, employing the rat TRPA1 ELISA kit (Abclonal, Wuhan, China) and assessed with a microplate reader at a wavelength of 450 nm.

Data analysis

Observational data were documented and expressed as mean±standard error of the mean (SEM). Data analysis was conducted using GraphPad Prism software version 10 (GraphPad Software, San Diego, CA, USA), employing ANOVA followed by Tukey's test. Statistical significance was defined as $p<0.05$.

Results

Results of balakka fruit extract

A total of 500 g of balakka fruit was macerated with 5 L of 70% ethanol for 24 hours. Subsequently, the mixture was concentrated using a rotary evaporator at 40°C until the ethanol evaporated, yielding a viscous balakka fruit extract. The balakka fruit extraction yield was 198 g (39.6%).

Characteristic examination of balakka fruit simplicia and extract

The results of the observation on the characteristics of balakka fruit simplicia and EEBF are presented in **Table 1**. The parameters tested included the percentage of water content, solubility in water, and solubility in ethanol. In addition, measurements of total ash residue and acid-insoluble ash were also carried out as part of the characteristic evaluation for both the simplicia and the extract of balakka fruit.

Table 1. Characterization of simplicia and ethanol extract of balakka fruit

Parameter	Simplicia	Extract
Water content	3.98%	18.40%
Solubility in water	55.74%	-
Solubility in ethanol	43.6%	-
Total ash residue	2.65%	1.27%
Acid-insoluble ash	1.01%	0.16%

Phytochemical screening of balakka fruit simplicia and extract

The results of the secondary metabolite compound examination of balakka fruit simplicia and EEBF are presented in **Table 2**. The groups of compounds analyzed included alkaloids, flavonoids, glycosides, and saponins. In addition to these four groups, the content of tannins and triterpenoids or steroids also served as parameters in the phytochemical examination of both the simplicia and the extract of balakka fruit.

Table 2. Phytochemical screening results of simplicia and ethanol extract of balakka fruit

Parameter	Results	
	Simplicia	Extract
Alkaloid	Present	Present
Flavonoid	Present	Present
Glycoside	Present	Present
Saponin	Present	Present
Tannin	Present	Present
Triterpenoid/steroid	Absent	Absent

The phytochemical test on balakka fruit simplicia and EEBF indicated that several groups of compounds were detected within them, namely alkaloids, flavonoids, glycosides, saponins, and tannins (**Table 2**).

Effect of balakka fruit extract on TRPA1 levels

TRPA1 levels varied across the treatment groups (**Figure 1**). The DOX group showed significantly higher TRPA1 levels than the normal group ($p<0.010$), indicating that doxorubicin administration increased TRPA1 expression. In contrast, TRPA1 levels in the DOX+QR group were significantly lower than those in the DOX group ($p<0.010$) and were not significantly different from the normal group ($p>0.050$) (**Figure 1**). This finding suggests that quercetin attenuated the doxorubicin-induced increase in TRPA1 levels and produced a TRPA1 profile comparable to that of untreated animals.

EEBF was also associated with lower TRPA1 levels in doxorubicin-induced rats (**Figure 1**). Compared with the DOX group, TRPA1 levels were significantly lower in the DOX+100 group ($p<0.050$), DOX+300 group ($p<0.050$), and DOX+500 group ($p<0.001$). Among the extract-treated groups, no significant differences were observed between the DOX+100, DOX+300, and DOX+500 groups ($p>0.050$), although the lowest TRPA1 level was observed in the DOX+500 group (**Figure 1**). These results indicate that TRPA1 levels were lower in the EEBF-treated groups

than in the doxorubicin control group, with the 500 mg/kg BW group showing the lowest mean TRPA1 level.

