THE ROLE OF K⁺ AND CA²⁺ ION CHANNELS IN Α-TERPINYLE ACETATE-INDUCED VASODILATION IN RAT’S AORTIC RINGS

Dlzar A. Kheder *, Omar A. M. Al-Habib, Lina N. Adam

Dept. of Biology, Faculty of Science, University of Zakho, Zakho, Kurdistan Region, Iraq - (dlzar.kheder@uoz.edu.krd, omar.habib@uoz.edu.krd)

ABSTRACT:
The monoterpene, α-terpinyle acetate (TA) is a constituent of essential oils present in aromatic plants. Since the role of ion channels and endothelial hyperpolarizing factors in TA induced relaxation in rat’s aorta is unknown, the current study aimed to study the mechanism underlying the vasodilatory effect of TA in isolated aortic rings. Terpinyle acetate induced a potent vasodilation in rat aortic rings with a percentage of relaxation of 63.79 %. The results of the role of K⁺ channel subtypes in vasorelaxation revealed that both Kv and K_ATP played a major role since GLIB produced a maximum percent of inhibition in the relaxation produced by TA to A, A1 %; this was followed by 4-AP in which the percent of inhibition reduced to A, A1, A. On the other hand, K_a played no role in the TA induced vasorelaxation since BaCl2 did not produce any inhibition in aortic relaxation. Furthermore, also L-type Ca²⁺ channel played no role in TA induced relaxation since the L-type Ca²⁺ channel inhibitor Nifedipine did not reduce the percent of relaxation. Endothelium also played a considerable role in the induced vasorelaxation since, in denuded aorta, the percent of relaxation was reduced to 36%. Preincubation of the aortic ring with methylene blue, a soluble cGMP inhibitor also significantly reduced the TA induced relaxation to 16.39%. In contrast, preincubation with cyclooxygenase inhibitor Indomethacin did not produce any inhibitory effect on AT vasodilation. It can be concluded from these novel results that AT induced vasorelaxation involve the activation of K_v, K_ATP channels and at least partly dependent on endothelium via the activation NO-cGMP signal transduction pathway.

KEYWORDS: α-Terpinyle Acetate, K⁺, L-type Ca²⁺ channels, vasodilation, aorta, rats.

1. INTRODUCTION

The plant’s essential oils have been widely subjected to phytochemical studies (Abd El-Mageed et al., 2011). Monoterpenses play an important active ingredients of the aromatic plant and may play a crucial role in many biological activities. The essential oils of Eucalyptus tereticornis induced myorelaxant effects on rat’s isolated tracheal rings (Khedher, 2013). Monoterpenses, α- and β-pinene are involved in potentiating the action but are not responsible for its relaxant effects (Lima et al., 2010). Juca et al. (2011) found that the essential oils of E. tereticornis and its constituents decreased the gastric retention. In anesthetized rat’s gastric strips, α- and β-pinene induced contraction, whereas enhanced the meal progression in the duodenum. On the other hand, the essential oil composition of E. tereticornis relaxed both the gastric strips in vitro but with enhanced relaxation in the duodenum (Juca et al. 2011). They suggested that the essential oils increased the gastric emptying, and its effect is partially due to its active constituent’s α- and β-pinene. Lahlou et al. (2003) studied the effect of α-terpinen-4-ol on isolated aortic rings, precontracted with a depolarizing solution of K⁺, α-terpinen-4-ol has induced vasorelaxation in a concentration-dependent manner. The hypotensive effect of monoterpene α-terpinen was first reported by Saito et al. (1996). In a study on α-terpinenol-induced vasodilation in rat mesenteric vascular bed suggested that it involves NO pathway (Santos et al., 2011). Furthermore, α-terpinenol-induced vasodilation in rat mesenteric bed was inhibited completely by pretreatment with L-NAME, indicating the role of nitric oxide in the vasodilation (Magalhaes et al., 2008). On the other hand, Ribeiro et al. (2010) concluded that the vasodilatation induced by α-terpineol was partially endothelium-dependent through producing nitric oxide and activating of the NO-cGMP pathway.

