GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Vasorelaxant effect of "6,8-diméthylhexadecane" isolated from *Passiflora edulis* (PASSIFLORACEAE) leaves in isolated rat thoracic aorta

Miora Diane RASOLOFONIAINA RANDRIAMPAMELONA [1, 2, *], Jean François RAJAONARISON [1], Donné Yodan RALAHIRAVO [2], Soaviherimbola Delore RAZAFIMAHAZORO [2], Nathaniel QUANSAH [2] and Patricia RANDRIANAVONY [2]

[1] *Laboratory of Biotechnology Research Environment and Health, Doctoral School of Engineering Living and Modeling, Faculty of Sciences Technology and Environment, University of Mahajanga, Madagascar.*
[2] *Department of Pharmacology, Sciences Faculty, University of Antananarivo, Antananarivo, Madagascar.*

GSC Biological and Pharmaceutical Sciences, 2025, 33(02), 145-152

Publication history: Received on 29 September 2025; revised on 08 November 2025; accepted on 10 November 2025

Article DOI: https://doi.org/10.30574/gscbps.2025.33.2.0440

Abstract

Passiflora edulis leaves are used traditionally in Madagascar for the treatment of various ailments including hypertension. This study was carried out to investigate the vasodilatory mechanism of a bioactive molecule isolated from P. edulis leaves "6,8-diméthyhexadecane" (D65). It was tested using isolated rat thoracic aorta contracted with norepinephrine at 1µM. Its mechanism of action was investigated using isolated thoracic aorta with endothelium and endothelium free. Involvement of prostacylin in vasodilation induced with D65 was investigated by pre incubating the organ in a bath containing indomethacin. The results indicate that D65 relaxes organ pre contracted with norepinephrine with EC_{50} of $1.74.10^{-7}$ M. Its vasodilating action is endothelium dependent. Pre incubating the organ in bath containing atropine before contracting with norepinephrine decreases the value of pD_2 from 6.77 in the absence of atropine to 6.62, 6.53, and 6.41 in the presence of atropine at 10^{-6}, 10^{-5}, and 10^{-4} M, respectively ($p < 0.05$). Pre incubating the organ in a bath containing indomethacin in concentrations of 10^{-7}, 10^{-6}, and 10^{-5} M decreases respectively the pD_2 from 6.74 in the absence of indomethacin to 6.61, 6.49 and 6.44 in the presence of indomethacin ($p < 0.05$).

Our results demonstrate that D65 induces vasodilation by mechanisms that involve prostacyclin signalling pathways. These findings indicate that this molecule has therapeutic potential in the treatment of cardiovascular diseases.

Keywords: *Passiflora edulis*; Leaves; Vasodilation; Prostacyclin

1. Introduction

Hypertension remains one of the most prevalent and significant risk factors for cardiovascular morbidity and mortality worldwide [1, 2]. Despite the availability of numerous classes of antihypertensive drugs, many patients in developing countries - such as Madagascar - continue to rely on medicinal plants for the management of high blood pressure. This is often due to limited access to healthcare services due to shortage of medical personnel. Medicinal plants have been used for centuries in traditional medicine systems and represent a rich source of structurally diverse bioactive compounds. Investigating these natural products offers promising opportunities for the discovery of novel, low-cost antihypertensive agents that may also present fewer side effects compared to conventional therapies.

Vasodilator is widely recognized as a primary therapeutic strategy for the management of hypertension [3]. Currently, numerous pharmacological agents exhibit vasodilatory effects through various mechanisms, including inhibition of

* Corresponding author: Miora Diane RASOLOFONIAINA RANDRIAMPAMELONA

angiotensin-converting enzyme (ACE), blockade of calcium channels, activation of potassium channels, and inhibition of cGMP-specific 3',5'-cyclic phosphodiesterase (PDE5) [4]. Despite their therapeutic potential, several limitations associated with these agents have been reported, such as the development of drug resistance, orthostatic hypotension, and compensatory increases in heart rate and myocardial contractility [5]. Consequently, the search for novel vasodilatory compounds remains an active area of research.

