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VASORELAXANT AND ANTIHYPERTENSIVE EFFECTS OF *RHUS PENTAPHYLLA (SEARSIA PENTAPHYLLA)*

Narjisse Messaoudi, Hassane Mekhfi, Mohammed Aziz, Abdelkhaleq Legssyer, Mohammed Bnouham and Abderrahim Ziyyat *

Laboratory of Physiology, Genetics and Ethnopharmacology, Department of Biology, Faculty of Sciences, University Mohammed First, Oujda-Morocco.

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Correspondence to Author:
Abderrahim Ziyyat

Professor,
Laboratory of Physiology, Genetics and Ethnopharmacology, Department of Biology, Faculty of Sciences, University Mohammed First, BP 717, Boulevard Mohamed VI, 60000, Oujda, Morocco.

E-mail: ziyyat@yahoo.fr

ABSTRACT: *Rhus pentaphylla* (Jacq.) Desf. (*Searsia pentaphylla* (Jacq.) FA. Barkley) is used for its colorant and tanning properties by the local population. The bark, leaves, roots, and fruits are employed in Moroccan traditional medicine to treat gastrointestinal disorders and diarrhea. Nevertheless, the pharmacological properties of *R. pentaphylla* on cardiovascular diseases have not yet been presented. This study was carried out to explore the vasorelaxant effect of the aqueous extract (decoction) from the leaves of *R. pentaphylla* (RpAE), its mechanism of action, and its antihypertensive effect. The results of this study demonstrate that RpAE induces a dose-dependent effect on isolated rat aorta. The vasorelaxation is endothelium-dependent *via* the muscarinic receptor, Calmodulin/eNOS/sGC/cGMP/PKG signaling pathway through the activation of SERCA pump, the inhibition of VOCC, and opening of K_{Ca}^{2+} channels. *In-vivo*, RpAE induces antihypertensive effect, ameliorates diuresis, and vascular reactivity on L-NAME induced hypertensive rats. The oral administration of RpAE reveals no mortality or toxicity. This is the first study in Morocco showing vasorelaxant and antihypertensive effects of species belonging to genus Rhus.

INTRODUCTION: Hypertension is one of the ultimate prevalent and modifiable risk factor for cardiovascular diseases (CVD) all over the world [1]. Certainly, it is an imminent risk factor for endothelial dysfunction [2], diabetes [3], atherosclerosis [4], renal dysfunction [5], coronary artery disease [6], congestive heart failure, and stroke [7]. It is also one of the important preventable causes of disability, morbidity, and mortality throughout the world [8].

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It reported to be responsible for about 6% of deaths globally, and the overall prevalence reveals to be around 30-45% of the population and increases with age [9]. High blood pressure is clinically determined as systolic blood pressure (SBP) ≥140 mmHg and diastolic blood pressure (DBP) ≥90 mmHg [9].

The present pharmacological treatment of hypertension includes a range of chemical drugs acting on vessels, heart, central nervous system, and kidneys, which successfully decrease blood pressure in many hypertensive subjects [10]. Even if multidrug therapy is selected, several people do not have its arterial blood pressure adequately adjusted by present antihypertensive drugs [11]. Moreover, these tools are characterized by limited efficacy,

and combination therapy [10], which discourages drug adherence and increase the risk of undesirable effect as well as drug-drug interactions [12].

Since ancient times, natural products have been used like crucial remedies for handling and treating many diseases and illnesses [13]. Nowadays, there is an increasing trend to discover new drugs *via* exploring natural products such as medicinal plants. In Morocco, the genus Rhusis represented by three species: *Rhus pentaphylla* (Jacq.) Desf., *Rhus albidum* (Schousb), and *Rhus tripartita* (Ucria) Grande [14].

Rhus pentaphylla (Jacq.) Desf. (Anacardiaceae) is a wild edible plant growing in the Mediterranean region [15, 16] and popularly known as Tizgha [14, 17-20], Azad [21, 22] or Tazzad [22]. It is broadly spread in Morocco [14, 17, 18, 23-25]. Since old times, *Rhus pentaphylla* (Jacq.) Desf. (*Searsia pentaphylla* (Jacq.) F.A. Barkley) is used for its colorant, and tanning properties by local population [14, 26]. The bark, leaves, roots, and fruits are used in Moroccan traditional medicine to treat gastrointestinal disorders and diarrhea [14, 18-22].

The crushed fresh leaves are used to treat injuries [17]. Previous investigations showed that *Rhus pentaphylla* possesses an antibutyrylcholinesterasic activity [27], antifungal [28] and antioxidant activity [29] in addition to its potential to color wood and silk as a new source of natural colorant [30].

Nevertheless, the pharmacological properties of *Rhus pentaphylla* from Oriental region of Morocco on cardiovascular diseases have not yet been studied. Therefore, the goal of this study is to investigate the vascular effect of the aqueous extract of leaves *via* decoction, to explore the possible mode of action of vasorelaxant activity and to assess the antihypertensive property of *Rhus pentaphylla*.

MATERIALS AND METHODS:

Plant Material: Fresh leaves from *Rhus pentaphylla* (Jacq.) Desf. were harvested from Berkane city (Oriental region of Morocco) in March 2016. A voucher specimen was identified and conserved in the Herbarium of the Faculty of Sciences, University Mohammed First (Oujda, Morocco) under the reference number (HUMPOM-208).

Preparation of Aqueous Extract: Hundred grams of dried leaves of *R. pentaphylla* were decocted with 600 ml of distilled water for 20 min. The obtained mixture was filtered and then evaporated using a rotary evaporator at 50-60 °C. The extract was retained at -20°C for further utilization.

Chemicals and Reagents: (R)-(-)-phenylephrine hydrochloride [PE], carbamylcholine chloride [carbachol, CCH], atropine, Nω-Nitro-L-arginine methyl ester hydrochloride [L-NAME], Rp-8-bromo-β-phenyl-1,N2-ethenoguanosine3′, 5′-cyclic monophosphorothioate sodium salt [Rp-8-Br-PET-cGMP], calmidazolium chloride, tetraethyl ammonium chloride hydrate [TEA], dimethyl sulfoxide [DMSO], thapsigargin, glibenclamide, D(+)-glucose anhydrous, sodium chloride [NaCl], potassium chloride [KCl], indomethacin, (±)-verapamil hydrochloride, and magnesium sulfate [$MgSO_4$] are obtained from Sigma Aldrich company. Sodium nitroprusside [SNP] and sodium hydrogen carbonate [$NaHCO_3$] are from Farco chemical. Others chemicals were obtained as follow: 1H-[1,2,4] Oxadiazolo[4,3-a]quinoxalin-1-one [ODQ] (Cayman Chemical, USA), 4-aminopyridine [4-AP] (Alfa Aesar), barium chloride dehydrate [$BaCl_2$] (AnalaR Normapur - VWR International), calcium chloride dehydrate [$CaCl_2$, $2H_2O$] (Scharlauchemie), potassium dihydrogen phosphate [KH_2PO_4] (Panreac), pentobarbital (Ceva Santé Animale), hydroxocobalamin hydrochloride (Fluka), and enalapril maleate [Renitec®20mg] (Afric-Phar).

The stock solution of indomethacin was prepared in 5% (w/v) sodium bicarbonate solution. Rp-8-Br-PET-cGMP, glibenclamide, thapsigargin, and ODQ were prepared in DMSO. However, all other drugs were dissolved in distilled water.

Experimental Animals: All procedures implicating animals were organized by the Guide for the Care and Use of Laboratory Animal published by the US National Institutes of Health (NIH publication no. 85-23, revised in 1996). Wistar rats and albinos mice were obtained from the local colonies of the department of Biology (Faculty of Sciences-Oujda, Morocco). Animals were kept under a 12 h / 12 h light/dark cycle and had free access to food and water.

Acute Toxicity Test: A total of 30 mice weighing between 23-37 g were randomly divided into five experimental groups of 6 mice each (3 males and 3 females per group). After fasting overnight, RpAE was administered to each treatment group at single doses of 1, 2, 5, 8, and 10 mg/kg respectively by oral gavage. The control group was supplied with distilled water. After oral administration, all animals were observed individually for mortality and changes in general behavior over the first 30 min, then at 4 and 48 h, and after two weeks following up the administration of RpAE.