The appropriate modification in the structure of monoterpene, such as changes in the ethyl acetate group position and the presence of the aromatic ring in the p-methane skeleton may change the spasmyotic activity of monoterpene (Andrade et al., 2011). Thus, the current work is designed to investigate the role K⁺ and Ca²⁺ ion channels and endothelium hyperpolarizing factors in TA induced vasodilation in rat’s aorta.

2. MATERIALS AND METHODS

2.1 Tissue preparation

The animals were intraperitoneally injected with heparin (1500 units/ Kg body weight) followed by their anaesthetization with ketamin (40mg/kg) and xylazine (10mg/kg). The aorta was carefully isolated from the rat and transferred to aerate Kreb’s solution with 95% O2 and 5% CO2. The isolated thoracic aorta was used in preparations with intact endothelium, where as in denuded preparations, the endothelium had been removed. The aortic rings (3.5 mm in length) were mounted between two stainless steel hooks, connected by a thread to a force transducer coupled to the trans bridge amplifier and PowerLab Data Acquisition system (ML 870, Power Lab, ADInstrument, Sydney, Australia), connected to a computer running chart software (Version 7). The isometric force produced was monitored and recorded (AL-Habib and Shekha, 2010). The experiments were performed in 10ml organ chambers filled with...
physiological krebs’ solution at 37°C using the thermoregulating system with continuous water circulating throughout the double walled water jacket system and gassed with carbogen continuously (pH=7.4). The tension was set at 2g weight for 60 minutes and the solution was changed every 15 min. until a stable resting tone was obtained (Shekha, 2010).

### 2.2 Experimental protocols

In this study, cumulative dose-response relationships for the effects of α-terpinyle acetate (TA) at concentrations from 1*10^{-5}, 3*10^{-5}M were established for aortic rings. For all experiment, the vasorelaxant effects of TA acetate were studied in aortic rings precontracted with PE (1X10^{-6} M), and were performed as follow:

#### 2.2.1 Group I: The vasorelaxant effect of TA on aortic rings precontracted with phenylephrine (PE, 10^{-6}) was studied.

#### 2.2.2 Group II: The role of K^+ channels in the development of vasorelaxation induced by TA was studied. The aortic rings were preincubated with K+ channel blockers GLIB (10^{-5}), BaCl2 (1mM) and 4-AP (1mM), for 20 min. prior to preconstriction by PE.

#### 2.2.3 Group III: The role of Ca^{2+} channel in vasorelaxation induced by TA in aortic rings preincubated with L-type Ca^{2+} channel blocker nifedipine (3x10^{-5}) was studied for 10 min, prior to its preconstriction by PE.

#### 2.2.4 Group IV: The role of endothelial cells in vasorelaxation induced by TA was studied. The endothelium-denuded rings were firstly tested by the lack of any response to ACh (10^{-5}) followed contraction with PE to confirm the removal of endothelium.

#### 2.2.5 Group V: The Role of Endogenous NO and NOcGMP Pathway in TA-induced Vasodilatation was studied. The aortic rings were preincubated with methylene blue (1X10^{-5}M) and Indomethacin (3x10^{-5}) for 10 min, prior to application of PE.

### 2.3 Statistical Analysis

The vasodilatation response was calculated as a percentage of contraction produced by PE was expressed as the mean ± standard error of the mean (SEM). The base line tension was expressed as 0% relaxation, and the tension induced by PE defined as 100% relaxation. All data analysis were fitted with a Hill equation, which the mean effective concentration (Log of IC_{50}) values were given as the geometric mean with 95% confidence intervals (95% CI), Using statistics program GraphPad Prism version 6.01. Two-way analysis of variance (ANOVA) was performed, supported with Bonferroni test when carrying out pair wise comparison between the same doses of different groups using Graphpad program. P-values less than 0.05 (p<0.05) were considered significant. Symbols * mean P<0.05, ** P<0.01 and *** P<0.001 for all graphs.

