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Original Research Article

Refined Camphor (*Kafura Pelebe*) Modulates Thoracic Aortae Vasomotor Tone of Rats

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ABSTRACT

Aqueous extract of refined camphor (AERC) is used in traditional medicine to manage various ailments including erectile dysfunction and to improve blood circulation. These uses are suggestive of possible involvement of refined camphor in vasomodulation. This study evaluated the vasomodulatory potential of AERC. Rings of rats' thoracic aortae were used to determine the vasomodulatory potential of AERC using isolated organ bath preparations coupled with PowerLab data acquisition system. The direct effect of AERC as well as its influence on noradrenaline-, atropine- and potassium chloride (KCl)-induced responses on the thoracic aortae of rats were investigated. The extract (390 µg/mL) significantly relaxed rat aortic rings, an effect that was significantly ($p<0.0001$) inhibited by atropine (0.33-3.30 µg/mL). It significantly ($p<0.0001$) inhibited noradrenaline (3.32×10^{-5} – 9.44×10^{0} µg/mL) induced vasoconstriction and significantly relaxed noradrenaline pre-contracted tissues. The extract also significantly ($p < 0.0001$) inhibited potassium chloride (KCl)-induced contraction. The results show that AERC is vasoactive and modulates vascular motor tone via vasodilatory mechanisms involving various pathways including adrenergic and cholinergic mechanisms.

Keywords: Cardiovascular, Vaso-relaxation, Camphor, *kafura*, Adrenergic, Cholinergic.

Introduction

Refined camphor is commonly found as slightly transparent fibrous blocks with characteristic scent and pungent aromatic taste that is usually followed by a cold sensation. Although originally thought to be sourced from medicinal plants, synthetic forms are now available. It is commonly used in traditional medicine practice in various parts of the world. In Nigeria, the exposure of herbal medicine users to this substance is fairly common. This is due to the fact that in addition to its consumption alone as a medication, refined camphor is also very commonly used in aqueous herbal preparations as a preservative to prolong their shelf life. Camphor was used in ancient Sumatra to treat sprains, swellings, and inflammation.[1] Camphor is used to increase local blood flow in the skin and muscle and is known to induce both cold and warm sensations.[2] Oil of refined camphor is used in treatment of cardiovascular dysfunction in India.[3] Personal communications with local users in Lagos, Nigeria reveal that the aqueous extract of refined camphor is used for gastrointestinal disturbances by people of all age groups, including infants. It is also used to manage erectile dysfunction. Its use in the management of nasal congestion has also been reported.[4] Some of the uses of camphor reveal that it has potential to influence motor tone of blood vessels, smooth muscles, an effect which remains to be demonstrated experimentally. Vasomotor tone refers to the degree of smooth muscle tension within blood vessels walls or the degree of constriction experienced by a blood vessel relative to its maximally dilated state.

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Ordinarily, under basal conditions, arteries and veins possess some level of smooth muscle contraction that determine their diameter, and hence their tone[5]. This vascular tone is largely maintained by the autonomous branch of the nervous system, especially the sympathetic nervous system, the effects of which are mediated by the neurotransmitter, noradrenaline. A number of other endogenous vasoactive substances have also been identified.[6] Vasomodulation is an important physiological process in the regulation of blood pressure, which is essential for maintenance of cardiovascular health and prevention of cardiovascular disorders and associated co-morbidities.[7] Identification of the vasomodulatory potential of xenobiotics is an important approach that could significantly impact the quest for measures to effectively combat the burden of cardiovascular disorders and related morbidity. The study examined the capability of the aqueous extract of refined camphor to induce changes in the thoracic aortae vasomotor tone of rats. The thoracic aorta is a section of the aorta, the largest artery in the body, within the chest region. It contains several receptors that could be interacted with by various endogenous and exogenous substances to modulate its vasomotor tone, which in turn impacts blood pressure and other heamodynamic measures of cardiovascular health.