Vascular Reactivity on Aorta Isolated from Normotensive Rats: After anesthesia with sodium pentobarbital (0.1 ml/100g, ip), the descending thoracic aorta of Wistar rats (200-320) was immediately excised and placed in Krebs-Henseleit solution (NaCl 119 mM, KCl 4.7 mM, $CaCl_2$ 2.6 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.2 mM, $NaHCO_3$ 25 mM, and Glucose 11 mM, pH 7.4). Fat and connective tissues were trimmed off, and the aorta was then cut to make sections of about 3-5 mm in length. The aortic rings were then hanged by platinum hooks under an optimal tension of 1g in organ bath (Emka technologies, France) having 11 ml of Krebs solution maintained at 37 °C and continuously aerated with 95% O_2, 5% CO_2.

A stability period of 30 min was allowed before the addition of any drug or tested extract. The recording of responses was ceased by washing the aorta rings with Krebs solution. The presence of functional endothelium was verified by the ability of Carbachol (CCH) (10^{-4}M) to induce more than 60% relaxation in rings pre-constricted with phenylephrine (PE) (10^{-6}M). In denuded aortic rings, the endothelium was mechanically removed.

Effect of RpAE on PE-Induced Tonic Contractions in Endothelium-Intact and Denuded Aortic Rings: To verify if it is the case of endothelium-dependent vasorelaxation, the effects of 10^{-3}, 10^{-2}, 10^{-1}, and 1mg/ml of RpAE were tested in the presence (n=6) and absence (n=6) of vascular endothelium. Aortic rings were contracted with PE (1µM) to acquire a maximal response. Once the plateau attained, RpAE was added cumulatively (10^{-3} to 1mg/ml) into the organ bath. Relaxations were expressed as the percentage of relaxation of PE-induced contraction.

Role of Muscarinic Receptors in RpAE-Induced Vascular Response: To assess whether or not RpAE is producing vasodilatation through the activation of muscarinic receptors, endothelium-intact aortic rings were incubated with atropine: a non-selective muscarinic receptor antagonist (10^{-5} M; n=6) for 30 min before exposure to PE (1µM). After the PE response had reached the plateau, a single dose of 0.1 mg/ml of RpAE was added, and the responses were recorded. The recording of the responses was ceased by washing the aortic rings with Krebs solution. The control group was not pre-incubated with muscarinic receptor antagonist.

Role of NO and Prostanoids in RpAE-Induced Vascular Response: To determine the role of NO and prostanoids in RpAE-induced vasorelaxation, a pre-incubation with NO synthase inhibitor: Nω-Nitro-L-arginine methyl ester (L-NAME; 10^{-4}M; n=6), the NO scavenger: hydroxocobalamin (3.10^{-5}M; n=6), and the non-selective cyclooxygenase inhibitor: indomethacin (10^{-5}M; n=6), for 30 minutes prior to the contraction with PE (10^{-6}M) was realized. Afterward, a single dose of RpAE (0.1 mg/ml) was added. The response of each test was compared with that recorded with control. In the case of pre-incubation with L-NAME, Sodium nitroprusside (SNP, 1µM) was added to produce an endothelium-independent relaxation.

Vasorelaxant Effect of RpAE in the Presence of Calmidazolium, ODQ, and 8-RP-Br-PET-cGMP: To define the mechanisms by which RpAE relaxes vascular smooth muscle, another series of experiments were undertaken. The endothelium-intact rings were incubated with; Ca^{2+}- Calmodulin binding to NOS blocker: calmidazolium chloride (10^{-3}M; n=6), the guanylyl cyclase inhibitor: 1H-[1,2,4] oxadiazole [4,3-a]quinoxalin-1-one (ODQ; 10^{-5}M; n=6), and the competitive cGMP-dependent protein kinase G (PKG) inhibitor: Rp-8-Br-PET-cGMP (3.10^{-6}M; n=6) for 30 min prior the contraction with PE (10^{-6}M). Then, maximal relaxation induced by a single dose of 0.1 mg/ml of RpAE was determined and compared with that obtained with untreated rings.

Role of K^+ Channels in RpAE-Induced Vascular Response: To investigate the involvement of potassium channels in the effects of RpAE, the voltage-dependent K^+ channel blocker: 4-

aminopyridine (4-AP; 10^{-4}M; n=6), the non-specific ATP-sensitive K^+ channel blocker: glibenclamide (10^{-5}M; n=6), the selective inwardly rectifying potassium channel blocker: barium chloride ($BaCl_2$; 10^{-4}M; n=6), and the Ca^{2+} -activated potassium channels blocker: tetraethyl-ammonium (TEA; 10^{-3}M; n=6) were applied to endothelium-intact rings, 30 min prior to pre-contraction by PE (10^{-6}M). Then, maximal relaxation induced by a single dose of 0.1 mg/ml of RpAE was determined and compared with those obtained in the case of non-treated rings with these inhibitors.

Vasorelaxant Effect of RpAE in the Presence of Thapsigargin or Verapamil: To explore the role of calcium channels in the vasorelaxant effect of RpAE, endothelium-intact rings were incubated with the Ca^{2+} -channel type VOC: verapamil (10^{-5}M; n=6) and the endoplasmic reticulum Ca^{2+}-ATPase (SERCA) inhibitor: thapsigargin (10^{-6}M; n=6) for 30 min prior to contraction with PE (10^{-6}M). Then, maximal relaxation induced by a single dose of 0.1 mg/ml of RpAE was determined and compared with that obtained with untreated rings.

Determination of Antihypertensive Effect: An adaptation phase of 3 days for vehicle administration was carried out, and blood pressure measurement was allowed before the launching of the treatment. Antihypertensive activity study of RpAE was conducted on L-NAME hypertensive rats (250-310 g). Thirty Wistar rats were allotted into 5 groups (6 animals each: 3 males and 3 females), control group received tap water, L-NAME group received L-NAME (32 mg/Kg/day), L-NAME + Enalapril was co-treated with L-NAME 32 mg/Kg/day and Enalapril 15 mg/Kg/day. Whereas, L-NAME + RpAE 50 and L-NAME + RpAE 150 received simultaneously L-NAME (32 mg/Kg/day) and respectively the doses 50 mg/Kg/day and 150 mg/Kg/day of RpAE.

Treated groups received daily and orally a dose of 1 ml/100g of body weight for 4 weeks. Throughout the experimental period, animals had free access to tap water and chow. The Systolic blood pressure (SBP) was indirectly recorded before the treatment and once a week during the treatment period, by the non-invasive tail-cuff method. The rats were kept on a holder controlled to keep immobilized. The SBP signal was detected after 5-10 min of stabilization by a transducer placed around the tail and related to an inflation-deflation system using plethysmograph apparatus (Innovators in Instrumentation, Landings, USA).

Statistical Analysis: Relaxant responses are expressed as a percentage relaxation of PE (1µM) pre-contraction levels unless otherwise described in the figure legends. The values were expressed as means ± SEM. Statistical analysis was carried out using one-way analysis of variance (ANOVA) and two-way ANOVA, followed by Bonferroni's post-hoc test, using GraphPad Prism version 5.0 Software, San Diego California, USA. Significance was measured at p-values of less than 0.05.

RESULTS:

Acute Toxicity Test: The doses of 1, 2, 5, 8 and, 10 g/Kg of RpAE given orally by gavage to the mice showed no signs of toxicity or decrease during the 2 weeks observations.

Vasorelaxant Effect on Aorta Isolated from Normotensive Rats:

Effect of RpAE on PE-Induced Tonic Contractions in Endothelium-Intact and Denuded Aortic Rings: As shown in **Fig. 1**, RpAE (10^{-3}, 10^{-2}, 10^{-1} mg/ml) induced vasorelaxation of 9.04 ± 2.86%, 26 ± 2.01% and 80.67 ± 0.77% respectively. However, the addition of 1mg/ml induced a contraction of about 52.75 ± 0.83% (n=6) in intact aorta pre-contracted by PE (10^{-6} M). The vasorelaxation produced by RpAE was abolished after denudation of aorta. In fact, a vasoconstriction was noticed by RpAE (10^{-3}, 10^{-2}, 10^{-1} and 1mg/ml) 8.66 ± 1.85%, 18.3 ± 0.35%, 28 ± 3% and 53.33 ± 2.18% respectively.

