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Evaluation of the Vasorelaxant Effect Induced by the Essential Oil Extracted from the Root Bark of Hazumalania voyronii (Jum.) Capuron (Hernandiaceae) in Wistar Rat Aorta Ring

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ABSTRACT

The present was carried out with the aim to investigate the vasorelaxant effect induced by essential oil extracted from the root bark of *Hazumalania voyronii* in order to validate its use scientifically in Malagasy folk medicine. The vasorelaxant activity of the essential oil was carried out using an *ex vivo* method based on the rat aortic rings pre-contracted with phenylephrine. Rings of rat aorta were suspended in organ bath containing Krebs solution at 37 °C. Isometric contractions were measured using a force transducer coupled to an amplifier. Essential oil induces concentration-dependent relaxation in pre-contracted aortic rings (EC_{50} = 160.87 ± 7 µg/ml; E_{max} = 91.13 ± 3.2%). This result indicates that *Hazumalania voyronii* essential oil (EOIH) possesses vasorelaxant activity on the isolated organs. The ability of EOIH to display vasorelaxant activity supports the use of this plant species as medicine by the traditional healers in Madagascar. However, further studies are necessary to evaluate better its safety and therapeutic margin before the wide use of the plant oil.

Keyword: Traditional medicine, Madagascar, *Hazumalania voyronii*, vasorelaxant activity**,** aortic ring, Wistar rat

INTRODUCTION

The essential oils (EO) are defined as a mixture of volatile substances composed mainly of terpenes in addition to some other non-terpene components commonly found in aromatic plants [1-3]. Recent findings revealed that EO has numerous therapeutic values such as antithrombotic, antiplatelet, endothelial protective and hypotensive effects [4]. They have reported to improve coronary flow and also to have bradycardic and vasorelaxant properties [5-7]. Hypertension constitutes a cardiovascular disease with the most epidemiological impact worldwide and represents a risk factor for developing additional diseases such as diabetes, renal dysfunction, coronary artery disease and stroke [8, 9]. In some African countries, the prevalence of hypertension is between 31.1% and 32.5%. A study conducted in Madagascar, revealed that 28.05% of adult populations are concerned by this disease with an average mean age of 49 years [10, 11].

Several plants in Malagasy flora were reported as having therapeutic properties and are largely used by the local communities to treat various ailments including vascular tone dysfunction or blood vessels. Indeed, it was reported in the literature that, nitric oxide causes relaxation by activating/stimulating guanylate cylase. Nitric oxide is synthesized by a Ca^{+2}-camodulin-activated nitric oxide synthase in endothelial cells. Being lipid soluble, it diffuses rapidly to the underlying smooth muscle cells, in which it activates the soluble guanylate cyclase. The resulting cGMP can induce relaxation/vasodilatation through protein kinase G activation (NO/cGMP biochemical signaling pathways) [12].

Recently, an ethno-pharmacological survey was conducted in the South of Madagascar with the aim of conserving the indigenous knowledge of people inhabiting this island [13, 14]. Survey reported that the decoction of the root bark of *Hazumalania voyronii* (Jum.) Capuron (syn. *Hernandia voyronii*) or “Hazomalagny” in Malagasy vernacular name is used in folk medicine by the local communities to treat malaria, hypertension, and bacterial infections and for wound-healing [14]. The plant species *Hazumalania voyronii* belongs to the Hernandiaceae family. This botanical family is largely distributed in the subtropical and tropical regions, with about five genus and 54

species. About 10 species are present in America, 42 are found in Malaysia and two species are originated from Madagascar [15].

The aim of the present research work was to evaluate the vasorelaxant effect induced by the EO extracted from the root bark of *Hazumalania voyronii* in rat aorta ring in order to validate scientifically their traditional use as source of vasodilatator agents.

MATERIALS AND METHODS:

Plant material

The samples of *Hazumalania voyronii* roots were collected in Mareanano village, district of Manja (Southwest of Madagascar) on December 2011. The plant sample was identified by comparison with reference specimens available at the Department of Botany, Tsimbazaza Zoological and Botanical Park, Antananarivo. Voucher specimens with assigned sample number FR-01 was deposited at the Herbarium of the Laboratory of Applied Chemistry, Layflaylle Street, University of Toliara, Madagascar as previously reported [14].