Medicinal plants have been used throughout human history for the treatment of various ailments, and their pharmacological potential continues to attract scientific interest. The global resurgence in the use of plant-based and natural products highlights the importance of validating their efficacy and safety through rigorous scientific investigation. Several studies have demonstrated the therapeutic value of herbal medicines, including their role in cardiovascular health [6]. However, to ensure the safety and effectiveness of such treatments, comprehensive pharmacological evaluations are essential.

Our previous investigations demonstrated that the hydroalcoholic extract of *Passiflora edulis* leaves exhibits significant antihypertensive activity in a rat model of hypertension induced by a high-salt diet. In addition, ex vivo studies revealed that this extract induces vasodilatory effects in isolated rat thoracic aorta, suggesting a direct action on the vascular smooth muscle and/or endothelium [7]. In addition, we have isolated a bioactive compound, designated as D65, from *Passiflora edulis* leaves. Preliminary pharmacological evaluations demonstrated that D65 possesses significant vasodilatory activity, indicating its potential contribution to the overall antihypertensive effects observed with the hydroalcoholic extract [8]. However, its mechanism of action has not yet been elucidated. Therefore, the present study aimed to investigate the vasodilatory properties of D65 in isolated rat thoracic aorta, to evaluate its potential as a novel antihypertensive agent.

2. Materials and Methods

2.1. Animal and aortic rings preparation

Male Wistar rats weighing between 200 and 250 g were used throughout the whole experiment (n = 10 for each experiment) and acclimated in the animal house of the "Laboratoire de Pharmacologie Générale, de Pharmacocinétique et de Cosmétologie' (LPGPC) at the Faculty of Sciences, University of Antananarivo, with 12-h light–dark cycles with free access to food and water at room temperature. The experiment was performed based on the Guideline in Care and Use of animals of Faculty of Sciences (Animal Ethics Approval/ 2024). Prior to the isolation of the aorta from the rats, Krebs-Henseleit (Krebs) solution (4.7 mM KCl, 118.0 mM NaCl, 2.5 mM $CaCl_2$, 25.0 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 11.0 mM glucose, and 1.2 mM $MgSO_4$, pH 7.4) was prepared and continuously aerated with carbogen (5% CO_2 and 95% O_2) in a Petri dish. The rats were sacrificed by injection of 100 mg/kg of phenobarbital intra peritoneally. The thoracic aorta was immediately isolated and placed in Krebs' solution. After the adipose tissues were removed, the aorta was trimmed into 3–4 mm ring segments and mounted in an organ bath containing Krebs' solution (5 ml) by using two needle hooks with the temperature maintained at 37 °C and continuous aeration with carbogen gas. One hook was fixed at the base of the organ bath, and the other hook was connected to the Statham force transducer (Gould - Statam Isometric Measurement). The mounted aortic rings were allowed to equilibrate for 45 min, and the Krebs' solution was renewed every 15 minutes. The resting tension was readjusted to 1.0 g after the change of Krebs' solution. Once the tension of the mounted aortic rings stabilized, the contractile agent, phenylephrine (1 µM), and the relaxing agent, acetylcholine (1 µM), were added. The integrity of the endothelium aortic rings was assured with at least 50% of responses achieved. Then, the aortic rings were rinsed three times with Krebs' solution at 15 minutes intervals before phenylephrine precontraction [9].

Then, the tissue was contracted with 10 µM norepinephrine, and cumulative concentration-response curves were obtained for each extract in the absence or presence of 10 µM indomethacin (a cyclooxygenase inhibitor) or 6 µM atropine (an antagonist of muscarinic receptors). Those antagonists were added to the organ bath 10 minutes before the second noradrenaline-induced contraction.

2.2. Vascular response to D65 on norepinephrine precontracted aortic rings

The endothelium-intact aortic ring was precontracted with PE (1 µM). After the tonic contraction became stable, increasing concentrations of D65 were added cumulatively in the bath from 10^{-9} M until total relaxation. The total of D65 volume injected did not exceed 10% of the organ bath volume. The vascular response was detected by isometric transducer. The concentration-response curve was reported on semi logarithmic scale, the half maximal effective concentration (EC_{50}) was calculated by linear regression using software Microsoft Office LTSC Excel [9].

