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RESEARCH ARTICLE

Antihypertensive and vasorelaxant effects of *Salvia tingitana* in rats

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Abstract

Salvia tingitana (S. tingitana) is a medicinal and aromatic plant that belongs to the Lamiaceae family with several medicinal properties. Secondary metabolites, including flavonoids, polyphenols and tannins, were identified through phytochemical analysis of the aerial parts of *S. tingitana*. In addition, pharmacological investigation revealed this plants' antihyperglycemic and antihyperlipidemic activities. This study aimed to investigate the antihypertensive and vasorelaxant activities of the aqueous extract of *S. tingitana* (AEST) in normal and L-NAME-induced hypertensive rats by assessing its effects on heart rate (HR), systolic blood pressure (SBP), mean blood pressure (MBP) and diastolic blood pressure (DBP). The study further investigated the vasorelaxant effect of AEST on isolated rat thoracic aorta and elucidated the underlying mechanisms. As a result, AEST significantly lowered SBP after single and repeated oral administration in normal rats without affecting MBP, DBP and heart rate. Interestingly, after repeated oral AEST administration, arterial blood pressure parameters (SABP, DBP and MBP) significantly decreased in hypertensive rats. The vasorelaxation effect induced by AEST in aortic rings was mediated through numerous pathways involving calcium channels, nitric oxide synthase, muscarinic receptors and inward-rectifying potassium channels. In contrast, the pretreatment with methylene blue, glibenclamide, indomethacin and propranolol did not significantly alter AEST-induced vasorelaxation. The study showed that AEST exhibits a significant antihypertensive effect in L-NAME-rats and exhibits vasorelaxant properties in rat aortic rings via mechanisms involving calcium channels, nitric oxide synthase, muscarinic receptors and inward-rectifying K^+ Channels. Interestingly, many further studies on the structure-activity relationship of *S. tingitana* are needed to explain the mechanisms of action that have been revealed.

Keywords: antihypertensive effect; hypertension; *Salvia tingitana*; vasorelaxant effect

Introduction

Globally, cardiovascular illnesses are the major reasons of death. Hypertension (high blood pressure) is a major cause of cardiovascular ailments (1). Interestingly, hypertension is classified as a s'ilent killer' and is primarily identified through screening programs and blood pressure monitoring (2). Hypertension is diagnosed when a patients' systolic and diastolic blood pressures exceed 140 mmHg and 90 mm Hg, respectively (3). Concerning the treatment, medicinal plants have a long history in the treatment and control of this ailment; especially the use of modern medication available for treating hypertension can present various side effects (4).

Lamiaceae are a group of herbal remedies traditionally used for centuries in conventional medicine to manage cardiovascular illnesses. In addition to essential oils, these herbs make extracts and isolates such as polyphenols, phenolic compounds, terpenes, iridoids and terpenes (5). Correspondingly, genus Salvia is one of the essential genera of the Lamiaceae family, which has important effects in controlling hypertension. Indeed, *salvia aucheri* revealed a potent antihypertensive and vasorelaxant activity mediated through nitric oxide and NO-cyclic guanosine monophosphate pathways (6). Other pharmacological studies have revealed that genus *Salvia* reduces blood pressure through inhibiting oxidative stress, inflammation and fibrosis (7).

Originally from the Middle East and North Africa, *S. tingitana* is a medicinal and aromatic plant of the Lamiaceae family (8). Phytochemical analysis of *S. tingitana* aerial parts revealed the presence of enormous secondary metabolites such as a labdanes, nor-sesterterpenoid, sesterterpenoids, sesquiterpenoid, flavonoids, diterpenoid, polyphenols and tannins (9, 10). Importantly, we have previously revealed that aqueous extract of this plant exhibits a remarkable diminution effect of plasma glucose values after treating diabetic and normal rats. Furthermore, the administration of this aqueous extract revealed a potent amelioration in lipoprotein and lipid profiles (10). In addition to antidiabetic and antidyslipidemic abilities, *S. tingitana* aqueous extract (AEST) is a potent antimicrobial agent

against important Gram-positive human pathogens (11). Although so far, until now no pharmacological work was carried out to reveal the antihypertensive and vasorelaxant effect of *S. tingitana*. Hence, the main goal of this work was to display the effect of AEST in L-NAME (L-NG-Nitro arginine methyl ester) model hypertensive and normal rats, as well as to discover the underlying mechanisms involved in vasodilation through *ex vivo* vascular reactivity experiments using isolated rat aortic rings.