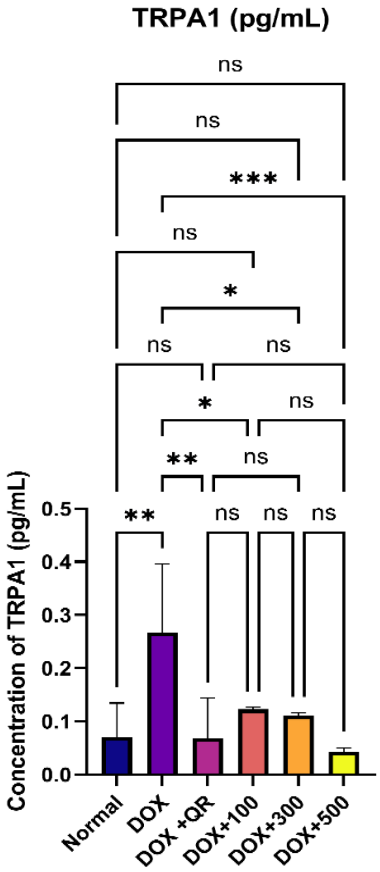

Figure 1. Effect of ethanol extract of balakka fruit on TRPA1 levels in doxorubicin-induced rats. TRPA1 levels were compared among the normal control, doxorubicin control, doxorubicin plus quercetin, and doxorubicin plus balakka fruit ethanol extract groups at doses of 100, 300, and 500 mg/kg BW. Data are presented as mean±SEM. Between-group comparisons were analyzed using one-way ANOVA followed by Tukey's post hoc test. Between-group comparison is statistically significant at *p<0.05, **p<0.01, *** p<0.001, **** p<0.0001; ns indicates not significant.

Discussion

Ethanol is a solvent capable of extracting polar, semi-polar, and non-polar compounds due to the presence of a hydroxyl (OH) group (polar) and an ethyl group (non-polar). This ability makes ethanol a universal solvent [27]. Additionally, ethanol has antibacterial effects, which help preserve samples for longer periods [28]. Maceration as an extraction method is used for thermolabile compounds (heat-sensitive). During this process, the container is tightly sealed to minimize solvent loss due to evaporation [29]. Therefore, researchers need to adjust the solvent, concentration, extraction method, and storage temperature according to the research objectives. Appropriate selection is an important effort to maintain good and stable sample quality throughout the process. A study conducted by [24] used balakka fruit macerated with 10 L of 96% ethanol from 1000 g of balakka fruit simplicia. The result obtained was 264.154 g, with a yield of 26.4%.

The water content determination of the simplicia showed a value of 3.98% for balakka fruit simplicia (**Table 1**). The obtained water content met the quality standard (≤10%). Water content measurement is closely related to simplicia purity [30]. Solubility in water and ethanol was determined to ensure the extractability of each solvent toward the simplicia content. Solubility in water was found to be 55.74%, while solubility in ethanol was found to be 43.6%. This comparison indicates that solubility in water is higher than solubility in ethanol, suggesting a greater affinity

of the compounds in balakka fruit simplicia toward water as a solvent. The preference for water as a solvent is due to the presence of more polar secondary metabolites compared to semi-polar secondary metabolite compounds in the simplicia; thus, these compounds will dissolve more easily in water than in ethanol [31].

The determination of total ash residue aims to determine the mineral content present in the sample. A high total ash residue indicates a large amount of inorganic material contamination. The test result for total ash residue was 2.65%, which meets the requirement of not more than 11.5% [32]. The acid-insoluble ash obtained was 1.01%. The higher the acid-insoluble ash, the more it indicates the presence of minerals and silicates derived from soil or sand [33].

Based on **Table 1**, the characterization of the extract includes the examination of water content, total ash residue, and acid-insoluble ash of the balakka fruit extract. The water content determination of the extract yielded a value of 18.40%. The obtained water content did not meet the quality standard (≤10%). The unsatisfactory water content result was due to prolonged storage time, causing the extract to absorb moisture from the surrounding air. High water content can lead to the growth of bacteria and fungi [34].