Effect of a Single Dose 0.1mg/ml of RpAE on PE-Induced Tonic Contractions in Endothelium-Intact and Denuded Aortic Rings and the Role of Muscarinic Receptors in RpAE-Induced Vascular Response: Fig. 2 shows that a single dose 0.1mg/ml of RpAE has produced 80 ± 0.81% of vasorelaxation. This effect was disappeared either after denudation with the vasoconstriction of 23.72 ± 1.55%; $p<0.001$, or pre-incubation with the muscarinic receptor antagonist atropine (10^{-5}M, n=6) with the negligible contraction 0.71 ± 1.66%; $p<0.001$.

These findings indicate that RpAE induces vasorelaxation *via* endothelium - dependent signaling pathway and *via* activation of muscarinic receptors.

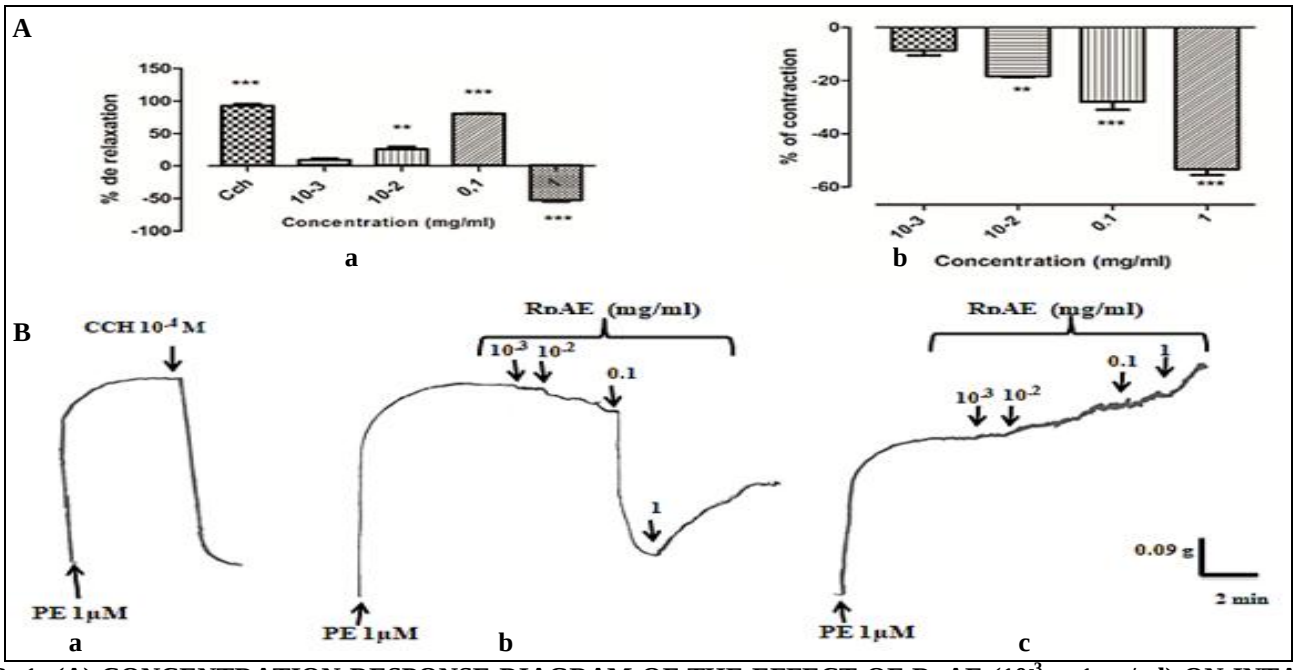

FIG. 1: (A) CONCENTRATION-RESPONSE DIAGRAM OF THE EFFECT OF RpAE (10^{-3} to 1mg/ml) ON INTACT (a) AND DENUDED (b) THORACIC AORTIC RINGS ISOLATED FROM NORMOTENSIVE RATS AND PRE-CONTRACTED WITH PE (10^{-6}M). (B) ORIGINAL TRACING SHOWING THE EFFECT OF CCH (10^{-4} M) ON PE PRE-CONTRACTED INTACT THORACIC AORTIC RINGS (10^{-6}M) (a), THE EFFECT OF RpAE ($10^{-3}$to 1mg/ml) ON PE (10^{-6}M) PRE-CONTRACTED INTACT THORACIC AORTIC RINGS (b), AND THE EFFECT OF RpAE ON PE PRE-CONTRACTED DENUDED THORACIC AORTIC RINGS (c). Values are mean ± SEM, n=6. One-way ANOVA followed by Bonferroni's post-hoc test; *** p<0.001 *vs.* control

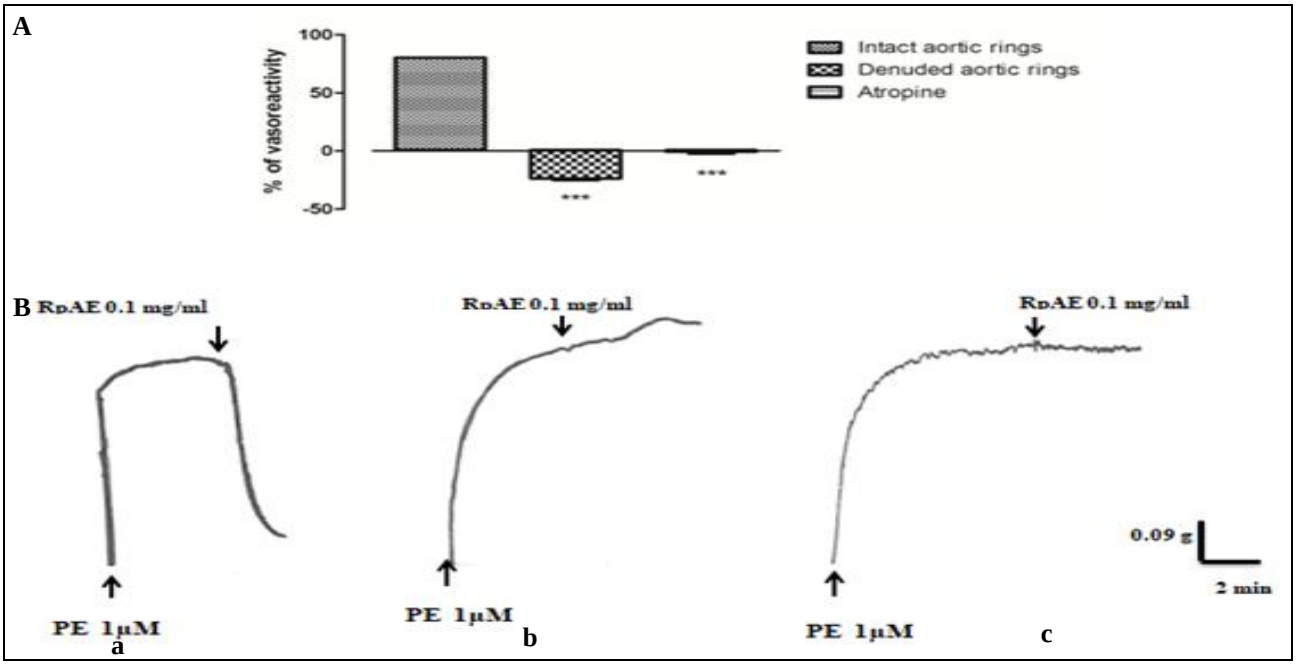

FIG. 2: (A) EFFECT OF A SINGLE DOSE 0.1mg/ml OF RpAE ON INTACT AND DENUDED THORACIC AORTIC RINGS ISOLATED FROM NORMOTENSIVE RATS AND PRE-CONTRACTED WITH PE (10^{-6} M) AND IN THE PRESENCE OF ATROPINE. (B) ORIGINAL TRACING SHOWING THE VASORELAXANT EFFECT OF A SINGLE DOSE 0.1mg/ml RpAE ON PE PRE-CONTRACTED INTACT THORACIC AORTIC RINGS (10^{-6} M) (a), THE EFFECT OF RpAE ON PE PRE-CONTRACTED DENUDED THORACIC AORTIC RINGS (b), AND IN THE PRESENCE OF ATROPINE (c). Values are mean ± SEM, n=6.One way ANOVA followed by Bonferroni's post-hoc test; *** p<0.001 *vs.* control

Role of NO and Prostanoids in RpAE-Induced Vascular Response: To evaluate the involvement of endothelium-derived relaxing factors in the RpAE-induced vasorelaxation, the effects of L-NAME (10^{-4}M; n=6) a non-selective NOS inhibitor, the NO scavenger: hydroxocobalamin (3.10^{-5}M; n=6), and the non-selective cyclooxygenase inhibitor: indomethacin (10^{-5} M; n=6) were examined. As illustrated in **Fig. 3**, RpAE-induced endothelium-dependent relaxation was vanished by pre-treatment with L-NAME and hydroxocobalamin with negligible values of contraction: 2.11 ± 1.26%; p<0.001 and 4.45 ± 0.34%; P<0.001 respectively. Pre-incubation with indomethacin did not affect RpAE-induced vasorelaxation: 79.15 ± 1.27%.