2.3. Vasodilation response to D65

To investigate the mechanism involved in relaxation induced by D65, the isolated aortic ring was pre incubated in a bath containing D65 at concentrations 6.10^{-8}, $0.12.10^{-6}$ and $0.2.10^{-6}$ M, for 10 minutes, before injecting norepinephrine in the bath, in a cumulative manner until maximal contraction.

2.4. Vasodilatory effect of D65 in endothelium free aortic rings

Investigation of endothelium involvement in vasodilation induced with D65 was done on aorta devoid of endothelium (endothelium-free aorta). The intimal surface of the isolated aortic rings was removed mechanically by gently rubbing with a stainless-steel stick. The absence of endothelium on the aortic rings was assured by exposure to phenylephrine with at least 60% of contraction achieved and no relaxation after injecting Ach in the bath. D65 at the concentration from 10^{-9} M was added to the organ bath cumulatively after PE precontraction, until 100% relaxation [10].

2.5. Investigation of the involvement of muscarinic receptors

To investigate the involvement of acetylcholine in the relaxation induced by D65, endothelium-intact isolated aortic rings were preincubated for 10 minutes in a bath containing atropine (1 µM) before norepinephrine injection. When the maximal tone was stable at the maximum contraction, D65 was then injected to the organ bath cumulatively, from 10^{-9} M until complete relaxation [11, 12].

2.6. Investigation of the relaxation pathway mechanism

To determine the mechanism involved in the relaxation induced by D65, endothelial intact isolated aortic rings were pre-incubated with indomethacin at different concentrations, an antagonist of cyclooxygenase (COX) for 10 minutes. After pre incubation period, norepinephrine was injected in the bath to obtain 1 µM. When the contraction was stable, D65 was then injected in the organ bath cumulatively from 10^{-9} M until maximal relaxation [13].

2.7. Statistical analysis

All the data were expressed as the mean ± S.E.M. of n animals. The results obtained were compared to the control by using one-way ANOVA and with $p < 0.05$. Concentration–response relationships were analyzed using two-way ANOVA followed by Student t-test; $p < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of D65 on aorta pre-contracted with norepinephrine

Injected in a bath containing isolated rat thoracic aorta, pre contracted with nor epinephrine at concentration of 1 µM in the bath, D65 relaxes the organ in a concentration dependent manner. It relaxes 100% the organ at the concentration of $3.6.10^{-7}$ M, with EC_{50} of $1.74.10^{-7}$ M (Figure 1). These results indicate that this molecule possesses a vasodilating activity.