Materials and Methods

Plant material

In March 2021, *S. tingitana* was purchased fresh from a local market in Moroccos' Tafilalet region (semi-arid region). A voucher specimen of the plant was deposited in Errachidias' Faculty of Sciences and Techniques herbarium after being taxonomically identified and authenticated (ST 25/35).

Preparation of the aqueous

Based to the conventional Moroccan approach, the decoction was used to prepare the *S. tingitana* aqueous extract (12). 4 grams of *S. tingitana* powder were boiled in 400 mL of distilled water for 10 minutes and then cooled for 20 minutes. A Millipore filter (Millipore 0.2 mm, St Quentin en Yvelines, France) was then used to remove particulate matter from the aqueous extract. After the filtration of extract, the last was lyophilized in a freeze dryer (Labcono, Boyer, Casablanca, Morocco) (13). In this work, a preliminary dose screening was carried out, a series of doses (10, 20, 40, 60 and 80 mg/kg) were tested to determine the appropriate dose that will be used. Each dose was administered to three rats. The dose of 80 mg/kg was identified as the minimum dose which in this case would induce significant antihypertensive activity.

Experimental animals

In Morocco, healthy albino male rats were obtained from the Experimental Center of Missour. The weight of rats ranges between 140 and 240 g (6 to 8 weeks old). Rats were housed in individual polyethene cages under conventional experimental conditions and provided with ad libitum consumption of a standard pellet diet (14). All of the tests were carried out following local ethics guidelines (FSTE/2015) after a three-week acclimation period to alleviate shipping stress.

Antihypertensive activity

At a dose of 60 mg/kg L-NAME body weight (b.w) is used in this study to induce hypertension in male rats by repeated oral administration (during 21 days). Distilled water was administered in this study concerning control group. L-NAME hypertensive and normal rats were randomly divided into three groups, each containing six rats. The first group received the aqueous extract of aerial parts of AEST (80 mg/kg b.w). The second group received lasilix (furosemide; 20 mg/kg b.w) and the last received distilled water (control group). Concerning single oral administration (six hours), AELP, lasilix and distilled water were administered orally and the heart rate (HR) and blood pressure parameters (DBP (diastolic blood pressure), SBP (systolic blood pressure) and MBP (mean blood pressure)) were followed after six hours of treatment. Identically, AELP, lasilix and distilled water were administered orally every day for 1 week and SBP, DBP, MBP and HR were detected. The measurements were taken throughout a period of repeated oral administration. To prevent immobilization stress induction, the rats were anaesthetized *via* the respiratory route with ether (50%) for approximately 2 mins and blood pressure was estimated as previously described (15).

Evaluation of vascular dilatation

The vascular relaxation measurement and mechanism evaluation were performed as described (16).

Statistical analysis

Following the blinded collection and data analysis, the statistical significance of data was tested between three groups using two-way ANOVA with Prism version 8 (Graph Pad SoftareInc, San Diego, CA, USA) followed by the Bonferroni multiple comparisons tests. The probability of $p<0.05$ established the significance of differences. Data were presented as mean ± SEM.

Results

Body weight changes after the administration of AEST

The study revealed that AEST administration at a dose of 80 mg/kg had no observable effect on b.w in any of the experimental groups during the subchronic study. (Data not shown).

Acute oral administration

Oral L-NAME administration revealed an augmentation effect of all the blood pressure parameters, including SBP, DBP and MBP, compared to baseline value. The impact of AEST on heart rate and blood pressure parameters in hypertensive and normal rats are presented in Table 1. In L-NAME rats, AEST induced a considerable reduction effect of SBP ($p<0.001$), DBP ($p<0.05$) and MBP ($p<0.001$) after six hours of administration. In the furosemide-treated rats, a considerable lowering effect of SBP ($p<0.05$), DBP ($p<0.05$) and MBP ($p<0.01$) was observed. However, the heart rate remained unchanged after six hours of oral furosemide administration compared to the baseline value. Nevertheless, there is no significant variation in heart rate after the same treatment period by AEST and standard medicament. Similarly, the control group also showed no changes in heart rate or arterial blood pressure parameters.

Repeated oral administration

Table 2 summarizes the effects of AEST on heart rate and SBP, MBP and DBP in normal and hypertensive rats after a seven-day treatment period. In this test, AEST (80 mg/kg) was administered orally to anaesthetized hypertensive rats over seven days.