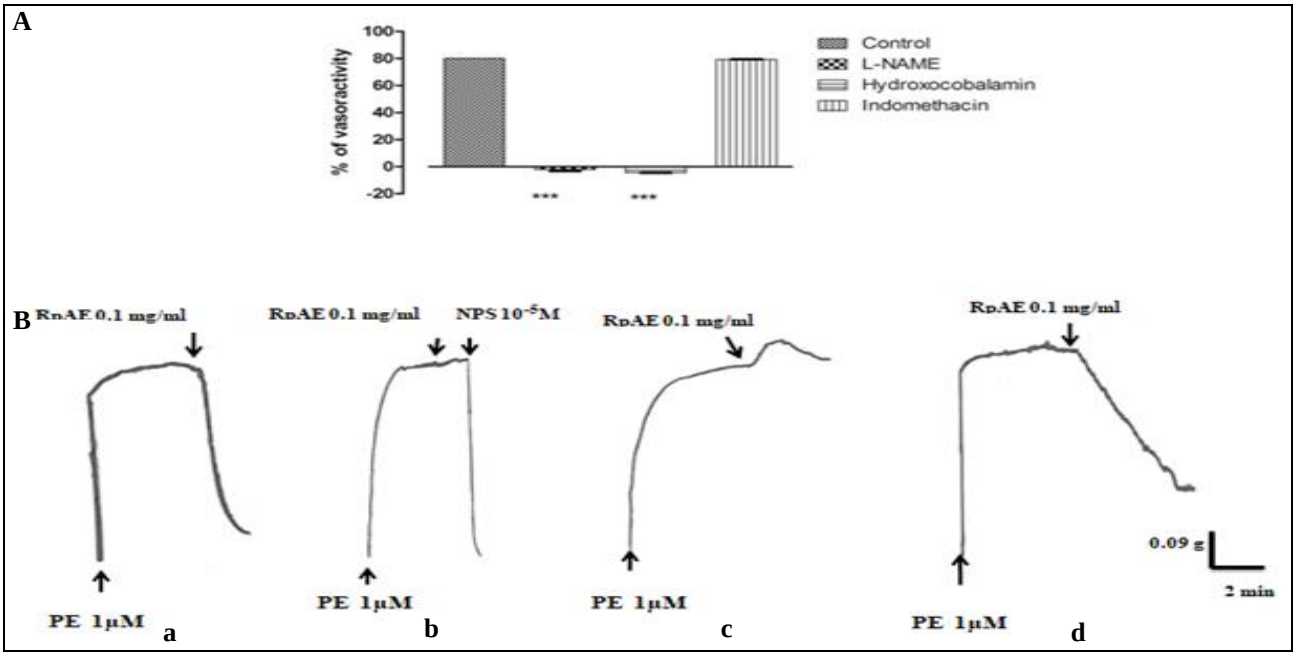

FIG. 3: (A) EFFECT OF A SINGLE DOSE 0.1mg/ml OF RpAE ON INTACT THORACIC AORTIC RINGS ISOLATED FROM NORMOTENSIVE RATS AND PRE-CONTRACTED WITH PE (10^{-6} M) AND IN THE PRESENCE OF L-NAME, HYDROXOCOBALAMIN, AND INDOMETHACIN. (B) ORIGINAL TRACING SHOWING THE EFFECT OF RpAE ON PE PRE-CONTRACTED INTACT THORACIC AORTIC RINGS (10^{-6} M) (a), IN THE PRESENCE OF L-NAME (b), HYDROXOCOBALAMIN (c), AND INDOMETHACIN (d). Values are mean ± SEM, n=6. One way ANOVA followed by Bonferroni's post-hoc test; *** p<0.001 *vs*. control.

The vasorelaxant effect of RpAE in the Presence of Calmidazolium, ODQ, and 8-RP-Br-PET-cGMP: In **Fig. 4**, pre-treatment of endothelium-intact aortic rings with the Ca^{2+} -Calmodulin binding to NOS blocker: calmidazolium chloride (10^{-3}M; n=6), or the guanylyl cyclase inhibitor: ODQ (10^{-5}M; n=6), or the competitive cGMP-dependent protein kinase G (PKG) inhibitor: Rp-8-Br-PET-cGMP (3.10^{-6} M; n=6) abolished RpAE-induced vasorelaxation. The maximum contraction values were 16.09 ± 1.67%, 6.42 ± 1.81%, and 17.36 ± 2.02% respectively. These findings suggest that RpAE induces vasorelaxation *via* NO-cGMP-PKG signaling.

Role of K^+ Channels in RpAE-Induced Vascular Response: As illustrated in **Fig. 5**, pre-treatment with the voltage-dependent K^+ channel blocker: 4-AP (10^{-4}M; n=6), or the non-specific ATP-sensitive K^+ channel blocker: glibenclamide (10^{-5}M; n=6), or the selective inwardly rectifying potassium channel blocker: $BaCl_2$ (10^{-4}M; n=6), neither of them had a significant influence on the RpAE-induced response. The maximal values were 78.65 ± 1.6%, 79.82 ± 1.65%, and 79.72 ± 1.29% respectively. However, pre-treatment with the Ca^{2+}-activated potassium channels blocker: TEA (10^{-3}M; n=6) annulled the RpAE-induced response: 0.39 ± 1.88 %; p<0.001.

Vasorelaxant Effect of RpAE in the Presence of Thapsigargin, or Verapamil: In **Fig. 6**, the vasorelaxant effect of a single dose 0.1mg/ml of RpAE was abolished after exposing aorta to the blocker of VOCC channel: Verapamil and to the inhibitor of SERCA: Thapsigargin with the percentage of vasocontraction of 43.24 ± 3.85%; p<0.001 and 19.59 ± 2.17%; p<0.001 respectively.