Essential oils isolation

Four hundred grams of *H. voyronii* were extracted for four hours by hydro-distillation using a Clevenger-type apparatus. Briefly, the plant roots were completely immersed in water (1000 ml) and heated to boiling after which the essential oil was evaporated together with water vapor and finally collected after decantation. The oil obtained was dried over anhydrous sodium sulphate and then stored under argon in a sealed vial, and kept at -10 °C until the time of further analysis. The percent yield was calculated relative to the dried mass of the initial sample as previously reported [14].

Ex vivo Pharmacological experiments

Animals

Wistar rats weighing between 270 ± 30 g were used for all experiments. Animals were kept under conditions of controlled temperature (24 ± 1 °C) and a 12 hr light/dark cycle. They freely access to food and tap water. All experimental procedures were approved by the Malagasy Institute of Applied Research (IMRA), Avarabohitra Itaosy lot AVB 77, P.O. BOX 3833, 102 Antananarivo, Madagascar.

Drugs/reagents

The following drugs were used: Essential oil from the root of the *H. voyroni* (EOIH), phenylephrine (PE), acetylcholine (Ach), N-nitro-L-arginine methyl ester (L-NAME), indometacin (IND), propranolol, dimethylsulfoxyde (DMSO) and ethyleneglycol-bis-N,N'-tetra acetic acid (EGTA) were purchased from Sigma Chemical Company (St. Louis, MO, USA). For all experiments, essential oil was diluted in dimethylsulfoxyde/deionized water (DMSO) (0.5% v/v) solution. All reagents used were of analytical grade.

Solutions

The composition of Krebs solution was (in mM): KCl : 4.75 ; NaCl : 118.5 ; $NaHCO_3$: 25 ; Glucose : 11.1 ; $MgSO_4$: 1.2 ; KH_2PO_4 : 1.2 ; $CaCl_2$: 1.36. The K^+-depolarizing solutions (KCl 60 and 80 mM) were prepared by replacing 60 or 80 mM KCl in the Krebs solution with equimolar NaCl. In nominally zero-Ca^{2+} solution, $CaCl_2$ was omitted and 0.5 mM EGTA was added as previously reported [16].

Preparation of isolated rat thoracic aorta artery ring

Vascular isometric tension was evaluated by organ bath technique as previously described elsewhere [17] with minor modifications. Briefly, Wistar rats were killed by cervical dislocation and then exsanguinated by carotid artery transaction. The thoracic aortas of Wistar rats were carefully removed and immediately placed in Krebs-Henseleit solution. This organ (thoracic aorta) was cleaned off adhering fat and connective tissue and cut into rings of 5 mm length each. All manipulations were carried out with rigorous care in order to prevent endothelium damage. The rings were placed in a borosilicate glass jacketed organ bath chamber of 20 ml capacity, and mounted on two small stainless steel hooks, one of which was connected to the organ bath and the other to a force transducer. The organ bath was filled with a Krebs-Henseleit solution maintained at 37 °C and gassed with a mixture of 5% CO_2 and 95% O_2. The Wistar rat rings were allowed to equilibrate for 1 hour under a resting tension of 1 g using micromanipulator until a constant base force was established. During this time, the bathing medium was measured every 15 min. the isometric tension was measured by the mean of a force transducer apparatus and recorded digitally using a Data acquisition system and then stored and analyzed with a computer program software. After the equilibration period, all isolated tissues were exposed repeatedly to 1 µM phenylephrine (PE) solution in order to test their contractile capacity. Once the response to PE had reached a stable plateau, the aortic rings were relaxed with acetylcholine (1 µM). Two series of experiments were carried out in order to assess the vasorelaxant activity of essential oil. The first ones used isolated rat aorta with the intact endothelium and the second series on endothelium-denuded aortic tissues. The presence of functional endothelium was checked by the ability of Ach to induce superior or equal to 80% relaxation of ring pre-contracted with PE. In experiment involved denuded aortic rings, a relaxation inferior or equal to 10% by Ach was considered as satisfying and were selected for the test.