In hypertensive rats, the finding showed that AEST produced a remarkable diminution of SBP and DBP at the fourth ($p<0.05$) and seventh ($p<0.0001$) days of administration. AEST provoked a significant lowering effect of MBP on the seventh ($p<0.0001$) day of oral administration. Still, no variation was recorded in the rats treated with the same extract concerning heart rate. The subchronic experiment demonstrated a substantial reduction of DBP and

Table 1. Effect of single oral administration (acute test) of AEST (80 mg/kg) on systolic, mean, diastolic arterial blood pressure (mm Hg) and heart rate (bmp) in normotensive and L-NAME hypertensive animals

Groups		Systolic blood pressure (mmHg)		
		Control	**AEST**	**Furosemide**
Normal	T_0	132±6	122.75±3.4	120±7
	T_6	138± 8	95±7.57*	111±7
L-NAME	T_0	180±5	175.5±5.91	169 ± 7
	T_6	175 ±7	135.25±5.80***	142±6*
		Mean blood pressure (mmHg)		
		Control	**AEST80mg/kg**	**Furosemide**
Normal	T_0	115± 7	92±6.3	108±6
	T_6	113±6	79.8±6.09	97±2
L-NAME	T_0	156 ±6	153.87±5.37	150±3
	T_6	157±6	121.5±4.36***	125±6**
		Diastolic blood pressure (mmHg)		
		Control	**AEST80mg/kg**	**Furosemide**
Normal	T_0	106±5	76.6±7.96	103 ±5
	T_6	101±6	72.25±5.78	91 ±4
L-NAME	T_0	144±7	142.9 ±5.36	152 ±5
	T_6	148±10	114.65±3.81*	129 ±4*
		Heart rate (bpm)		
		Control	**AEST**	**Furosemide**
Normal	T_0	320 ±10	332±3	349 ±19
	T_6	306 ±8	322±5	321 ±9
L-NAME	T_0	312 ±15	334±1	348 ±22
	T_6	318 ±12	349±8	311 ±17

Data are expressed as Mean±SE, **n**=6. *p<0.05, **p<0.01, ***p<0.001 when compared to the baseline value (T_0). **T**: time (hour).

Table 2. Effect of repeated oral administration (chronic test) of AEPG (120 mg/kg) on systolic, mean, diastolic arterial blood pressure (mm Hg) and heart rate (bmp) in normotensive and L-NAME hypertensive rats

		Systolic blood pressure (mm Hg)		
		Control	**AEST**	**Furosemide**
Normal	D_0	132 ± 6	122.75±3.4	120 ± 7
	D_2	136 ± 5	128.25±1.1	115 ± 5
	D_4	140 ± 3	97.5±4.75	108 ± 3
	D_7	135 ± 6	92.25± 6.22 *	99 ± 4**
L-NAME	D_0	180 ± 5	175.5±5.91	169 ± 6
	D_2	176 ± 5	154.25±10.29	122 ± 2****
	D_4	173 ± 9	149.75±4.75*	120 ± 4****
	D_7	172 ± 6	130±3.97****	109 ± 3****
		Mean blood pressure (mm Hg)		
		Control	**AEST**	**Furosemide**
Normal	D0	115 ± 7	92±6.37	108 ± 6
	D2	112 ± 5	113.25±2.32	107 ± 5
	D4	117 ± 6	81.25±6.92	101 ± 3
	D7	112 ± 4	78.25±7.25	96 ± 2
L-NAME	D0	156 ± 6	153.87±5.37	150 ± 3
	D2	149 ± 6	141±9.74	148 ± 5
	D4	148 ± 9	130±5.61	103 ± 5****
	D7	147 ± 6	111±5.01****	95 ± 4****
		Diastolic blood pressure (mm Hg)		
		Control	**AEST**	**Furosemide**
Normal	D0	106 ± 5	76.62±7.96	103 ± 5
	D2	101 ± 5	105.75±2.93	98 ± 5
	D4	106 ± 3	73±3.71	97 ± 6
	D7	102 ± 8	99.75±1.65	95 ± 4
L-NAME	D0	144 ± 7	142.9 ±5.36	152 ± 5
	D2	135 ± 7	134±9.57	103 ± 4****
	D4	135 ± 6	120±6.61*	100 ± 4****
	D7	136 ± 6	94±5.63****	98 ± 3****
		Heart rate (bpm)		
		Control	**AEST 80 mg/kg**	**Furosemide**
Normal	D0	320 ± 10	312±5	349 ± 19
	D2	320 ± 14	299±6	341 ± 16
	D4	312 ± 16	305±8	341 ± 17
	D7	319 ± 8	302±4	305 ± 8*
L-NAME	D0	312 ± 15	315±5	348 ± 22
	D2	310 ± 15	317±6	343 ± 13
	D4	307 ± 13	324±4	315.00 ± 11
	D7	318 ± 18	298±7	311.00 ± 10

Data are expressed as mean ±SEM, **n**= 6. *p<0.05, **p<0.01, ***p<0.001 ****p<0.0001 when compared to the baseline value (T0). **D**-. day.