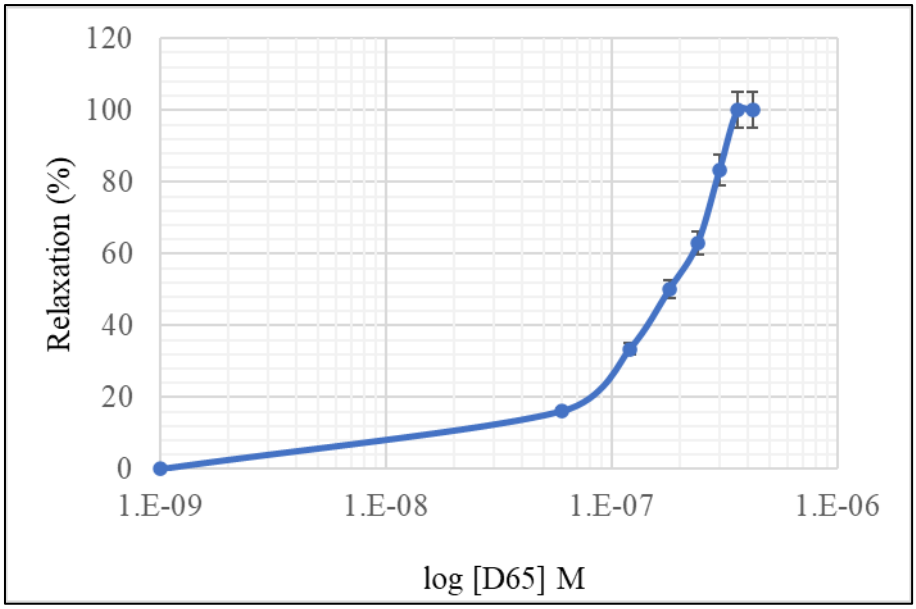

Figure 1 Relaxation of isolated rat aorta contracted with norepinephrine at 10^{-4} M, induced by D65, added in a cumulative manner in the bath ($\bar{x} \pm \bar{\sigma}$; n = 10; $p < 0.05$)

3.2. Interaction between D65 and norepinephrine in isolated aortic tissue

Pre-incubating the isolated aorta in a bath containing increasing concentrations of D65 before contracting with norepinephrine reduces the maximal effect of this vasoconstrictor mediator. The response decreased from 100% in the absence of D65 to 84.31 ± 0.51 %, 71.61 ± 0.48 %, and 58.80 ± 0.32 % in the presence of D65 at concentrations of 6×10^{-8} M, 0.12×10^{-6} M, and 0.2×10^{-6} M, respectively (Figure 2) ($p < 0.05$). The reduction of the maximal effect of norepinephrine in the presence of D65 indicates that this molecule acts as a non-competitive antagonist of norepinephrine.

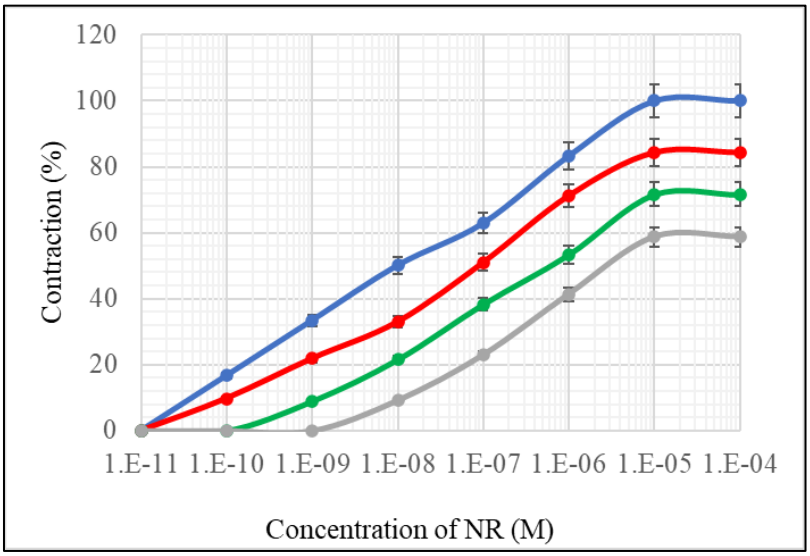

Figure 2 Contraction of isolated rat aorta induced with norepinephrine injected in a cumulative manner in the bath, in the absence ( ) and presence of D65 at concentrations of 6.10^{-8} ( ), $0.12.10^{-6}$ ( ) and $0.2.10^{-6}$ M ( ) in the bath ($\bar{x} \pm \bar{\sigma}$; n = 10; $p < 0.05$)

3.3. Vasodilatory effect of D65 in denuded endothelium aortic rings

Removal of the endothelium decreased the relaxant effect of D65 in thoracic aorta: It induces 100% relaxation in the presence of endothelium, and with the same concentrations, maximal relaxation is reduced to 18% in denuded

endothelium aortic rings (Figure 3). These results suggest that vasorelaxation induced by D65 is endothelium dependent.