Materials and Methods

Chemicals and reagents

Sodium chloride (J.T. Baker, USA), sodium bicarbonate (BDH laboratory suppliers), potassium chloride (May & Baker ltd, England), calcium chloride, magnesium sulphate (J.T. Baker, USA), monopotassium phosphate (May & Baker ltd, England), 95% oxygen (O_2), 5% carbon dioxide (CO_2) (BOC Gases Nigeria plc.), norepinephrine (CELON, India), glucose (J.T. Baker, USA).

Equipment

Radnoti organ bath, 50 g force transducers and PowerLab data acquisition system were all obtained from AD Instruments in New Zealand. The Microsoft windows computer used was installed with LabChart data acquisition software.

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Experimental animals
Male and female albino rats of average weight of 150 g were obtained from the laboratory animal care centre of the College of Medicine, University of Lagos. The rats were housed in polyethylene animal cages placed in well-ventilated rooms at 25-27ºC under suitable environmental conditions. Animals had access to standard rodent diet (Animal Care Service Consult, Ogun State, Nigeria) and drinking water *ad libitium*. They were acclimatized for 14 days before commencement of the experiment. The experimental procedures were carried out in accordance with the United States National Institute of Health's Guide for the care and use of laboratory animals (2011).[8]

Procurement of refined camphor and preparation of extract
Refined camphor was purchased from Ojuwoye market, Mushin, Lagos State, Nigeria in June 2018. Tablets of ingestible camphor weighing 27.69 g, was soaked in 750 ml of distilled water for 7 days at room temperature. The preparation was then filtered and the filtrate was stored in the refrigerator for use in the study. The difference between the initial weight of refined camphor (before extraction) and its final weight (after filtration i.e. the residue weight) was used to determine the percentage yield of the extract.

GC-MS analysis of AERC
Phytochemical analysis of refined camphor was performed with 7890A gas chromatography system coupled to VL/MSD 5975C mass spectrometer (GC-MS Agilent Technologies, Santa Clara, USA). The set-up comprised HP5MS fused silica capillary column [30 m (length) x 0.32 mm (diameter) x 0.25 µm (film thickness)]; helium gas (99.99%) used as carrier gas at constant flow rate of 1 ml/min and an injection volume maintained at 1 µl. The injector temperature was kept at 250^{o}C and pressure at 8.802 psi. Oven temperature initially set at 80^{o}C was later increased by 5 ^{o}C/min to 120 ^{o}C/min and eventually by 10 ^{o}C/min to 240 ^{o}C/min. Total running time was 30 min. Data interpretation was done using the National Institute of Standard and Technology (NIST) database.

Preparation of rat thoracic aortic rings
This was done following a slight modification of an earlier described procedure by Anthelme et al.[9] Rats that had been fasted overnight were humanely sacrificed by stunning and cervical dislocation. Their thoracic aortae were excised carefully and immediately transferred to a dish containing ice cold physiological Krebs-Henselett solution (NaCl 118.4 mM, KCl 4.7 mM, $CaCl_2$ 1.3 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.18 mM, $NaHCO_3$ 24.9 mM and glucose 11.1 mM; pH 7.4). The aortae were cleaned of adhering fat and connective tissue and then cut into 3-5 mm rings. The rings were mounted in isolated tissue baths filled with physiological solution aerated with 5% CO_2 and 95% O_2 and connected to a force transducer (ADInstruments, Australia) connected to a Microsoft Windows PC with data acquisition software, Labchart 7 installed. The rings were stretched progressively to an initial tension of 2 g and allowed to equilibrate for 60 min with washing (by draining and refilling with physiological solution) at 15 minutes interval. They were then contracted with noradrenaline (3.32 x 10^{-1} µg/ml) to ascertain their viability, washed and allowed to attain steady baseline tracing each time before the procedures described below were carried out respectively. Absence of acetylcholine-induced relaxation of the aortic ring tissues pre-contracted with noradrenaline was considered evidence for endothelium denudation.