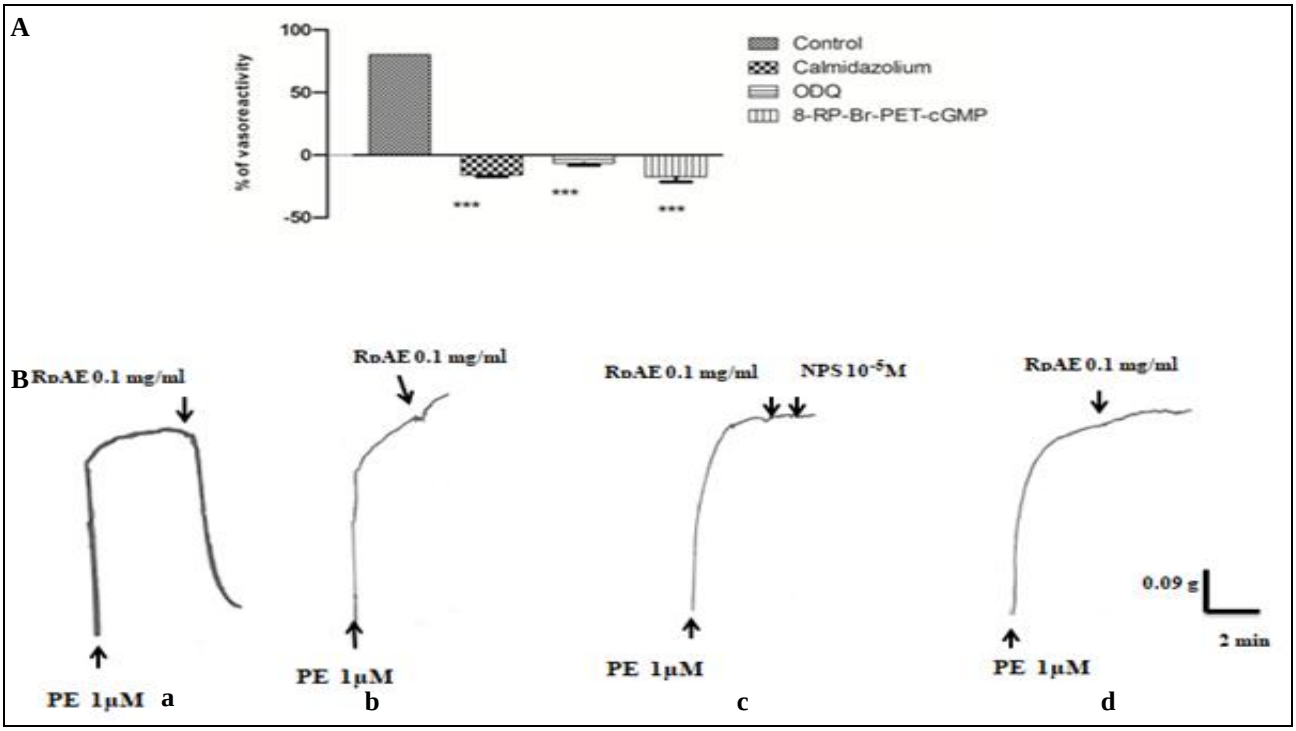

FIG. 4: (A) EFFECT OF A SINGLE DOSE 0.1mg/ml OF RpAE ON INTACT THORACIC AORTIC RINGS ISOLATED FROM NORMOTENSIVE RATS AND PRE-CONTRACTED WITH PE (10^{-6} M) AND IN THE PRESENCE OF CALMIDAZOLIUM, ODQ, AND 8-RP-Br-PET-cGMP. (B) ORIGINAL TRACING SHOWING THE EFFECT OF RpAE ON PE PRE-CONTRACTED INTACT THORACIC AORTIC RINGS (10^{-6} M) (a), IN THE PRESENCE OF CALMIDAZOLIUM (b), ODQ (c), AND 8-RP-Br-PET-cGMP (d). Values are mean ± SEM, n=6. One way ANOVA followed by Bonferroni's post-hoc test; *** p<0.001 *vs*. control.

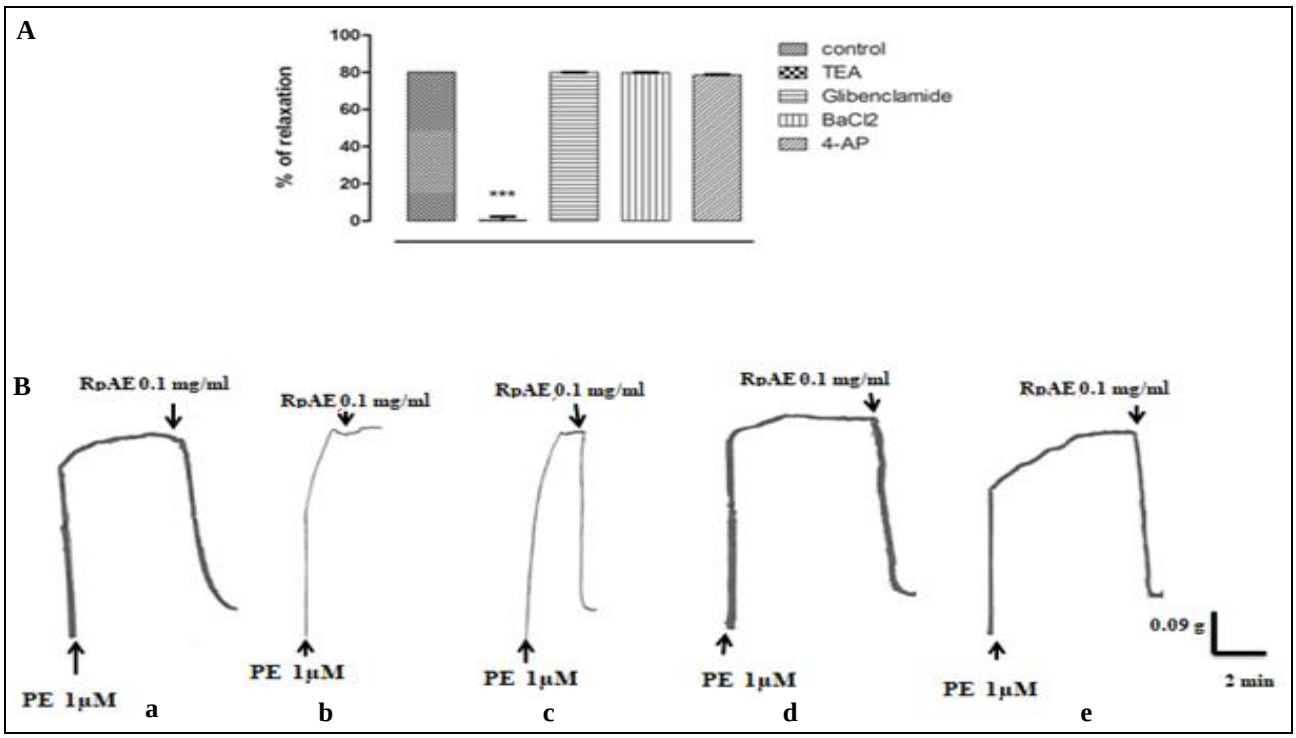

FIG. 5: (A) VASORELAXANT EFFECT OF A SINGLE DOSE 0.1mg/ml OF RpAE ON INTACT THORACIC AORTIC RINGS ISOLATED FROM NORMOTENSIVE RATS AND PRE-CONTRACTED WITH PE (10^{-6} M) AND IN THE PRESENCE OF TEA, GLIBENCLAMIDE, $BaCl_2$, AND 4-AP. (B) ORIGINAL TRACING SHOWING THE EFFECT OF RpAE ON PE PRE-CONTRACTED INTACT THORACIC AORTIC RINGS (10^{-6} M) (a), IN THE PRESENCE OF TEA (b), GLIBENCLAMIDE (c), $BaCl_2$(d), AND 4-AP (e). Values are mean ± SEM, n=6. One way ANOVA followed by Bonferroni's post-hoc test; ***p<0.001 *vs.* control.