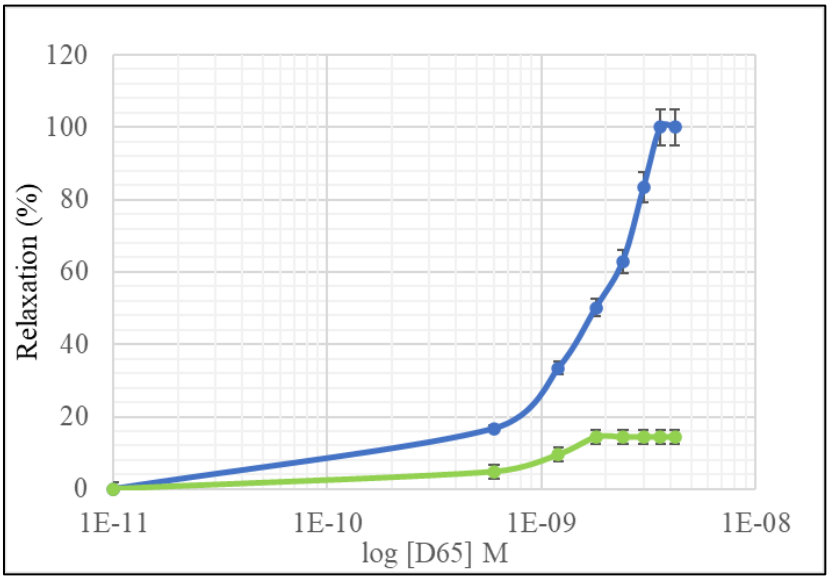

Figure 3 Relaxation of isolated rat aorta contracted with 10^{-4} M norepinephrine induced by D65, added in a cumulative manner in the bath, in the presence ( ) and absence ( ) of endothelium ($\bar{x} \pm \bar{\sigma}$; n = 10; $p < 0.05$)

3.4. Roles of muscarinic receptors in D65 induced vasorelaxation

The action of D65 on M3 muscarinic receptors was evaluated on the relaxation it induced on isolated aortic rings pre-contracted with norepinephrine and pre-incubated in a bath containing atropine at increasing concentrations. In the absence and presence of atropine, D65 was still able to induce relaxation of the norepinephrine-contracted aorta with a slight rightward shift of the concentration–response curve (Figure 4). However, its pD_2 value decreases with increasing concentrations of atropine in the bath, from 6.77 in the absence of atropine to 6.62, 6.53, and 6.41 in the presence of atropine at concentrations of 10^{-6}, 10^{-5}, and 10^{-4} M, respectively ($p < 0.05$). These results indicate that D65 and atropine act as competitive antagonists, suggesting that the vasodilatory effect of D65 is mediated through muscarinic receptors.

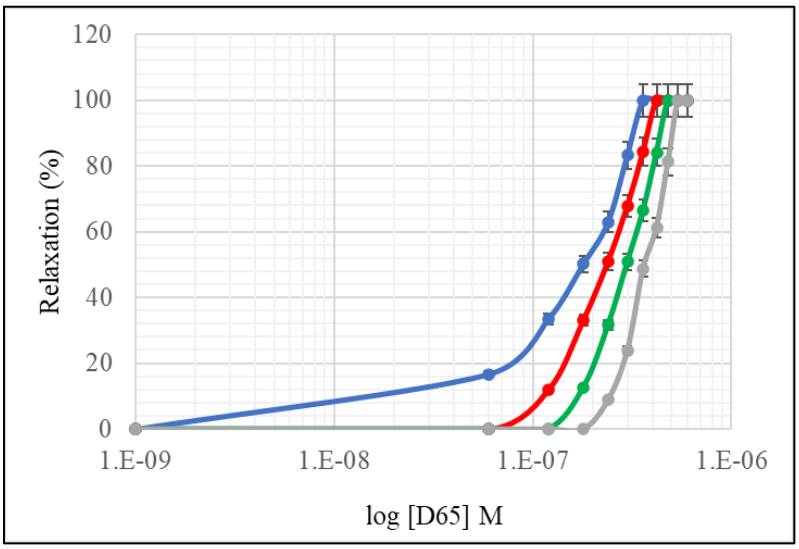

Figure 4 Relaxation of isolated rat aorta contracted with norepinephrine at 10^{-4} M, induced by D65, added in a cumulative manner in the bath, in the absence ( ) and presence of atropine at concentrations of 10^{-6} ( ), 10^{-5} ( ) and 10^{-4} M ( ) in the bath ($\bar{x} \pm \bar{\sigma}$; n = 10; $p < 0.05$)