Evaluation of the direct effect of AERC on rat thoracic aortic rings
The effect of AERC (13, 130 and 390 µg/ml) on vascular tone of the aortic ring tissues was assessed by adding increasing concentrations respectively, to the tissue mounted in the isolated tissue bath. The response of the tissue to each concentration was observed for 1 hour. Following the addition of each concentration and response observation, the tissue was washed and allowed to attain steady baseline tracing before the next addition.

Effect of atropine on AERC-induced vasomodulation
Atropine at 0.33 µg/ml was added to the bath to be in contact with tissue for 5 minutes, after which 390 µg/ml AERC was added and left in contact with the tissue for 1 hour. This was then followed by washing and attainment of steady baseline tracing. The same procedure was repeated with 0.99 and 3.3 µg/ml of atropine respectively.

Evaluation of the effect of AERC on noradrenaline induced response
The response of aortic ring tissues to cumulative additions of noradrenaline (3.32 x 10^{-5} – 3.32 x 10^{-1} µg/mL) was determined. This was then followed by pretreatment of the tissues with AERC (13 µg/ml) for 10 minutes before the cumulatively adding noradrenaline (3.32 x 10^{-5} – 9.44 x 10^{0} µg/mL). Following washing and attainment of steady baseline tracings, the latter procedure was repeated with AERC at 130 and 390 µg/mL, respectively. In another aspect of this study, aortic ring tissues were pre-contracted with noradrenaline (3.32 x 10^{-1}) and allowed to achieve steady contraction plateau before the addition of 13, 130 and 390 µg/mL of AERC cumulatively.

Effect of AERC on potassium chloride (KCl)-induced response
The response of the aortic tissues to cumulatively added KCl (29.97 x 10^{0}– 9.77 x 10^{3} µg/mL) was determined. This was then followed by pretreatment of the tissues with AERC (13 µg/mL) for 10 minutes before the cumulatively adding KCl (29.97 x 10^{0} – 9.77 x 10^{3} µg/mL). Following washing and attainment of steady baseline tracings, the latter procedure was repeated with AERC at 130 and 390 µg/mL respectively.

Statistical analysis
Statistical analysis was carried out via one or two-way analysis of variance followed by Tukey or Bonferoni's multiple comparison post hoc test, using Graphpad prism 7 software. Data are expressed as mean ± SEM. Results were considered statistically significant at p value less than 0.05.

Results and Discussion

GC-MS analyses of AERC
GC-MS analysis revealed the presence of compounds known to be chemically synonymous to or derivatives of refined camphor. They include (+)-2-Bornanone; α-campholenal; and Bicyclo[2.2.1]heptane-2-one, 1,7,7-trimethyl-,(1S). One unrelated compound identified was Benzenamine, 4-methoxy-N-(triphenylphosphoranylidene) as shown in Figure 1 and Table 1.

Direct effect of AERC on rat's thoracic aorta
A time-dependent decrease in vasomotor tone resulting in reduction of tissue tension (g) was observed at 13 µg/ml of AERC for up to 40 minutes, at which time point, the response was significant ($p < 0.01$), after which this response was reversed. However, at 130 and 390 µg/mL there was more prolonged concentration and time dependent decrease in the tone with varying level of significance for the entire 1 hour observation period. The peak of this response was noted for 390 µg/ml of AERC at 40 minutes (Figure 2).

Effect of atropine on AERC-induced vasomodulation
Figure 3 shows the relationship between AERC at 390 µg/mL and its response in the absence and presence of atropine at 0.33 – 3.30 µg/mL. At 0.33 µg/mL, atropine significantly ($p < 0.0001$) reversed AERC action, an effect which though maintained, appeared to diminish at the higher concentrations of atropine tested.