SBP in L-NAME rats treated with the standard drug (furosemide). This reduction was noted at the second, fourth and seventh ($p<0.0001$) days of treatment. Identically, during the subchronic treatment essay, MBP significantly decreased in hypertensive rats treated with furosemide on the fourth and seventh days ($p<0.0001$). In contrast, the heart rate remained unchanged during the treatment period by furosemide. No notable modifications were noticed in blood pressure measurements or heart rate in the non-treated group over the administered duration.

In normal rats, the results demonstrated that the AEST caused a remarkable decline effect of SBP at the seventh ($p<0.05$) day of treatment. In contrast, no observable changes impact heart rate, DBP and MBP after seven days of treatment. Furosemide-treated rats showed a substantial lowering effect of SBP and heart rate on the seventh day from the treatment in the regular group ($p<0.01$) and ($p<0.05$), respectively.

Vasorelaxant effect and mechanisms implicated

Vasorelaxant effect of AEST

AEST was tested using isolated aortic rings pre-contracted with KCL or norepinephrine (EP; 10-5) to determine its effect on vascular relaxation. The relaxation effects of five different concentrations were investigated in a series of experiments (0.0625, 0.125, 0.187, 0.3125, 0.5 mg/mL) in a dose-dependent method. It has been shown that AEST induced a vasorelaxation effect in aortic rings that were pre-contracted by KCL or EP (Fig. 1). Indeed, AEST demonstrated a significant concentration-dependent vasodilatation effect on aorta pre-contracted with EP (Fig. 1A), with considerable dilation observed from the initial dose of 0.0625 mg/mL ($p < 0.01$) to the other doses (0.125, 0.187, 0.3125, 0.5 mg/mL, $p<0.0001$). The vasorelaxation effect induced by AEST was achieved at an EC50 value of 0.133±0.04 mg/mL and a Rmax value of 128.5±26.04 %. In addition, AEST did not change significantly the contraction produced by KCL (Fig. 1B) concerning the first two doses (0.125, 0.187 mg/mL). The relaxant effect in these aortic rings (pre-contracted by KCL) was observed after the incubation of aortic rings by the third (0.3125 mg/mL, $p<0.01$) and the fourth (0.5 mg/mL, $p<0.0001$). Notably, the results demonstrate that AEST induced an antihypertensive effect by the vasodilation of vascular smooth muscle. To define the mechanism responsible for this vasorelaxant activity caused by the incubation of AEST, several drugs were employed in this work after a pre-contract with EP.

Responses of the aorta to AEST without and with glibencla-mide or propranolol

To investigate the integration of β-adrenergic receptors or potassium channels in AEST-induced relaxation, aortic rings were pre-incubated with propranolol (a β-adrenergic receptor blocker) or glibenclamide (a potassium channel inhibitor) before being contracted with EP (EP). The preincubation with neither glibenclamide (Fig. 2A) nor propranolol (Fig. 2B) significantly altered the vasoactivity effects of AEST in comparison with the control.

Responses of the aorta to AEST without and with indomethacin or L-NAME

The aortic rings with intact endothelium were pre-treated with NO synthase inhibitor (L-NAME) or prostaglandin synthesis inhibitor (indomethacin). The analysis results are depicted in Fig. 3. Correspondingly, the results show that L-NAME and indomethacin treatment didn't alter the vasorelaxation caused by AEST compared to the baseline value.

Analysis of aortic responses to AEST with or without nifedipine or methylene blue

To evaluate whether NO-cyclic guanosine monophosphate (cGMP) and Ca2+ channels are involved in the AEST vasodilatation activity. Methylene blue or nifedipine are pre-treated of aortic rings. The data were analyzed and are graphically represented in Fig. 4. The findings confirm that incubation of methylene blue altered the vasocontractility ability produced by AEST from the second to the fourth dose ($p<0.01$; Fig. 4A). In the same vein, the preincubation of nifedipine changed the vasocontractility effect produced by the same extract at the same doses ($p<0.05$; Fig. 4B).