The total ash residue test result obtained for the extract was 1.27%, which meets the requirement (≤11.5%). The acid-insoluble ash obtained was 0.16%. The amount of total ash residue in the extract indicates how many minerals the balakka fruit extract contains. Meanwhile, the determination of acid-insoluble ash is to evaluate the extract for contamination by materials such as soil and sand [34].

This finding is in line with previous research reports regarding the phytochemical content of balakka fruit. In previous studies, balakka was known to contain various secondary metabolites such as fixed oils, phosphatides, essential oils, tannins, minerals, vitamins, amino acids, fatty acids, and glycosides [25]. The flavonoid content in balakka fruit is closely related to antioxidants due to its ability to break down free radicals, whereby flavonoids slow down ROS formation, break down ROS, and regulate/protect with antioxidants; thus, flavonoids in balakka fruit play a role in reducing TRPA1 levels [26].

Administration of EEBF to rats with doxorubicin-induced hypertension has the potential to lower blood pressure by affecting TRPA1 parameters. Doxorubicin, a common chemotherapy drug, often causes high blood pressure as a side effect, which can be dangerous for patients. Studies indicate that doxorubicin can cause excessive activation of TRPA1 receptors, contributing to high blood pressure. However, treatment with EEBF may prevent TRPA1 overactivation, leading to vasodilatory effects that can lower blood pressure. EEBF may help protect blood vessel tissues from damage caused by doxorubicin and reduce the risk of associated cardiovascular complications [25].

The lowest TRPA1 level was shown in the DOX+500 group. In an investigation conducted by [35], the antihypertensive effects of *P. emblica* were studied using a randomized, triple-blind, placebo-controlled trial method. A total of 92 patients were randomly divided into 2 groups. Each group received *P. emblica* at a dose of 500 mg three times daily after meals or a placebo, in addition to their regular antihypertensive medication. After 8 weeks of treatment, the *P. emblica* group showed a reduction in systolic blood pressure of 15.6±8.23%, compared to a reduction of 6.3±7.49% in the placebo group. Similarly, diastolic blood pressure in the *P. emblica* group decreased by 12.3±7.87%, compared to a decrease of 3.88±7.98% in the placebo group. This proves that the most effective result aligns with the study, namely in the treatment group receiving 500 mg/kg BW of EEBF. Improvement in TRPA1 levels can help stabilize blood pressure by balancing vasodilation and vasoconstriction responses.

This study has some limitations that need to be acknowledged. The effect of EEBF was evaluated mainly through TRPA1 expression, while direct cardiovascular parameters, such as systolic and diastolic blood pressure, heart rate, vascular function, and cardiac histopathology, were not comprehensively assessed. Although phytochemical screening identified several secondary metabolite groups, the specific active compounds responsible for the observed effect were not isolated or quantified. Antioxidant and inflammatory markers were also not measured, making it difficult to confirm the mechanistic pathway linking balakka fruit extract, oxidative stress, and TRPA1 modulation. Toxicity testing and long-term safety evaluation were not performed; therefore, further studies with larger sample sizes, standardized extracts, additional

cardiovascular and biochemical markers, and safety assessments are needed to confirm the therapeutic potential of balakka fruit extract.

Conclusion

Balakka fruit ethanol extract reduced TRPA1 levels in doxorubicin-induced rats, suggesting its potential protective effect against doxorubicin-related cardiovascular alterations. The reduction in TRPA1 levels appeared to increase with higher extract doses, with the 500 mg/kg BW dose showing the most effective result. These findings support the potential role of balakka fruit extract as a natural antioxidant source in modulating TRPA1-associated cardiovascular responses. Further studies are required to evaluate its toxicity, long-term safety, active compounds, antioxidant activity, inflammatory markers, and direct cardiovascular effects.

Ethics approval

The procedure of this study has received approval from the Ethics Commission of Universitas Sumatera Utara, Indonesia (No.0460/KEPH-FMIPA/2024).

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None to declare.

Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

No new data were generated in this study.

Declaration of artificial intelligence use

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

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