Effect of AERC on noradrenaline-induced vasoconstriction
Figure 4 shows the effect of noradrenaline in the absence (3.32 x10^{-5} – 3.32 x 10^{-1} µg/mL) and presence (3.32 x10^{-5} – 9.44 x 10^{0} µg/mL) of AERC (13 – 390 µg/mL). The extract significantly inhibited the vasoconstrictive action of noradrenaline at 13 and 130 µg/mL and completely reversed its effect at 390 µg/mL. It also significantly ($p< 0.0001$) enhanced the relaxation of noradrenaline pre-contracted rat thoracic aortic rings at all the concentrations tested (Figure 5).

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Effect of AERC on KCl-induced vasoconstriction

Cumulative addition of KCl (29.97 x 10^0 – 9.77 x 10^3 µg/mL) induced contraction of rats' thoracic aorta. The extract significantly ($p < 0.0001$) in a concentration-dependent manner, inhibited the contractile response to KCl (Figure 6). The GC-MS analysis of refined camphor showed the presence of (+)-2- bornanone and bicyclo[2.2.1]heptane-2-one, 1,7,7-trimethyl-,(1S); which are known to be synonymous to camphor and like camphor are also slightly soluble in water. α – campholenal used for flavouring food and manufacturing processes was also identified. Although it has similar molecular formula and weight as camphor, it is insoluble in water, as a result, it may be said to not contribute to the actions of AERC observed.[10] A relatively less known compound, benzenamine, 4-methoxy-N-(triphenylphosphoranylidene), was also identified. Its contribution to the effect of AERC in the study remains to be investigated. Camphor is either used alone or in combination with other components for managing cardiovascular disorders, especially in relation to blood circulation,[11] which is largely influenced by vascular motor tone. The action of AERC as well as its influence on known modulators of this tone were observed in the study. Upon investigation of the direct effect of AERC on rats' thoracic aortae, the extract (13 - 390 µg/mL) induced a significant concentration- and time-dependent vasodilatation, particularly at 130 and 390 µg/mL. This was noted for both endothelium-denuded and endothelium-intact (data not shown) aortic ring tissues. Although the presented data shows an hour-long vasodilation, this effect in fact lingered on for over an hour. Following this "prolonged" response however, the tissue recovered after washing to dislodge AERC from interaction sites. The vasodilatory activity of the extract as observed in endothelium denuded aortic rings indicates that it is capable of endothelium-independent vaso-relaxation. Interestingly, endothelium as well as nitric oxide independent mechanisms have also been reported for vasodilating nitric oxide donors.[12-14] Vascular motor tone mediators beyond the endothelium include M_1, M_2 and M_3 muscarinic receptors as well as calcium channel receptors in the tunica media smooth muscle cells of blood vessels.[15] Tunica adventitia adipocytes derived substances are also known to mediate vasodilation.[16] Investigation of the interaction of AERC with these mediators will provide further insight on the effect of AERC in the vascular motor tone and its consequent impact on cardiovascular health. Meanwhile in the study, atropine (0.33 – 3.3 µg/mL) significantly ($p < 0.0001$) inhibited the vasodilatory effect of AERC (390 µg/mL) throughout the 1hour observation period. As a nonselective muscarinic antagonist, it is not clear which muscarinic receptor is being blocked by atropine. However, an involvement of muscarinic receptor pathway in the action of AERC has thus been demonstrated. A ceiling effect of atropine at 0.33 µg/mL was noted as it failed to produce greater inhibition at higher concentrations, 0.90 and 3.3 µg/mL, rather a diminishing effect was observed. This could be due to saturation of muscarinic receptor occupancy in the region of the blood vessel involved. The extract also significantly inhibited noradrenaline-induced contraction, with a noradrenolytic or vasomotor reversal-like response at 390 µg/mL. Such response is usually due to blockade of vasoconstriction mediating α_1-adrenoceptors, thus leaving the vasodilating β_2-adrenoceptors to be stimulated by noradrenaline. Although noradrenaline has a rather low affinity for β_2-adrenoceptors, on the occasion of an exhaustive blockade of α_1-adrenoceptors, the vasorelaxant response to noradrenaline via β_2-adrenoceptors ensues.[17,18] The extract shifted in a non-parallel manner the concentration-response curve of noradrenaline to the right. Although it also obliterated the maximum response and reduced the slope of the curve, it did not alter the potency of noradrenaline. This indicates that AERC has non-competitive antagonistic effect on noradrenaline. Following wash out, normal response to noradrenaline at 3.32 x 10^{-1} µg/mL resumed, indicating the reversibility of the antagonistic action of AERC on noradrenaline. The extract also relaxed noradrenaline pre-contracted aortic rings by a yet to be determined mechanism. Previous reports indicate that Ca^{2+} influx via opened voltage dependent calcium channels subsequent to cell membrane depolarization is involved in potassium chloride-induced smooth muscle contraction.[19,20] Inhibition of potassium chloride-induced contraction by AERC demonstrates its possible inhibitory action on voltage-dependent calcium channel pathway. Although the observations reported here were made for endothelium-denuded aortae, similar findings were noted for endothelium intact aortic ring tissues (data not shown). While these observations appear beneficial in conditions that require relaxation of blood vessels, it is important to note that these responses were observed in blood vessels of normotensive rats. This indicates that AERC may be non-selective in its vasorelaxant action and can have far reaching effects on the cardiovascular system.