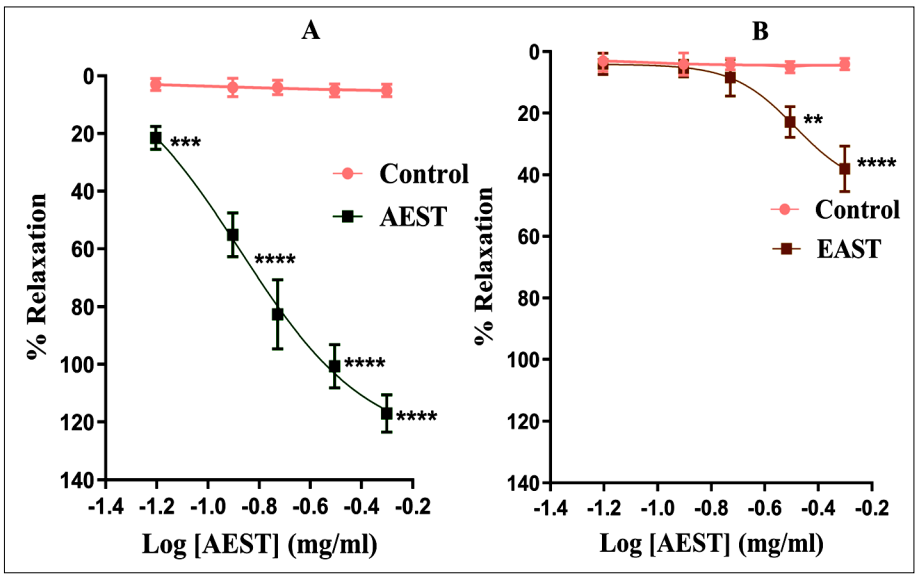

Fig. 1. Effect of AEST on EP and KCl-induced contraction in thoracic aortic rings. Cumulative dose-dependent curves for AEST-induced relaxation and distilled water in rat aortic rings. **n** = 6; (**A**). pre-contraction with norepinephrine (10 µM); (**B**). pre-contraction KCl (80 mM). **p < 0.01, ****p < 0.0001.

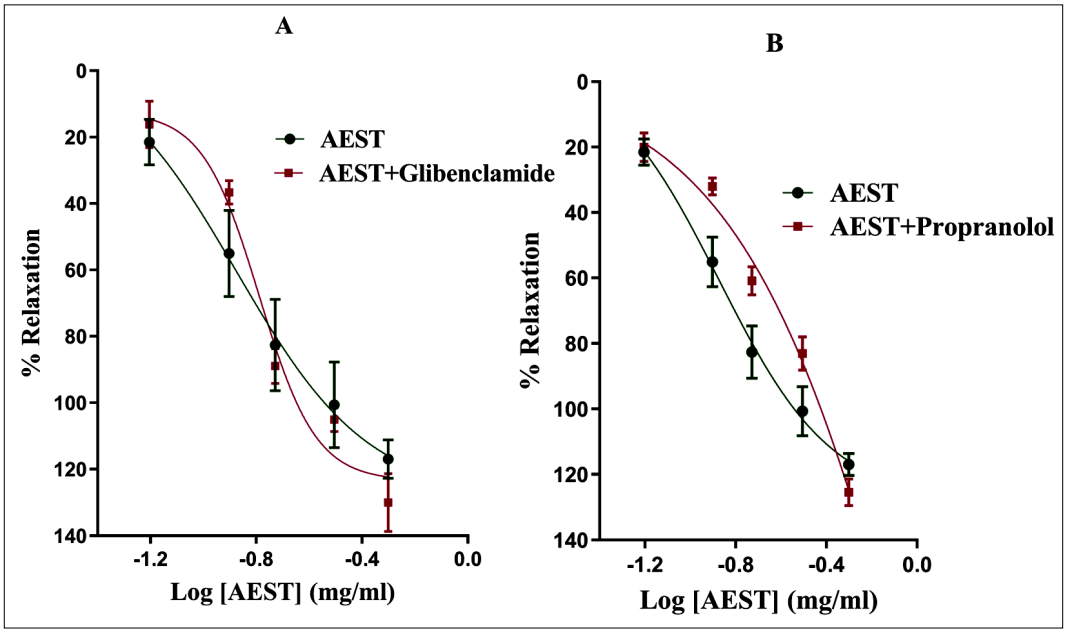

Fig. 2. Effect of glibenclamide and propanolol incubation on AEST-induced vasorelaxation. Aortic rings were pre-incubated with cumulative doses of AEST in the absence or presence of the following drugs: (**A**) Glibenclamide; (**B**) Propanolol. Data represent mean ± SEM.