3.5. Role of prostacyclin in D65-induced vasodilation

Pre-incubating the aorta pre-contracted with norepinephrine in a bath containing increasing concentrations of indomethacin prior to relaxation with D65, it is still able to induce complete relaxation (100%) of the aorta pre-contracted with norepinephrine. However, the pD_2 of D65 decreased with the concentration of indomethacin in the bath. In the absence of indomethacin, the pD_2 is 6.74, and it decreases to 6.61, 6.49 and 6.44 in the presence of indomethacin at concentrations of 10^{-7}, 10^{-6}, and 10^{-5} M, respectively ($p < 0.05$) (Figure 5). These results indicate a competitive antagonism between indomethacin and D65.

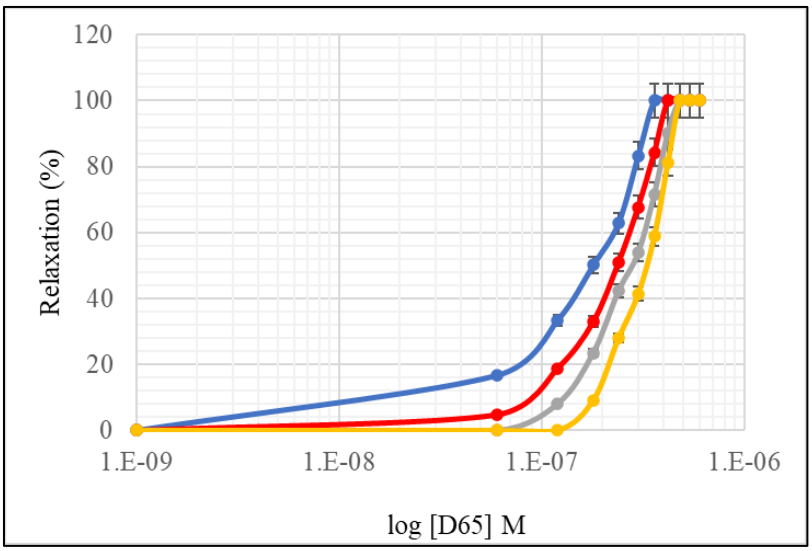

Figure 5 Relaxation of rat isolated aorta contracted with norepinephrine at 10-4 M, induced by D65, added in a cumulative manner in the bath, in the absence ( ) and presence of indomethacin at concentrations of 10^{-7}( ), 10^{-6} ( ) and 10^{-5} M ( ) in the bath ($\bar{x} \pm \bar{\sigma}$; n = 10; $p < 0.05$)

4. Discussion

Passiflora edulis leaves are widely used in the high plateau of Madagascar traditional medicine practise to relieve the symptoms of high blood pressure. Our previous studies have confirmed its anti-hypertensive [7] and vasodilating activities in rat's aorta and identified a vasodilator molecule D65 [8]. However, its mechanism of action has not been investigated so far. In the present study, we aimed to determine it using isolated rat thoracic aorta, with endothelium and without endothelium contracted with norepinephrine. Our results demonstrated that D65-induced vasorelaxation is endothelium-dependent and was significantly blocked in the presence of indomethacin. Endothelial cells are an intimate modulator for the control of vascular homeostasis. They respond to humoral and physical stimuli by releasing endothelium-dependent vasodilators including prostacyclin [14].

Prostacyclin (PGI_2) is a vasodilatory eicosanoid synthesized by the action of cyclooxygenase (COX) on arachidonic acid within endothelial cells. Its biosynthesis is typically triggered by various endogenous stimuli, including acetylcholine. Once produced, PGI_2 acts on specific G-protein-coupled receptors (IP receptors) located on vascular smooth muscle cells, leading to the activation of adenylyl cyclase (AC). This cascade elevates intracellular cyclic adenosine monophosphate (cAMP) levels, resulting in vascular smooth muscle relaxation and consequently, vasodilation [15].