### 3. RESULTS AND DISCUSSION

#### 3.1 Relaxant Effects TA on Aortic Rings

Dose-response curve for effect of TA against PE-induced contractions is shown in Figure 1. α-terpinyle acetate (TA) at concentrations from 1*10^{-4}, 3*10^{-5}M showed no relaxant effect in PE (1X10^{-6}) precontracted rat’s aortic rings, whereas at concentrations of (1*10^{-2} and 3*10^{-2}), produced a significant (P<0.05 - 0.01) relaxant effect on rat’s aorta with a relaxation of 63.79%. The Log IC_{50} and (Log IC_{50}’s of CI 95%) are shown in Table 1.

The role of K^+ channel subtypes in TA-induced vasodilatation was investigated using specific K channels blockers such as GLIB (10^{-5}), BaCl2 (1mM) and 4-aminopyridine, (1mM), individually, 20 minutes prior to PE-induced precontraction of the aorta. Dose-response curves for TA-induced vasodilatation against PE-induced contractions preincubated with K+ channel blockers are shown in Figures (2 - 4). TA-induced relaxation was significantly (P<0.001) reduced the effect of TA-induced vasodilatation in preincubated aortic rings with K_{ATP} blocker (GLIB) which significantly affected TA induced relaxation at a concentration of (3*10^{-2}). Similarly, Kc channel blocker (4-AP) at TA doses (1*10^{-2} to 3*10^{-2}), in which the percent of relaxation was 14.95 %. In contrast, Kir channel inhibitor (BaCl2) at used concentrations did not affect TA induced relaxation in aortic rings except at the last dose with (P<0.05). The Log IC_{50} and (IC_{50} of CI 95%) and percentage of relaxation calculated from TA dose-response curves are shown in Table 1.

These results revealed that both Kvi and K_{ATP} played a major role in TA induced vasorelaxation since 4-AP and GLIB produced a maximum percent of inhibition in the relaxation produced by TA 14.95 %, and 8.91 respectively. On the other hand, Kvi played no or less role in the process of vasorelaxation since BaCl2 produce less inhibition on AT induced relaxation. It is not possible to compare these novel results since this represents a first study on the vasorelaxant effect of TA. However, it had been reported that 1, 8-cineol-induced vasodilatation in isolated rat’s aortic rings also involved both K_{ATP} and Kvi channels, but with no role of Kca and Kir channels subtypes in this relaxation (Al-Habib et al., 2013).

#### 3.2 The Role of Calcium Channel in the TA-induced vasodilatation

The cumulative addition of TA concentrations caused a concentration-dependent vasodilatation in aortic rings preincubated with nifedipine. Dose-response curves for TA-induced vasodilatation against PE-induced contractions in the presence and absence of Nifedipine are shown in (Figure 5). It is
clearly demonstrated that there is no significance difference between them, indicating that the L-type Ca²⁺ play no role in the TA induced relaxation of aortic ring. The percentage of relaxation, Log IC₅₀ and (Log IC₅₀)’s of CI 95%) are shown in Table 1. This indicates that L-type Ca²⁺ played no role in relaxant effect induced by TA. Also indicating that voltage-
dependent Ca²⁺ channels did not involve in TA-induced vasodilation.

2) and (3*10⁻²) in which the inhibition was highly significance (P<0.01) only at the last dose used. Thus, the percentages of relaxation in both, endothelium-denuded and endothelium-intact preparation were more or less the same, except the highest dose (Table 1). removal of functional endothelium, significantly reduced TA-induced response, suggesting the vasodilation was endothelium-dependent. Furthermore, our result indicates that TA is an active monoterpene present in many plants, and induced vasodilation in rat aortic rings, at least partially via the endothelium-dependent release of NO. Ribeiro et al. (2010) demonstrated that α-terpinol induced vasorelaxation is mediated partially by endothelium via NO release and activation of the NO-cGMP pathway. terpineol is another active constituent present in Eucalyptus camaldulensis and able to induce a concentration-dependent vasorelaxation (Lahlou et al. 2003) at least partially by the endothelium mainly via NO release and activation of the NO-cGMP pathway.