Effects of the essential oil on isolated rat aortic ring

Two tonic responses to PE or KCl, which stabilized in 15 min, were registered. After a third response, different concentrations of the essential oil (10-1000 µg/ml) were added cumulatively to Wistar rat isolated aortic preparations. Some experiments in which PE was added to the tissues and left to stand for at least 30 min in order to observe whether the tension was maintained during the period were also carried out. The effect of essential oil on the resting tone of Wistar rat aorta was also evaluated. The relaxation was evaluated by comparing the developed tension before and after addition of essential oil.

Influence of β-adrenergic receptor on the vasorelaxant effect of the essential oil against PE-induced contractions

In order to evaluate the implication of β-adrenergic receptor on the vasorelaxant effect of the essential oil, two tonic responses to PE, which have been stabilized for 15 min, were registered. Wistar rat aortic rings were then exposed to propranolol (10^{-5} M) and a third response to PE was obtained and a graded concentration of essential oil (10-1000 µg/ml) was added cumulatively to the isolated aortic preparations. The effectiveness of the β-adrenergic receptor activity was verified by the loss of relaxing response of the preparation to PE (10^{-6} M) in the presence of propranolol (10^{-5} M).

Influence of endothelium on the vasorelaxant effect of the essential oil against phenylephrine-induced contractions

To evaluate the role on endothelium in the vasorelaxant effect of the essential oil, phenylephrine-induced sustained contractions were obtained in endothelium-denuded rings or in endothelium-intact preparation in the presence L-NAME (10^{-5} M) and IND (10^{-5} M). L-NAME and IND were added 30 min before

the addition of PE. After a third PE-induced contraction, the essential oil (10-1000 µg/ml) was added cumulatively to isolated aortic preparation.

Investigation of the essential oil effect on $CaCl_2$-induced contraction

To evaluate whether the inhibition of extracellular Ca^{2+}influx was involved in EOIH-induced relaxation, the experiments were carried out in Ca^{2+}-free Krebs solution. After the stabilization period, the effect of $CaCl_2$-induced contraction in endothelium-denuded rings was assessed as previously reported [18]. Cumulative concentration-response curves for $CaCl_2$ were obtained in endothelium-denuded rings exposed to nominally without Ca^{2+} (10^{-7} to 10^{-2} M) solution with KCl 60 mM before and after pre-incubation, in the presence of essential oil at different concentrations (100-300 µg/ml) for 30 min. The results were expressed as percentages of the maximal response for only $CaCl_2$-induced response, and curves were statistically compared.

Statistical analysis

The results the study were expressed as percent inhibition. The percent inhibition of each tested dose was averaged and expressed as mean ± SD. Biological experiments were carried out in triplicate. The IC_{50} values were calculated by regression analysis. The statistical analysis was processed using paired t-Student's test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

The vasorelaxant activity of the essential oil from the root bark of *Hazumalania voyronii* was investigated in Wistar rat aortic rings. EO from *H. voyronii* induced relaxation of the PE pre-contracted aorta in a concentration dependent manner (Fig. 1). The concentration of the essential oil to induce 50% of the maximal relaxation (EC_{50}) in the PE-induced contraction of endothelium intact aortic rings was 160.87 ± 7 µg/ml and the E_{max} = 91.13 ± 3.2%.

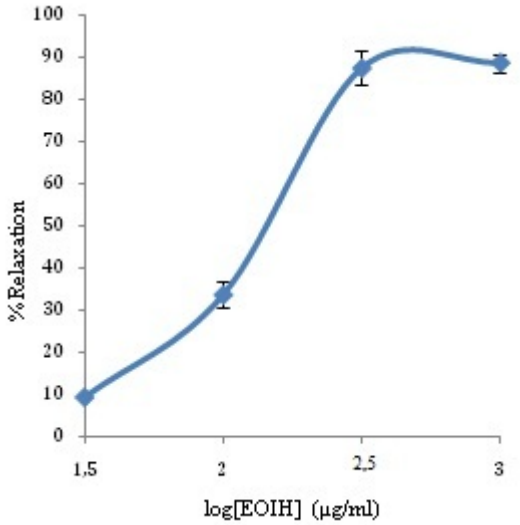

Fig. 1 -The concentration-response curves for EOIH in endothelium intact aortic ring, pre-contracted with PE (10^{-6} M). Data are mean ± SD, EOIH = Essential oil of *Hazumalania voyronii.*

EOIH displayed a good vasorelaxant effect on phenylephrine pre-contracted rat aortic rings and on endothelium-denuded aortic rings (data not shown). The contribution of endothelium in the vasorelaxant activity of EOIH was remarkable. This result conducted us to undertake further studies for elucidating the mode of action of EOIH.