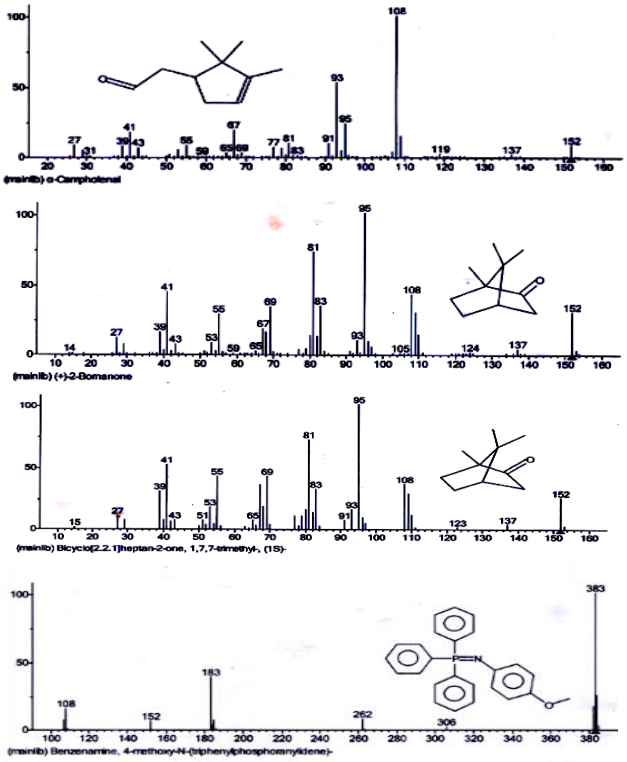

Figure 1: Mass spectra of the compounds identified in the GC-MS analysis of refined camphor.

Table 1: Results of GC-MS analysis of refined camphor

S/N	Retention time (min)	Compound name	% Composition	Chemical formula	Molecular weight (g/mol)	Chemical Abstract Service no.
1.	8.191	α, -Campholenal	2.26	$C_{10}H_{16}O$	152.23	91819-58-8
2.	8.941	(+)-2-Bornanone	28.98	$C_{10}H_{16}O$	152.23	000464-49-3
3.	9.267	Bicyclo[2.2.1]heptane-2-one, 1,7,7-trimethyl-,(1S)	5.40	$C_{10}H_{16}O$	152.23	000464-48-2
4.	33.722	Benzenamine, 4-methoxy-N-(triphenylphosphoranylidene)	3.41	$C_{25}H_{22}NOP$	383.40	014796-89-5