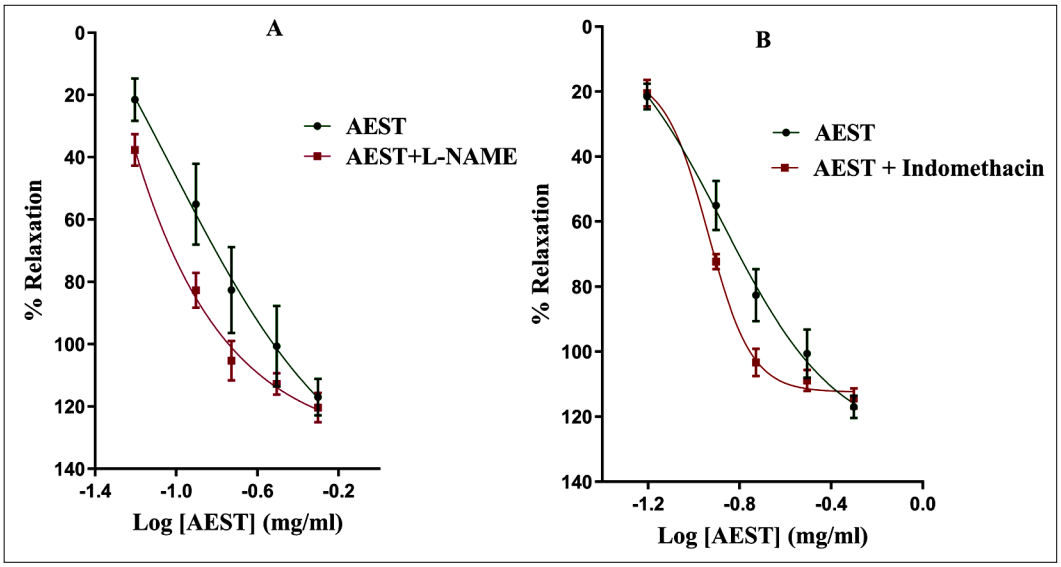

Fig. 3. Effect of different drugs incubation on AEST-induced relaxation. Aortic rings were pre-incubated with cumulative doses of AEST in the absence or presence of the following drugs: (**A**) L-NAME; (**B**) Indomethacin. Data represent mean ± SEM.

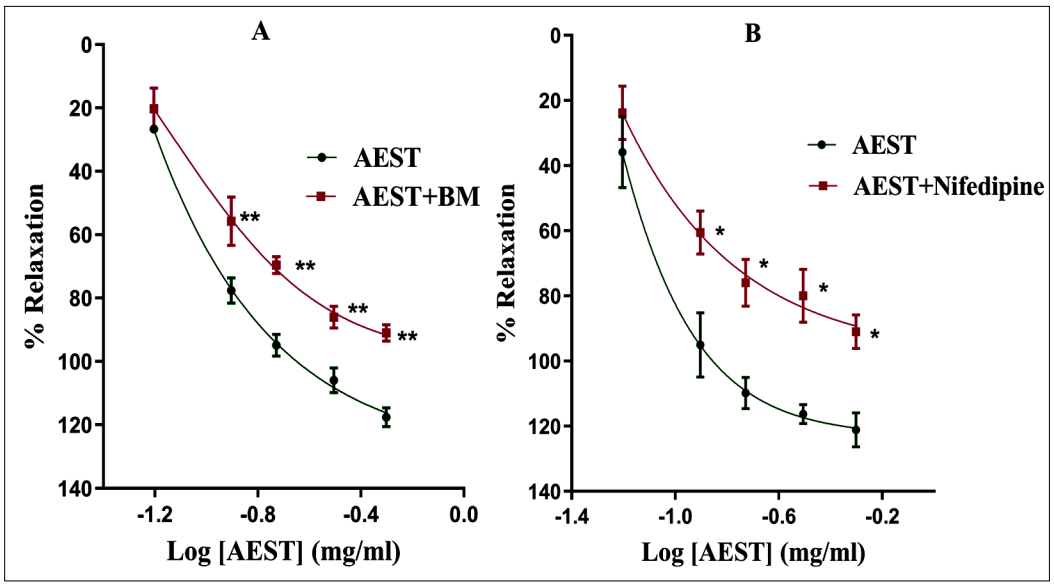

Fig. 4. Effect of different drugs incubation on AEST-induced relaxation. Aortic rings were pre-incubated with cumulative doses of AEST in the absence or presence of the following drugs: (**A**) Methylene blue; (**B**) Nifedipine. *p<0.05, **p<0.01. Data represent mean ± SEM.