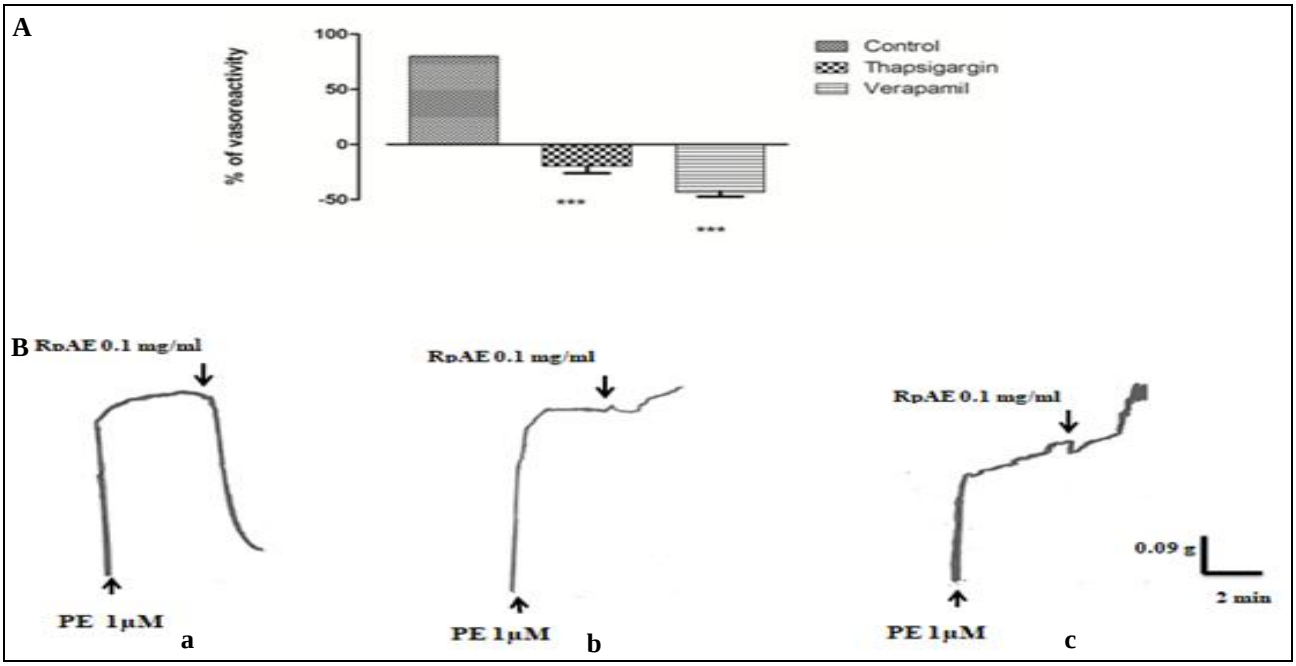

FIG. 6: (A) EFFECT OF A SINGLE DOSE 0.1mg/ml OF RpAE ON INTACT THORACIC AORTIC RINGS ISOLATED FROM NORMOTENSIVE RATS AND PRE-CONTRACTED WITH PE (10^{-6} M) AND IN THE PRESENCE OF THAPSIGARGIN AND VERAPAMIL. (B) ORIGINAL TRACING SHOWING THE EFFECT OF RpAE ON PE PRE-CONTRACTED INTACT THORACIC AORTA (10^{-6} M) (a), IN THE PRESENCE OF THAPSIGARGIN (b) AND VERAPAMIL (c). Values are mean ± SEM, n=6. One way ANOVA followed by Bonferroni's post-hoc test; *** p<0.001 *vs.* control.

Antihypertensive Effect:

Effect of RpAE on L-NAME Hypertensive Rats

SBP: The treatment of the adult Wistar rats with RpAE (50 mg/kg/day and 150 mg/kg/day) did not affect the body weight of the rats. As illustrated in **Fig. 7**, oral administration of L-NAME (32 mg/Kg/day) showed a progressive increment in SBP, which became significant from the first week. Effectively, SBP increased from 115 ± 1.82 mmHg to 170 ± 1.82 mmHg compared to the control group, which indicate the installation of arterial hypertension.

Enalapril is an ACE inhibitor, significantly prevents the elevation of SBP, which remained constant at 131±1.05 mmHg; p<0.001 after four weeks of (L-NAME 32 mg/kg/day + Enalapril 15 mg/kg/day) treatment. The administration during the same period and conditions with (RpAE 50 mg/kg/day + L-NAME 32 mg/kg/day) and (RpAE 150 mg/kg/day + L-NAME 32 mg/kg/day) denoted a significant and persistent reduction of blood pressure all along the treatment to a normotensive level at the end of the treatment. Indeed, SBP remained constant at around 114.1 ± 1.53 mmHg; p<0.001 and 122.5 ± 2.14 mmHg; p<0.001 respectively following four weeks of treatment.

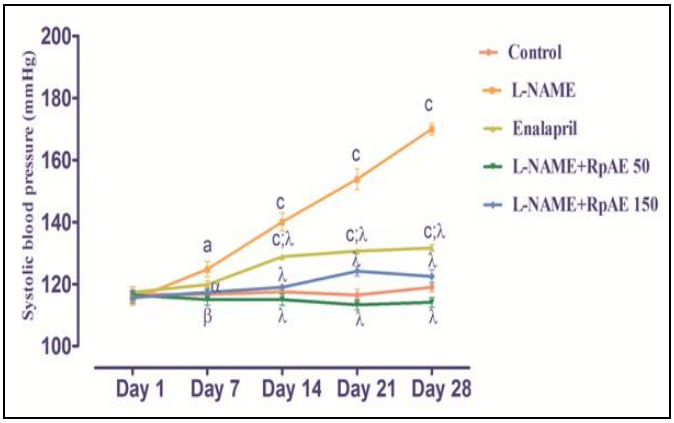

FIG. 7: EFFECT OF FOUR-WEEK ORAL TREATMENT ON SBP OF RATS FED WITH L-NAME AT 32 mg/kg/day (L-NAME GROUP), ENALAPRIL AT 15 mg/kg/day (L-NAME + ENALAPRIL GROUP), AND RpAE AT 50 mg/Kg/day (L-NAME + RpAE 50 GROUP), AND AT 150 mg/Kg/day (LNAME + RpAE 150 GROUP). Data are mean ± SEM (n=6 rats per group). Two way ANOVA followed by Bonferroni's post-hoc test; a: p<0.05; c: p<0.001 *vs.* control group; α: p<0.05; β: p<0.01; λ: p<0.001 *vs.* L-NAME group.

Effect of Different Treatments on Vascular Sensitivity to Phenylephrine and Carbachol: To test the effect of different group on carbachol-induced relaxation, endothelium-intact aorta from each group (n=6) was mounted in an organ bath containing Krebs solution continuously bubbled

with 95% O_2–5% CO_2. When the contraction with PE (1µM) reached a plateau, concentration-response curves to increasing concentrations (10^{-9} to 10^{-4}M) of CCH were constructed in a cumulative manner. Relaxation data were expressed as a percentage of the maximal Phenylephrine-induced contraction.

As shown in **Fig. 8**, the relaxation induced by the cumulative concentration of CCH (expressed as % of the maximal contractile response to PE) was significantly declined in aortic rings from the group treated by L-NAME only (the maximal relaxation Rmax = 33.72 ± 0.6 %; p<0.001 *vs*. 97.59 ± 0.01% in control rats). Aortic rings exposed to a cumulative concentration of CCH showed an increased vasorelaxation in (L-NAME 32 mg/kg/day + Enalapril 15 mg/kg/day) group; the maximal relaxant effect was 61.22 ± 3%; p<0.001 *vs*. L-NAME group.

The deterioration in vascular response caused by L-NAME was significantly enhanced by RpAE. Certainly, 91.1 ± 1% and 96.33 ± 1.02%; p<0.001 were the maximal relaxant effects in (RpAE 50 mg/kg/day + L-NAME 32 mg/kg/day) and (RpAE 150 mg/kg/day + L-NAME 32 mg/kg/day*)* groups respectively *vs*. L-NAME group suggesting that RpAE improved the vascular damage caused by L-NAME.

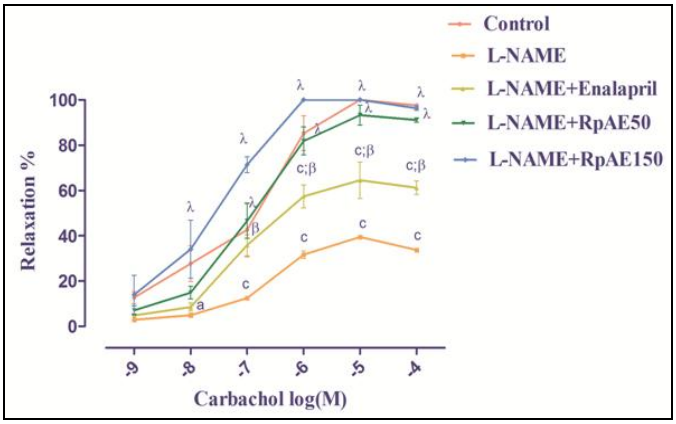

FIG. 8: VASORELAXATION INDUCED BY CARBACHOL IN CONTROL (TAP WATER), L-NAME (L-NAME AT 32 mg/kg/day), L-NAME + ENALAPRIL (L-NAME AT 32 mg/kg/day AND ENALPRIL AT 15 mg/kg/day), L-NAME + RpAE 50 (L-NAME AT 32 mg/kg/day AND RpAE AT 50 mg/kg/day) AND L-NAME + RpAE150 (L-NAME AT 32 mg/kg/day AND RpAE AT 150 mg/kg/day) GROUPS. Data are mean ± SEM (n=6 rats per group). Two way ANOVA followed by Bonferroni's post-hoc test; a: p<0.05; c: p<0.001 *vs*. control group; α: p<0.05; β: p<0.01; λ: p<0.001 *vs*. L-NAME group.