Vasorelaxant effect of the essential oil in the presence of propranolol, indometacine and L-NAME

The addition of propranolol (10^{-5} M, 30 min) to the organ bath system failed to prevent the relaxation induced by essential oil on phenylephrine-induced contractions (Fig. 2a). Similarly, the addition of indometacine (10^{-5} M, 30 min) could not prevent the essential oil induced relaxation in PE-induced contraction of intact aortic preparation (Fig. 2b)

The addition of L-NAME (10^{-5} M, 30 min) to the organ bath system, prevented the essential oil induced relaxation significantly to the extent of 270 ± 11 µg/ml and 170.14 ± 6 µg/ml respectively (Fig. 2c).

In a nominally without Ca2+ depolarizing Krebs solution, the vasorelaxant activity of the EOIH was not modified in denuded endothelium aorta ring pre-treated with CaCl2 (Fig. 2d).

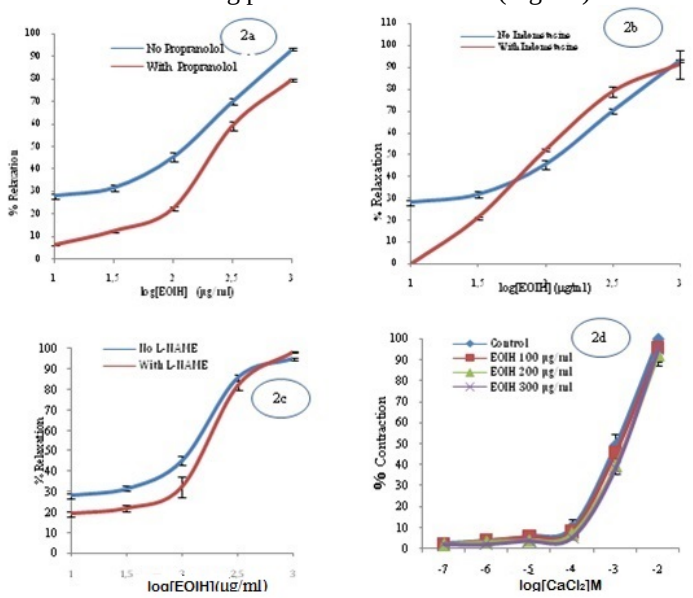

Fig. 2 – Effects of essential oil from the root bark of *H. voyronii* in presence of various blockers in isolated aorta rings contracted with PE (10^{-6} M). Typical tracing showing the sustained and concentration-dependent relaxant reponse of EOIH. Cumulative concentration of EOIH was added when the earlier response exhibited a plateau. Effects of EOIH in presence of (2a) propranolol (10^{-5} M), (2b) indomethacin (10^{-5} M), (2c) L-NAME (10^{-5} M) and (2d) inhibitory effect of EOIH (100-300 µg/ml) on calcium-induced contraction in endothelium-removed rat thoracic aorta rings in nominally without Ca^{2+} Depolarising krebs solution. Data are expressed as mean±SD, a value of $P < 0.05$ was considered statistically significant.