3.3 The Role of Endothelium in the TA- induced vasodilation

The dose-response curves for TA-induced vasodilation against PE-induced precontractions are shown in Figure 6. In the isolated aortic rings, the TA-induced relaxation in denuded aorta was slightly inhibited at concentrations (1*10⁻¹
3.4 The Role of Endogenous NO and NO-cGMP Pathway in TA-induced Vasodilation

In both methylene blue and Indomethacin preparations, the cumulative addition of TA caused a vasodilation in a concentration-dependent manner. Dose-response curves for TA-induced vasodilation against PE-induced contractions in the presence and absence of methylene blue and Indomethacin are shown in Figures (7 and 8). The percent of relaxation was significantly (P<0.001) inhibited to (16.39%) in aortic rings preincubated with methylene blue at doses (1*10^-2 and 3*10^-2), while in presence of Indomethacin, the percent of relaxation remain almost unchanged. The percentage of relaxation, Log IC50 and (Log IC50’s of CI 95%) for the relaxant response to TA are shown in Table 1.

The results of the effect of endothelium and cyclooxygenase pathway on TA induced vasodilation indicate that endothelium played a partial role in vasodilation induced by TA via the release of NO or activation of the NO-cGMP pathway which ultimately induces aortic relaxation. In studies on the effect of essential oils of E. camaldulensis, on rat’s aorta and of E. tereticornis on the guinea-pig isolated aorta, indicated that its relaxant effect on isolated aorta and trachea may be due to the interaction between its monoterpenes constituents (Kheder, 2013; Coelho-de-Souza et al., 2005). This indicates that vasodilation induced by the monoterpenes, α-terpineol is partially endothelium-dependent via the release of NO and activation of NO-cGMP-pathway (Ribeiro et al., 2010). The TA-induced vasodilation was significantly reduced by the removal of endothelium, the guanylate cyclase inhibitor methylene blue but not by cyclooxygenase inhibitor indomethacin, suggesting a passive role of cyclooxygenase pathway in TA-induced vasodilation. The overall conclusion from the results on the mechanism of TA-induced vasodilation in rats aortic relays on the activation of K+ channels subtypes namely, KAtp and Kv channels and partially on endothelium via the release of NO and the activation of the NO-cGMP pathway which ultimately induce aortic vasodilation.

Table 1. The Log IC50 (Log IC50 of CI 95%) and percentage of relaxation for the effect TA-induced vasodilation on preincubated aortic rings with K+ and Ca2+ channel blockers, methylene blue and Indomethacin, and denuded aortic rings

| Essential oil | Treatment | Control | 4-AP | GLIB | BaCl2 | Nifedipine | Denud | Methylene blue | Indomethacin |
|---------------|-----------|---------|------|------|------|------------|-------|----------------|--------------|
|               | IC50      |         |      |      |      |            |       |                |              |
| 95% CI IC50   | 0.005035  | 0.0002  | 9780e-007 to 1206 | 0.003445 to 0.0073 | 0.006 to 0.016 | 0.004349 to 0.054 | 0.004637 to 0.0273 | 0.005 to 0.023 |
| Relaxation (%) ± SEM | 63.79 ± 14.95 ± 8.91 ± 39.14 | 64.58 ± 9.37 | 36 ± 15.94 | 16.39 | 65.16 ± 13.995 |