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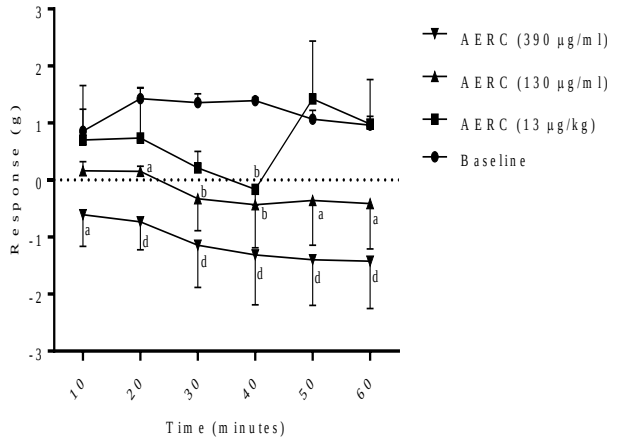

Figure 2: The vasomodulatory effect of AERC (13 - 390 µg/mL) on rats' thoracic aortae.
Points on the line graph represent mean ± SEM (n = 6). $^{a,b,d}p < 0.05$, 0.01, 0.0001 vs baseline tracing (Two way ANOVA followed by Bonferoni's multiple comparison post hoc test). AERC- aqueous extract of ingestible camphor

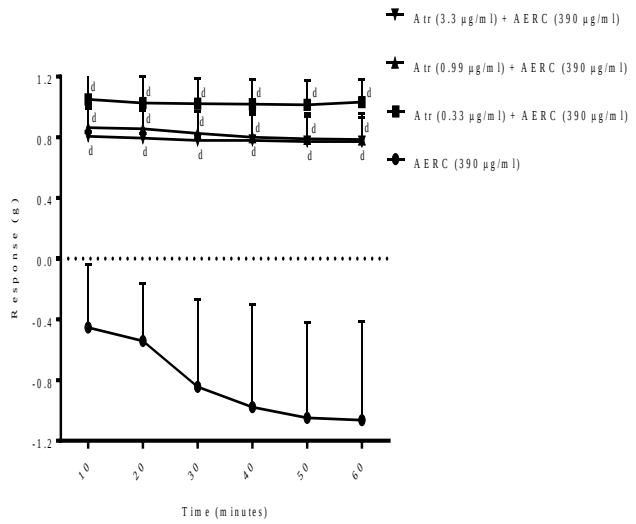

Figure 3: The effect of atropine on the vasomodulatory effect of AERC (390 µg/mL) on rats' thoracic aortae.
Points on the line graph represent mean ± SEM (n = 6). $^{d}p < 0.0001$ vs AERC at 390 µg/ml (Two-way ANOVA followed by Bonferoni's multiple comparison post hoc test). AERC- aqueous extract of ingestible camphor, Atr - atropine

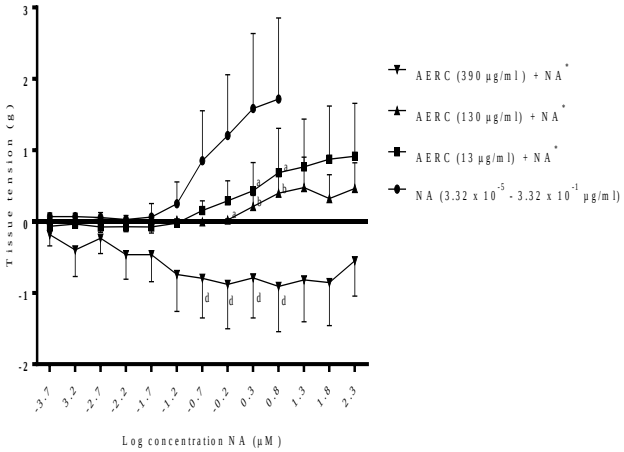

Figure 4: The effect of noradrenaline in the absence (3.32 x 10^{-5} – 3.32 x 10^{-1} µg/mL) and presence (3.32 x 10^{-5} – 9.44 x 10^{0} µg/mL) of AERC (13-390 µg/mL) on rats' thoracic aortae.
Points on the line graph represent mean ± SEM (n=8). $^{a,b,d}p < 0.05$, 0.01, and 0.0001 vs NA in the absence of AERC i.e. at 3.32 x 10^{-5} – 3.32 x 10^{-1} µg/mL (Two way ANOVA followed by Bonferoni's multiple comparison post hoc test). AERC- aqueous extract of ingestible camphor, NA-noradrenaline, *noradrenaline at 3.32 x 10^{-5} – 9.44 x 10^{0} µg/mL in the presence of AERC.