DISCUSSION

It is well known that the contractile response produced by phenylephrine attained a maximum within 10 min and is relatively well sustained over the subsequent 30 min [18]. The present study indicated the EOIH relax the resting tension slightly. This essential oil could inhibit phenylephrine contraction of Wistar rat aorta in a concentration dependent manner. This vasorelaxant effect of the oil was not modified by propranolol. This result revealed that the action of EOIH is not mediated through a β-adrenoceptor. EOIH also antagonized the phenylephrine in a concentration-dependent manner. Nitric oxide (NO) is reported in the literature as a major endothelium derived relaxing factor (EDRF). The release of such chemical compound from

endothelial cells induces the relaxation of vascular smooth muscle cells and plays a key role in the maintenance of vascular tone [19]. In order to verify whether the vasorelaxant effect induced by EOIH in isolated aortic rings was due to NO, experiments were assessed in intact aortic ring preparation (pre-contracted with 10^{-6} M PE) in the presence of L-NAME (10^{-5} M), a competitive inhibitor of NO-synthase [19]. Under such experimental conditions, EOIH-induced vasorelaxation was significantly attenuated to the extent of 270 ± 11 µg/ml but not completely abolished. The results of the present work revealed also that, the vasorelaxant effect induced by EOIH was not modified in aortic ring pre-treated with IND. This means that prostacyclins biosynthesis pathway is not involved in such relaxation. Prostacyclins are the biologically active products derived from eicosanoids through the cyclooxygenase (COX) pathway. These interesting findings demonstrate clearly that EOIH could induce vasodilatory effect by stimulating the release of NO [20-21]. However, because of the poor affinity of L-NAME and relative arginine derivatives towards nitric oxide synthase (NOS), the use of a guanylate cyclase inhibitor inhibitors such as 1H-[1,2,4]oxadiazolo- [4,3-a]quinoxalin-1-one (ODQ), or even other NO scavengers such as 2-Phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (PTIO), hydroxocobalamin for future researches may increase the validity of the present study findings [22].

It is scientifically validated that high KCl solution induces the opening of voltage-operated calcium channels [23]. Artery contraction induced by high KCl solution results from an increase in cytosolic calcium ions caused by the opening of voltage-dependent Ca^{2+} channels (VDCs). The probable involvement of VDCs in the relaxing activity of EOIH was evaluated by measuring the effect of EOIH on $CaCl_2$ induced contraction of Wistar rat denuded endothelium aorta ring in depolarizing solution. When the $CaCl_2$ at different concentrations was used to induce contraction of the Wistar rat denuded endothelium aorta ring, essential oil extracted from the root bark of *Hazumalania voyronii* has no effect on the contraction of denuded endothelium aorta ring. These results indicated clearly that the vasorelaxation effect induced by EOIH in Wistar rat aorta ring is not mediated through VDCs indicating the pivotal role of endothelium in the vascular tone regulation. Indeed, it is known that the high level of calcium in endothelial cells relaxes the underlying vascular smooth muscle because they stimulate the synthesis of nitric oxide. On the other hand, by removing the endothelium, vascular smooth muscle is contracted by agents that raise calcium levels. To validate this hypothesis, the future research may evaluate the vasorelaxant effect of EOIH in the presence of known calcium channel blocker such as nifedipine [12].

The role of essential oil components such as borneol and caryophyllene oxide in the vasorelaxant effect on rat isolated aorta ring was previously reported [24, 25]. Borneol and caryophyllene oxide were found in EOIH. Indeed, we recently analyze the chemical composition of the essential oil extracted from the root bark of *H. voyronii* by GC-FID and GC/MS. Results revealed that *H. voyronii* essential oil contains 13.4% of monoterpens hydrocarbons, 0.08% of sesquiterpens hydrocarbons, 36.6% of oxygenated monoterpens and 42.2% of oxygenated sesquiterpens [14]. The biologically active compounds such as borneol and caryophyllene oxide which are present in the studied essential oil could be responsible for the displayed vasorelaxant effect as it can be noticed for the essential oils extracted from other aromatic and medicinal plants reported in the literature [26-31].

CONCLUSION

The results of the present study have demonstrated that EOIH induces vasorelaxation in Wistar rat aorta ring. This vasorelaxant effect is not modified by propranolol and indometacine and is significantly attenuated but not completely abolished by L-NAME. The ability of *Hazumalania voyronii* essential oil to display vasorelaxant activity supports the use of this plant species as vasodilatator medicine by the traditional healers in Madagascar. However, further studies are necessary to evaluate better its safety and therapeutic margin before the wide use of the plant oil.

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