Another array of experiments based on changes in developed tension in responses to increasing concentrations (10^{-9} to 10^{-4} M) of PE have also been carried out. As shown in **Fig. 9**, the maximal contractile response of aorta to Phenylephrine was attenuated in L-NAME group (Cmax = 0.75 ± 0.09g) compared to control group (Cmax = 1.27 ± 0.12g). Enalapril ameliorates the response of aorta to PE (Cmax = 1.06 ± 1.03g). This parameter was normalized by RpAE. Which means that the maximal contractile response to Phenylephrine was: Cmax = 1.22 ± 0.01g and Cmax = 1.26 ± 0.03g in (RpAE 50 mg/kg/day + L-NAME 32 mg/kg/day) and (RpAE 150 mg/kg/day + L-NAME 32 mg/kg/day) group respectively.

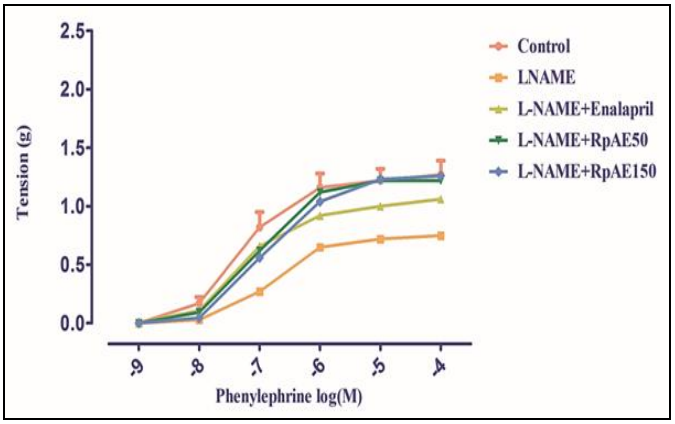

FIG. 9: CONTRACTION INDUCED BY PHENYL-EPHRINE IN CONTROL (TAP WATER), L-NAME (L-NAME AT 32 mg/kg/day), L-NAME + ENALAPRIL (L-NAME AT 32 mg/kg/day and ENALPRIL AT 15 mg/kg/day), L-NAME + RpAE 50 (L-NAME AT 32 mg/kg/day AND RpAE AT 50 mg/kg/day) AND L-NAME + RpAE150 (L-NAME AT 32 mg/kg/day AND RpAE AT 150 mg/kg/day) GROUPS. Data are mean ± SEM (n=6 rats per group). Two way ANOVA followed by Bonferroni's post-hoc test.

Effect of Different Treatment on Urinary Volume: Before any treatment (Day 0), diuresis is of the same magnitude among the different groups. Four weeks of treatment with L-NAME alone lowered urinary volume; however, this parameter was augmented by Enalapril in association with L-NAME (8 ± 1.27 ml/24h on day 30). Diuresis remained constant at around 5.16 ml/24h after 30 days of treatment in (RpAE 50 mg/kg/day + L-NAME 32 mg/kg/day) group. The urinary volume was significantly increased in (RpAE 150 mg/kg/day + L-NAME 32 mg/kg/day) group (10.41 ± 4.96 ml / 24 h, p<0.01) compared to L-NAME group and control group.

TABLE 1: EFFECT OF 4 WEEK ORAL TREATMENT WITH TAP WATER (CONTROL GROUP) OR L-NAME ALONE AT 32 mg/kg/day (L-NAME GROUP) OR ASSOCIATED WITH RpAE AT 50 mg/kg/day (L-NAME + RpAE 50 GROUP), RpAE AT 150 mg/kg/day (L-NAME + RpAE 150 mg/kg/day GROUP) OR WITH ENALAPRIL AT 15 mg/kg/day (L-NAME + ENALAPRIL GROUP) ON DIURESIS (ml/24h)

	Control	L-NAME			
Diuresis (ml/ 24h)		**alone**	**+Enalapril**	**+RpAE 50**	**+RpAE 150**
Day 0	4.66 ± 0.49	5 ± 0.43	4.54 ± 0.69	5.5 ± 0.4	5.5 ± 0.6
Day 15	3.66 ± 0.33	3.66 ± 0.54	4.16 ± 0.69	4.08 ± 0.37	4.75 ± 0.61
Day30	3.83 ± 0.33	3.66 ± 0.35	8 ± 1.27	5.16 ± 0.77	$10.41 \pm 4.96^{\beta b}$

Data are mean ± SEM (n=6 rats per group). Two way ANOVA followed by Bonferroni's post-hoc test, β: $p<0.01$ *vs*. L-NAME group; b: $p<0.01$ *vs*. Control group.

DISCUSSION: Modern medicine has benefited considerably from traditional medicine in searching for novel drugs in combination with new technology [31]. This study was conducted to determine the toxicological profile of the aqueous extract of *Rhus pentaphylla* (Jacq.) Desf. (RpAE) collected from Oriental Morocco by performing acute oral toxicity in mice, to clarify the effects of the aqueous extract, and the mechanism by which RpAE elicits vascular actions on isolated rat aorta. The antihypertensive effect of the extract given orally by gavage to L-NAME induced hypertensive rats over four weeks was also investigated. To confirm the safety of RpAE, the extract was tested for it oral acute toxicity; thus, the median lethal dose (LD_{50}) was greater than 10 g/Kg, also revealing a positive safety profile clarified by the absence of any behavioral disorders during the 2 weeks following the administration.

Results showed that RpAE had a dose dependent effect on isolated rat aorta. Certainly, it induced both vasorelaxation and vasoconstriction in PE-evoked vasoconstriction in endothelium-intact aorta rings of normotensive rats; thus, showing for the first time its effect on smooth vascular vessels. Therefore, RpAE relaxes vascular smooth muscle via endothelium-dependent NO-PKG signaling through activation of Ca^{2+}-calmodulin-eNOS-sGC-cGMP-PKG. Furthermore, it was demonstrated that muscarinic receptors are implicated in RpAE induced vasorelaxation. The activation of K^+ channels, especially, Ca^{2+}- activated K^+ channels (K_{Ca}^{2+}), activation of sarco-endoplasmic reticulum Ca^{2+}-ATPase pump (SERCA) and inhibition of L-type Ca^{2+} channels (VOC) are also involved.

The regulation of the vascular tone is principally intended for the convenient control of blood pressure [32]. It has been revealed that an endothelium dysfunction is related to hypertension [33] besides other cardiovascular illnesses [34, 35]. Endothelial cells (ECs) are an inward modulator for the control of vascular homeostasis through synthesizing and releasing a set of vasoconstrictor factors such as endothelin and thromboxane in addition to vasodilator factors such as endothelium-derived relaxing factors (EDRFs), nitric oxide (NO) and prostacyclin (PGI_2) [36]. A drastic role is played by a balance between vasodilators and vasoconstrictors in the control of blood flow and pressure in healthy persons and patients with cardiovascular diseases. A wide range of vascular actions are evoked by a biological signaling molecule: nitric oxide (NO); essentially, vasorelaxation [37], anti-inflammatory activity [38], antiplatelet activity [39], and its effect to counteract malaria infection [40].

NO is formed in endothelium by endothelial nitric oxide synthase through the reaction of oxygen and L-arginine; then, it diffuses into the vascular smooth muscle whither it will exert its vasorelaxant effect by stimulating soluble guanylate cyclase (sGC) to catalyze the process of the conversion of guanosine triphosphate (GTP) to the intracellular second messenger cyclic 3', 5'-guanosine monophosphate (cGMP) [41, 42, 43]. The increased cGMP concentration that turns cGMP-dependent protein kinase (PKG) on, mediates variety of physiological mechanism events in vascular smooth muscle cells (VSMC), thus decreases the intracellular Ca^{2+} levels, causing membrane hyperpolarization, and inhibition of myosin light chain phosphorylation, producing vasorelaxation, and thereby decreasing blood pressure [41, 42, 44].