Some plant extracts have been reported to exert their vasodilatory effects through activation of the prostacyclin (PGI_2) pathway, although this mechanism has only been reported in a limited number of studies. We can cite as exemples ethyl cinnamate from *Kaempferia galanga* (aromatic ginger) [16], *Psoralea corylifolia* [17], *Garcinia cowa* leaf extract [10], and Labdane-302 (diterpene) *Xylopia langsdorffiana* Involves endothelium-dependent PGI_2 signaling [18].

The involvement of this pathway is commonly confirmed using indomethacin, a non-selective COX inhibitor, which blocks PGI_2 synthesis and thereby attenuates the vasodilatory response [15]. In this study, when the functional endothelium was removed from the aortic strips, vasorelaxant action of D65 was highly reduced. This observation indicates that D65 modulates vascular tone by acting on smooth muscle cells via the endothelium pathway. The results of this study also showed that D65 induced relaxation of the aortic artery was attenuated in the presence of indomethacin, where its concentration-response curve was shifted significantly to the right. These observations suggest

the involvement of prostacyclin in the vascular relaxation induced by D65 through the activation of cAMP signalling. Increase in intracellular adenosine 3',5'-cyclic monophosphate (cAMP) levels lead to the reduction of Ca^{2+} entry from extracellular space through ionic channels reduced release of Ca^{2+} from intracellular stores; and decrease of cytoplasmic Ca^{2+} concentration, resulting in relaxation of vascular smooth muscle [19, 20, 21, 22]. The present study provides strong evidence on vasorelaxant effect of D65, compound isolated from *Passiflora edulis* leaves, justifying in part the traditional use of this plant in the treatment of hypertension.

5. Conclusion

The present study focused on the mechanisms of action responsible for the vasodilator activity of D65. Our results suggest that D65 promotes vasodilation by stimulating PGI_2 release from endothelial cells. This mechanism aligns with previous studies on some plant-derived vasodilators, suggesting the therapeutic potential of *Passiflora edulis* in managing hypertension through natural, PGI_2-mediated vasodilatory pathways.

Compliance with ethical standards

Acknowledgments

All authors have contributed according to their speciality to the work

Disclosure of conflict of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Statement of ethical approval

All experiments conducted in this study were approved by the Sciences Faculty of Antananarivo Animal Ethics Committee with the approval (Reg. N° ECFS-03/24).

References

[1] Mills KT, Stefanescu A, He J. (2020). The global epidemiology of hypertension. Nat Rev Nephrol. 2020; 16(4):223-237.

[2] WHO (World Health Organization). Hypertension. 2021. https://www.who.int/news-room/fact-sheets/detail/hypertension.

[3] Whelton PK, Carey RM, Aronow WS, Casey DE, Collins KJ, Himmelfarb CD, DePalma SM, Gidding S, Jamerson KA, Jones DW, MacLaughlin EJ, Muntner P, Ovbiagele B, Smith SC, Spencer CC, Stafford RS, Taler SJ, Thomas RJ, Williams KA, Williamson JD, Wright JT. Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults. J Am Coll Cardiol. 2017; 71(19):127-248.

[4] Kaczorowski DJ, Schwartz GG. (2006). Pharmacology of vasodilators. In Elsevier eds, Hypertension: A Companion to Braunwald's Heart Disease. Paris(France); 2006.p. 233-245.

[5] Oparil S, Acelajado MC, Bakris G, Berlowitz D, Cífková R, Dominiczak A, Grassi G, Jordan J, Poulter N, Rodgers A, Whelton P K. Hypertension. Nat Rev Dis Primers. 2018; 4(1): 1-48.

[6] Ernst E. The efficacy of herbal medicine – an overview. Fundam Clin Pharmacol. 2005; 19(4):405-409.

[7] Rasolofoniaina RMD, Rajaonarison JF, Ralahiravo DY, Razafimahazoro SD, Quansah N, Randrianavony P. Evaluation of antihypertensive and vasorelaxant activity of ethanolic extract of *Passiflora edulis* leaves in rats. World J Biol Pharm Health Sci. 2024; 20(03):390-395.