Analysis of aortic responses to AEST with or without $BaCl_2$ or atropine

To understand if the pathways of $BaCl_2$ or Atropine are implicated in the vasorelaxant action involved in AEST on endothelium-intact rat aortic rings, $BaCl_2$ or Atropine preincubation was used in a series of experiments. The results are shown in Fig. 5. The presence of $BaCl_2$ significantly reduced AEST-induced dilatation after the incubation of aortic rings by the last four doses (0.125, 0.187, 0.3125,0.5 mg/mL, p<0. 01) in comparison to the control (Fig. 5A). Identically, the incubation of atropine before EP-evoked contraction significantly attenuated AEST-induced relaxation after the incubation of aortic rings by the third, fourth and higher doses ($p < 0.01$; Fig. 5A) when the responses were compared to that of aortic rings pre-contracted exclusively with EP.

Discussion

One-week studies were conducted on normal and L-NAME-rats to evaluate the antihypertensive effect of *S. tingitana* extract. In addition, the vasorelaxant ability has been discovered to determine the possible mechanisms involved in the antihypertensive response using isolated rat aorta. An experimental hypertensive state was induced in Wistar rats by administering L-NAME over seven days. In the present study, AEST decreased the parameters of arterial blood pressure without affecting the heart rate in hypertensive rats. AEST significantly lowered SBP after six hours of administration in normal rats without affecting MBP, DBP and heart rate. Interestingly, following repeated oral administration of AEST, arterial blood pressure parameters (SABP, DBP and MBP) significantly decreased in hypertensive rats; however, heart rate did not change. Repeated oral AEST treatment over seven days had no effects on heart rate or the parameters of arterial blood pressure in normal rats. Secondary metabolites are beneficial in the treatment of hypertension in several studies. In L-NAME-induced hypertensive rats, these secondary metabolites may act individually or synergistically to lower blood pressure (17). In the same vein, several works revealed the existence of numerous phytochemical compounds like tannins, alkaloids, flavonoids and polyphenols in *S. tingitana* (9,10). Notably, the consumption of foods with high percentages of polyphenolic is linked with diminution incidence of cardiovascular illness; this effect is probably due to the capacity of polyphenols to prevent leukocyte adhesion and platelet aggregation, to promote relaxation of vascular smooth muscle and to protect LDL (low-density lipoprotein) from oxidation (18,19). In addition, polyphenols may act on the metabolism of cells such as nitric oxide (NO), thrombus development and vascular tone (20-22).

It is possible to lower arterial pressure by reducing cardiac output or systemic vascular resistance (23). AEST was shown to be a potent vasorelaxant in aortic rings pre-contracted with EP, as evidenced by the experimental results; this effect was also attenuated after the precontraction of aortic rings by KCL. Importantly, it induced vasoconstriction by stimulation of voltage-operated Ca^{2+} channels (24), suggesting the intervention of Ca^{2+} channels in the vasorelaxant effect observed after the incubation of AEST.

The observed vasodilatory capacity of AEST likely plays a significant role in its antihypertensive effects. An *ex vivo* study was undertaken to elucidate the potential mechanisms associated with this vasodilation activity. Indeed, a total of eight reference drugs, were pre-incubated in rat aortic rings, including indomethacin (a prostaglandin synthesis inhibitor), glibenclamide (an ATP-sensitive K+ channel blocker), methylene blue (a cyclic guanosine monophosphate blocker), atropine (muscarinic receptors blocker), L-NAME (a nonselective nitric oxide synthase inhibitor), propranolol (a beta-blocker), nifedipine (a calcium channel blocker) and $Bacl_2$ (inward-rectifying K+ channels (KIR) inhibitor). The findings revealed that preincubation of aortic rings with propranolol, glibenclamide, methylene blue and indomethacin did not alter the vasorelaxation effect produced by the AEST. In contrast, nifedipine, L-NAME, atropine and $BaCl_2$ have reduced the vasorelaxant ability of AEST. Medicinal plant extracts have previously reported calcium channel inhibition (25). In general, calcium entry into the cell cytosol can occur through two primary mechanisms: the influx from the extracellular space via voltage-gated calcium channels. The second is calcium release from the intracellular sarcoplasmic reticulum stored in the cytosol (26). Nifedipine, a member of the dihydropyridine group of a selective antagonists of calcium channels, is indicated for