In this study, RpAE elicited potent relaxation in endothelium-intact aortic rings from normotensive rats pre-contracted with phenylephrine. This effect was related to the production of endothelium-derived vasodilators since the removal of

endothelium abolished the relaxant effect of RpAE. Thus, L-NAME (NOS inhibitor), hydroxocobalamin (NO scavenger), and indomethacin (the non-selective COX inhibitor) were employed to examine the possible involvement of NO and prostaglandins. The results showed that RpAE-induced vasorelaxation was inhibited by L-NAME and hydroxocobalamin but not by indomethacin, which indicated that the NO system might be implicated and which suggested that PGI_2, a major vasodilator cyclooxygenase (COX) product, would not take part in the vasorelaxation induced by the RpAE. We also found that pre-incubation of endothelium-intact aortic rings with calmidazolium (a Ca^{2+}- Calmodulin binding to NOS blocker), or ODQ (the GC inhibitor), or with Rp-8-Br-PET-cGMP (the competitive cGMP-dependent protein kinase G (PKG) inhibitor) eliminated the vasorelaxant effect. These findings revealed that the vascular relaxation evoked by RpAE was mediated by the endothelium and vascular smooth muscle system *via* Ca^{2+}-calmodulin complex-NO-sGC-cGMP-PKG signaling pathway.

Also, the current study showed that pre-treatment of aortic rings with atropine (muscarinic receptor antagonist) annulled RpAE-induced relaxation. In common, the muscarinic vasorelaxation is chiefly intervened across NO production in endothelial cells resulting in the activation of endothelial nitric oxide synthase (eNOS) yielded by Ca^{2+}-calmodulin complex [42, 45, 46]. Thereby we suspect that the muscarinic receptor activation is one of the mechanisms responsible for the vasoactive properties of RpAE that may contain one or more compounds acting as agonists of muscarinic receptors on endothelial cells.

K^+ channels evenly play crucial roles in the management of vascular tone [47]. Many vascular bioactive agents and drugs produce their vasodilator or vasoconstrictor effects through unlocking or closing K^+ channels [47]. The K^+ channels exist in blood vessels indirectly regulate vascular tension *via* changing the membrane potential of vascular smooth muscle in rest thereby; modulate the relaxant response of blood vessel [47]. Four categories of potassium channels are found in arterial smooth muscle: Ca^{2+}-activated K^+ channels (K_{Ca}^{2+}), voltage-dependent K^+ channels (K_V), ATP-sensitive K^+ channels (K_{ATP}), and inward rectifier K^+ channels (K_{ir}) [47]. Our study demonstrated that RpAE-induced relaxation of the endothelium-intact aortic ring was abolished by TEA, the Ca^{2+}-activated potassium channels blocker. It has been reported that the K_{Ca}^{2+} are directly activated by NO contributing to hyperpolarization by extrusion of K^+ and leading to vasorelaxation due to a drop in intracellular Ca^{2+} level [48, 49]. Pre-treatment with 4-AP the voltage-dependent K^+ channel blocker, or glibenclamide the non-specific ATP-sensitive K^+ channel blocker, or $BaCl_2$ the selective inwardly rectifying potassium channel blocker, did not influence the RpAE-induced response though. Therefore, we suspected that one or more components in RpAE would be acting as K_{Ca}^{2+} channel operators.

Contraction and dilation of blood vessels as feedback to the physiological requirement is controlled by changes in cytosolic Ca^{2+} levels in vascular smooth muscle (VSM) [50]. The Ca^{2+} used for muscle contraction includes intracellular and extracellular sources [50]. Ca^{2+} ions occur in four different compartments: extracellular, cytoplasmic, mitochondrial, and non-mitochondrial (sarcoplasmic reticulum) [50]. The sarcoplasmic reticulum is the primary store of intracellular Ca^{2+} ions [50]. Experiments conducted with the thapsigargin; the SERCA (sarco/endoplasmic reticulum Ca^{2+}-ATPase) inhibitor demonstrate that uptake of Ca^{2+} by the sarcoplasmic reticulum is required for RpAE-induced relaxation because this relaxation was abolished in the presence of thapsigargin.

The Ca^{2+} levels are important to activate NO-sGC-cGMP signaling and vasorelaxation. A prior study has shown that the SOCE (store-operated Ca^{2+} entry) affects the eNOS activation and vasorelaxation [51]. The movement of Ca^{2+} from the cytoplasm to intracellular stores is achieved by SERCA pumps, and in this way, cytosolic calcium levels return to baseline after a contraction [52]. Previously, it has been pointed out that SOCE is inhibited by cGMP *via* a protein kinase G-dependent mechanism by phosphorylating SERCA pumps irreversibly in vascular endothelial cells [54]. The cytosolic Ca^{2+} concentration is controlled by both extracellular Ca^{2+} influx and Ca^{2+} release from intracellular stock [52]. Voltage-dependent Ca^{2+} channels (VOC), also known as L-type Ca^{2+} channels and receptor-operated Ca^{2+} channels

(ROC) situated in the plasma membrane deal the setting of Ca^{2+} influx [52]. Based on our results, we believed that RpAE inhibited the Ca^{2+} influx through VOC since the RpAE induced vasorelaxation vanished after pre-incubation of endothelium-intact aortic rings with verapamil the Ca^{2+} channel type VOC inhibitor.

It has been reported that PKG inhibits Ca^{2+}influx, augments Ca^{2+} sequestration, and decrease the responsiveness of contractile elements to Ca^{2+} [53]. It seems distinctly possible that emphasis of endothelial NO output by RpAE inhibits Ca^{2+} entry of vascular smooth muscle cells *via* sGC–cGMP signaling for the reason that Ca^{2+} entry *via* L-type Ca^{2+} channels is under the potency of cGMP-PKG signaling pathway [54], thereby guiding to a fall in intracellular Ca^{2+} level and hence cause relaxation.

The L-NAME hypertensive rat model is one of the rat models used for mimicking the role of NO in the pathogenesis of high blood pressure. L-NAME which is a non-selective inhibitor of NO synthase inhibits the NO synthase isoforms, cause endothelial dysfunction, and a significant increase in arterial blood pressure [55, 56, 57]. In addition to that, we investigated the antihypertensive action of RpAE on L-NAME induced hypertensive rats.

The results demonstrate that the treatment of rats with RpAE at doses 50 and 150 mg/kg/day, co-administrated with L-NAME, prevent significantly the rise in SBP induced by L-NAME and, both of them apply about the same level of preventive action. This is the first time that such beneficial effects of *Rhus pentaphylla* are reported.

The kidney plays a chief role in the regulation of the body salt and water balance and any disturbed regulation of renal functions could distort this balance in pathophysiological states including hypertension [58, 59]. As previously reported, chronic inhibition of NOS by L-NAME bring out a decrease of diuresis [60]. Furthermore, an improvement of the diuresis has been revealed by RpAE at 150 mg/kg/day. It is possible that such beneficial effects of RpAE on renal function are mediated through increasing NO synthesis. Indeed several studies have demonstrated that endogen NO or NO released from NO donors, inhibits some transporters in renal tubular epithelial cells [61].

In addition, an evident lack of vasorelaxation in response to CCH and an impairment reaction of the aorta to PE were correlated with chronic L-NAME hypertension and which may translate the deficiency of NO synthesis, giving rise to increased vasoconstrictor factors. However, the association of RpAE with L-NAME improved a full amelioration of vascular reactivity. Therefore, the anti-hypertensive effect of RpAE *via* enhancement of the NO system is likely involved.

CONCLUSION: Considering all the above results and discussions, the present finding proves the vasorelaxant and the antihypertensive effects of RpAE. Our results suggest that RpAE induces dose-dependent effect (both relaxation and contraction) in rat aortic rings. The vasorelaxant effect is through endothelium-dependent signaling through activation of muscarinic receptors and implication of Calmodulin/eNOS/sGC/cGMP/ PKG signaling pathway involving the opening of K_{Ca}^{2+}, activation of SERCA pump, and inhibition of VOC channels. Additionally, this vasorelaxant effect is translated *in-vivo* into a significant antihypertensive effect, a high-ameliorated vascular reactivity and diuresis in L-NAME induced hypertensive rats.

Further, cardiovascular studies are needed to better clarify these beneficial effects of *Rhus pentaphylla* extract, especially on the heart and renal system. Moreover, phytochemical studies are necessary to determine and isolate the bioactive components accountable for these observed effects.

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CONFLICT OF INTEREST: None

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