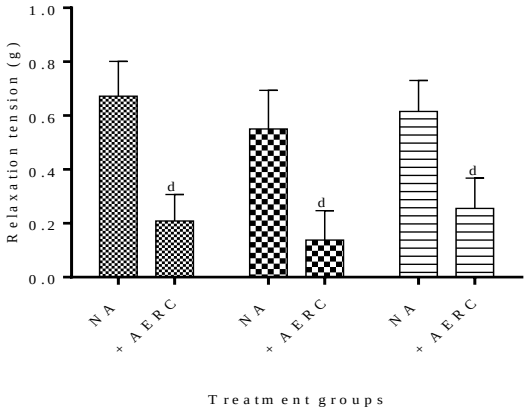

Figure 5: The vasomodulatory action of AERC on NA pre-contracted rats' thoracic aortae.
Bars represent mean ± SEM (n = 8). $^{d}p < 0.0001$ vs NA treatment. (One-way ANOVA followed by Tukey's multiple comparison post hoc test). AERC- aqueous extract of ingestible camphor, NA - noradrenaline

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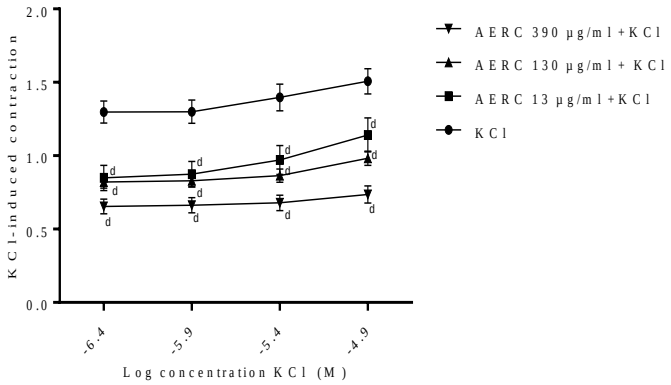

Figure 6: The effect of AERC on KCl-induced vasoconstriction of rats' thoracic aortae. Points on the line graph represent mean ± SEM (n = 11). $^{d}p < 0.0001$ (Two-way ANOVA followed by Bonferoni's multiple comparison post hoc test). AERC- aqueous extract of ingestible camphor, KCl – potassium chloride