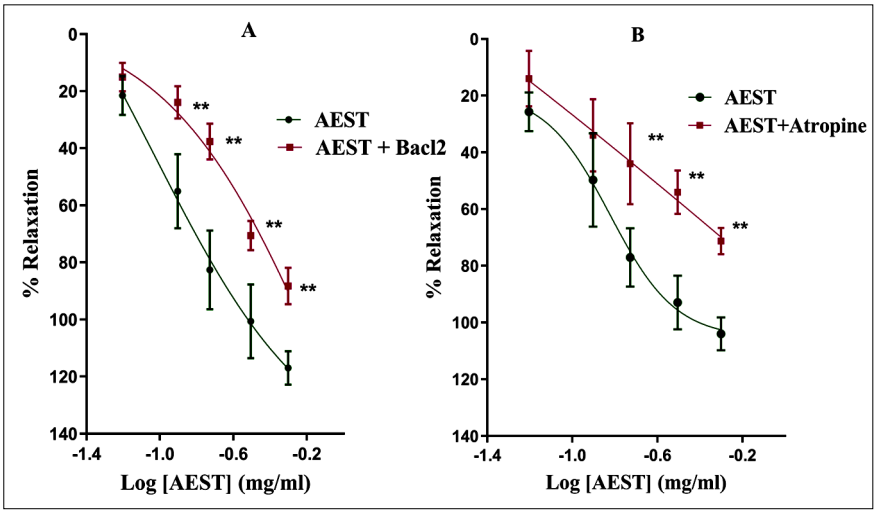

Fig. 5. Effect of different drugs incubation on AEST-induced relaxation. Aortic rings were pre- incubated by cumulative doses of AEST leaves in the absence or presence of the following drugs: (A) $BaCl_2$; (B) Atropine. **p<0.01. Data represent mean ± SEM.

treating chronic stable angina and vasospastic. As well as, nifedipine is effective in treating essential hypertension (relaxed vascular smooth muscle), acute hypertensive crises and achalasia (27). In the present study, nifedipine has partially inhibited the vasorelaxant activity of AEST, indicating the role of calcium channels in this pharmacological activity.

In endothelium, the continual production of NO keeps the balance between vasoconstriction and vasodilation (28). Indeed, NO plays a crucial role in cardiovascular function, primarily by inducing vasorelaxation and regulating vascular tone (29). Reduced NO production has been implicated in various cardiovascular diseases, including hypertension, coronary vasospasm and increased systemic vascular resistance (30). In contrast, augmentation of NO production causes inflammation and neurodegenerative diseases (31). This finding suggests the critical role of NO in the vasodilatory effect of AEST, as evidenced by the partial inhibition of this effect by L-NAME.

In addition, the results show that the presence of atropine attenuated significantly the vasorelaxant effect provoked by AEST, so it is hypothesized that the impact of this plant may be mediated partially by the muscarinic receptor. By blocking muscarinic receptors, atropine can inhibit the inhibitory effects of acetylcholine (30). In addition, it has been suggested that at the dose of 10-8 M (lower concentrations), atropine blocks muscarinic receptors, leading to inducing contraction effects. In contrast, at a dose of 10-3 M (large dose), atropine increased intracellular calcium sequestration, leading to the induced relaxation effect (32). The activity of potassium ion (K^+) channels directly impacts vascular smooth muscle membrane potential, which in turn determines vascular tone. Inward-rectifying potassium (K^+) channels in vascular smooth muscle exhibit distinct characteristics compared to other types of K^+ channels, including voltage-dependent K^+ channels (KV channels), ATP-sensitive K^+ channels (KATP channels) and Ca^{2+}- activated K^+ channels. In addition, inward-rectifying K^+ channels mediate K^+-induced relaxation in rat arteries and contribute to basal vasodilation in human forearms (28, 33-37). $BaCl_2$, in the present study, can partially inhibit the vasorelaxant effect of AEST, indicating the implication of inward-rectifying K^+ channels in this vasorelaxant effect.

Conclusion

The current study demonstrates clearly that AEST provokes a pronounced antihypertensive activity in L-NAME rats. Interestingly, the vasodilation effect of AEST appears to be mediated by the activation of nitric oxide synthase, calcium channels, muscarinic receptors and inward-rectifying K^+ channels. The phytocompounds responsible for this pharmacological property need to be identified in further studies.

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Authors' contributions

AA conducted the investigation, interpreted the data, and drafted the manuscript. AQ performed the analysis, investigation and data interpretation. IB assisted in the investigation and contributed to the manuscript's drafting. ME conceptualized the study, critically revised the manuscript and supervised the research. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: No humans were involved in the study. All animal procedures were performed per the local ethical committee guidelines, Faculty of Sciences & Techniques Errachidia, Morocco, N° FSTE/205.

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