[8] Donkor PO, Rasolofoniaina RMD, Ralahiravo DY, Randrianavony P, Rajaonarison JF. Structural determination of an isolated vasodilatory molecule from *Passiflora edulis* Sims (Passifloraceae). Int J Adv Chem Res. 2025; 7(7):7-11.

[9] Assaidi A, Dib I, Tits M, Angenot L, Bellahcen S, Bouanani N, Legssyer A, Aziz M, Mekhfi H, Bnouham M, Frederich M, Ziyyat A. *Chenopodium ambrosioides* induces an endothelium-dependent relaxation of rat isolated aorta. J Integr Med. 2019; 17(2):115-124.

[10] Somruedee Y, Somchai S, Watchara C. Vasorelaxing effect of *Garcinia cowa* leaf extract in rat thoracic aorta and its underlying mechanisms. J Tradit Complement Med. 2022; 13(3):219-225.

[11] Ajagbonna OP, Sofola OA. Endothelium dependent relaxation of aorta to *Samorin sokoto*. J Vet Sci. 1999; 1(1):21-28.

[12] Sun T, Liu R, Cao YX. Vasorelaxant and antihypertensive effects of formononetin through endothelium-dependent and independent mechanisms. Acta Pharmacol Sin. 2011; 32:1009-1018.

[13] Ajagbonna OP, Mojiminiyi FBO, Sofola OA. Relaxant effects of the aqueous leaf extract of *Cassia occidentailis* on rat aortic rings. Afr J Biomed Res. 2001; 4:127-129.

[14] Vanhoutte PM, Shimokawa H, Feletou M, Tang EHC. Endothelial control of vasomotor function - role of prostacyclin. Acta Physiol. 2017; 219(1):22-96.

[15] Fries S., Grosser T. The cardiovascular pharmacology of COX-2 inhibition. Hematol Am Soc Hematol Educ Program. 2005; 445-451.

[16] Othman R, Ibrahim H, Ali Mohd M, Awang K, Gilani AH, Mustafa MN. Vasorelaxant Effects of Ethyl Cinnamate Isolated from *Kaempferia galanga* on Smooth Muscles of the Rat Aorta. Planta Med. 2002; 68(7):655-657.

[17] Gebremeskel AK, Wijerathne TD, Kim JH, Kim MJ, Seo CS, Shin HK, Lee KP. *Psoralea corylifolia* extract induces vasodilation in rat arteries through both endothelium-dependent and -independent mechanisms involving inhibition of TRPC3 channel activity and elaboration of prostaglandin. Pharm Biol. 2017; 55(1):2136-2144.

[18] Dos Santos PF, Duarte MC, Bezerra DAC, Filho JMB, Tavares J, Agra MDF, Da Silva MS. Diterpenes from *Xylopia langsdorffiana*. Helvetica Chimica Acta. 2013;96(6):1085-1092.

[19] Ishikawa T, Hume JR, Keef KD. Regulation of Ca^{2+} channels by cAMP and cGMP in vascular smooth muscle cells. Circ Res. 1993; 73:1128-1137.

[20] Orlov SN, Tremblay J, Hamet P. cAMP signalling inhibits dihydropyridine-sensitive Ca^{2+} influx in vascular smooth muscle cells. Hypertension. 1996; 27:774-780.

[21] Yang CM, Chiu CT, Wang CC, Tsao HL, Fan LW. Forskolin inhibits 5-hydroxytryptamine-induced phosphoinositide hydrolysis and Ca^{2+} Mobilisation in canine cultured aorta smooth muscle cells. Cell Signal. 1999; 11:697-704.

[22] Akata T. Cellular and molecular mechanisms regulating vascular tone. Part 2: Regulatory mechanisms modulating Ca^{2+} mobilization and/or myofilament Ca^{2+} sensitivity in vascular smooth muscle cells. J Anesth. 2007; 21:232-242.