REFERENCES

Abd El-Mageed AA, Osman AK, Tawfik AQ and Mohammed HA (2011). Chemical Composition of the Essential Oils of four Eucalyptus Species (Myrtaceae) from Egypt. J Phytochem 5(2): 115-122.
Al-Hlabi OA, Kheder DA, Vidari G and Gilardoni G (2013). Relaxant effect of essential oils of Eucalyptus camaldulensis on aortic rings in male albino rats. JUOZ 1(1): 139-146.
AL-Hlabi, O.A.M. and Shekha M.S., (2010). Vasorelaxant Effect of Aqueous Extract of Crataegus azarolus aronia and Quercetin on Isolated Albino Rat’s Thoracic Aorta. J. Duho Univ. 13 (1): 7-13.
Andrade LN, Batista JS and de Sousa DP (2011). Spasmolytic activity of p-menthane esters. J Med Plant Res 5(32): 6995-6999.
Coelho-de-Souza LN, Leal-Cardoso JH, de Abreu Matos FJ, Lahlou S, Magalhães PJ (2005). Relaxant effects of the essential oil of Eucalyptus tereticornis and its main constituent 1,8-cineole on guinea-pig tracheal smooth muscle. Planta Med. 71(12):1173-5.
Jucá DM, da Silva MT, Junior RC Jr, de Lima FJ, Okoba W, Lahlou S, de Oliveira RB, dos Santos AA and Magalhães PJ (2011). The essential oil of Eucalyptus tereticornis and its constituents, α- and β-pinene show accelerative properties on rat gastrointestinal transit. Planta Med. 77(1):57-9.
Kheder DA (2013). Physiological Effects of Essential Oils of Eucalyptus camaldulensis Dehn Fractions on Isolated Aorta and Trachea in Male Albino Rats. PhD Thesis. University of Zakho, Kurdistan-Region/ Iraq.
Lahlou S, Interaminense LF, Leal-Cardoso JH, Duarte GP 2003. Antihypertensive effects of the essential oil of Alpinia zerumbet and its main constituent, terpinen-4-ol, in DOCA-salt hypertensive conscious rats. Fundam Clin Pharmacol 17:323-330.
Lima JF, Brito TS, Freire WB, Costa RC, Linhares MI, Sousa FC, Lahlou S, Leal-Cardoso JH, Santos AA, Magalhães PJ (2010). The essential oil of Eucalyptus tereticornis, and its constituent’s alpha- and beta-pinene potentiate acetylcholine-induced contractions in isolated rat trachea. Fittoterapia. 81(6):649-55.
Magalhães PJ, Lahlou S, Jucá DM, Coelho-De-Souza LN, Da Frota PT, Da Costa AM, Leal-Cardoso JH (2008). Vasorelaxation induced by the essential oil of Croton nepetifolius and its constituents in rat aorta are partially mediated by the endothelium. Fundam Clin Pharmacol 22: 169-177.
Ribeiro TP, Porto DL, Menezes CP, Antunes AA, Silva DF, De Sousa DP, Nakao LS, Braga VA, Medeiros IA (2010). Unraveling the cardiovascular effects induced by alpha-terpineol: a role for the nitric oxide-cGMP pathway. Clin Exp Pharmacol Physiol. 37(8):811-6.
Saito K, Okabe T, Inanomi Y, Tsujibo H, Miyake Y, Hiraoka K, Ishida N (1996). The biological properties of monoterpenes: Hypotensive effects on rats and antifungal activities on plant pathogenic fungi of monoterpenes. Mokuzai Gakkaishi 42: 677-680.

Santos, MRV, Moreira FV, Fraga BP, De Sousa DP, Bonjardim LR and Quintans-Junior LJ (2011). Cardiovascular effects of monoterpenes: a review. Brazilian J Pharmacognosy 21(4): 764-771.

Shekha, M.S.S., (2010). Physiological Effects of Crataegus aronia Fractions on Isolated Smooth Muscle and Perfused “Langendorff” Heart in Albino Rats. PhD thesis. Duhok University. Kurdistan region-Iraq.

**Gorani, L.**

α-terpineyl acetate

α-terpineyl acetate is a common constituent of essential oils and has been shown to have hypotensive effects in several animal models. It is believed to act by blocking the influx of calcium ions through voltage-sensitive calcium channels, leading to a decrease in vascular tone.

**Summary**

The study aimed to investigate the mechanism of the cardiovascular effects of α-terpineyl acetate. The results showed that this compound significantly reduced blood pressure and increased the heart rate in isolated smooth muscle preparations. These effects were associated with a decrease in cGMP levels and an increase in intracellular calcium concentrations.

**Conclusions**

α-terpineyl acetate is a promising candidate for the development of new hypotensive drugs. Further studies are needed to elucidate the exact mechanism of action and to determine the optimal dose for clinical use.