Conclusion

The aqueous extract of refined camphor is capable of significant relaxant action on rats' thoracic aorta. Its modes of action involve endothelium-independent, muscarinic, adrenergic, and voltage-dependent calcium channel pathways. This finding indicates that AERC has potential to impact the cardiovascular system.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Miller C. An Account of the Island of Sumatra. London: The Royal Society; 1778. 121 p.
2. Kotaka T, Kimura S, Kashiwayanagi M, Iwamoto J. Camphor induces cold and warm sensations with increases in skin and muscle blood flow in human. Biol Pharm Bull. 2014; 37(12):1913-1918.
3. Madan U. Camphor: This is how you can use its medicinal benefits to the fullest! [Online]. 2015 [cited 2021 Jun 6] Available from: https://zeenews.india.com/news/health/tips/camphor-this-is-how-you-can-use-its-medicinal-benefits-to-the-fullest_1814892.html.
4. Drugs.com. Camphor inhalation liquid. [Online]. 2020 [cited 2021 Jan 16] Available from http://www.drugs.com/cdi/camphor-liquid.html.
5. Klabunde RE, Cardiovascular Physiology Concepts. [Online]. 2016 [cited 2021 Jan 16] Available from https://www.cvphysiology.com/.
6. Jaimes L, Vinet R, Knox M, Morales B, Benites J, Laurido1 C, Martínez JL. A review of the actions of endogenous and exogenous vasoactive substances during the estrous cycle and pregnancy in rats. Anim. 2019; 9(6):288.
7. Ong P and Seitz A. The importance of assessing coronary vasomotor function in patients with SIHD with unobstructed coronary arteries. [Online]. 2020 [cited 2021 Jun 6]. Available from: https://www.acc.org/latest-in-cardiology/articles/2020/12/18/13/54/the-importance-of-assessing-coronary-vasomotor-function-in-patients-with-sihd.
8. United States National Institute of Health. Guide for the care and use of laboratory animals. (8th ed). Washington DC: National Academic Press; 2011. 184-202
9. Anthelme NS, Pathak P, Kanshana JS, Kanuri BN, Rebello SC, Kumar B, Yadav PP, Traore F, Jagavelu K, Dikshit M. Vasomodulatory effects and mass spectral analysis of *Bridelia ferruginea* Benth, Indian J Exp Biol. 2018; (56):621-628.
10. National Institutes of Standards and Technology (NIST). (+)-2-Bornanone [Online]. 2018 [cited 2021 June 06] Available from: https://webbook.nist.gov/cgi/cbook.cgi?ID=000464-49-3&Units=SI
11. Sweetman SC (ed.) Martindale-The Complete Drug Reference. (36th ed). London: The Pharmaceutical Press; 2009. 2274 p.
12. Mendes-Júnior LG, Guimarães DD, Gadelha DDA, Diniz TF, Brandão MCR, Athayde-Filho PF, Lemos VS, França-Silva MS, Braga VA. The new nitric oxide donor cyclohexane nitrate induces vasorelaxation, hypotension, and antihypertensive effects via NO/cGMP/PKG pathway. Front Physiol. 2015; 6(243):1-9.
13. Zhou K and Parker JD. The role of vascular endothelium in nitroglycerin-mediated vasodilation. Br J Clin Pharmacol. 2019; 85(2):377-388.
14. Kleschyov AL, Oelze M, Daiber A, Huang Y, Mollnau H, Schulz E, Sydow K, Fichtlscherer B, Mülsch A, Münzel T., Does nitric oxide mediate the vasodilator activity of nitroglycerin? Circ Res. 2003; 93:e104-e112.
15. Saternos HC, Almarghalani DA, Gibson HM, Meqdad MA, Antypas RB, Lingireddy A, Translational Physiology Distribution and function of the muscarinic receptor subtypes in the cardiovascular system. Physiol Genomics. 2018; 50:1-9.
16. Cheng, CK, Bakar HA, Gollasch M, Huang Y. Perivascular adipose tissue: the sixth man of the cardiovascular system. Cardiovasc Drugs Ther. 2018; 32: 481–502.
17. Dugdale AH, Beaumont G, Bradbrook C, Gurney M. Veterinary Anaesthesia: Principles to Practice, West Sussex: John Wiley and Sons; 2020. 58 p.
18. Rang HP, Dale MM, Ritter JM, Flower RJ, Henderson G. Rang and Dale's Pharmacology. China: Elsevier; 2012. 174 p.
19. Hu G, Li X, Zhang S, Wang X. Association of rat thoracic aorta dilatation by astragaloside IV with the generation of endothelium‐derived hyperpolarizing factors and nitric oxide, and the blockade of Ca^{2+} channels. Biomed Rep. 2016; 27-34.
20. Niazmand S, Fereidouni E, Mahmoudabady M, Mousavi SM. Endothelium-independent vasorelaxant effects of hydroalcoholic extract from *Nigella sativa* seed in rat aorta: the roles of $Ca^{2+}$and K^{+} channels. BioMed Res Internat. [Online]. 2014 [cited 2021 Feb 19]. Available from http://dx.doi.org/10.1155/2014/24705.