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Vasorelaxant Effect of Ethanolic Extracts from *M. Vulgare*: Mexican Medicinal Plant as Potential Source for Bioactive Molecules Isolation

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ABSTRACT: To investigate vasorelaxant effect of ethanol extracts from *M. vulgare*, it is part of a group of plants subjected to pharmacological and phytochemical study with the purpose of offering it as an ideal source for obtaining lead compounds for designing new therapeutic agents with potential vasorelaxant and antihypertensive effects. In this context, all extracts caused concentration-dependent relaxation in -precontracted aortic rings with and without endothelium; the most active extract was EERMv. These results suggest that secondary metabolites responsible for the vasorelaxant activity belong to a group of compounds of high polarity and the roots were the main tissues of the plant where the vasorelaxant compounds are stored. In conclusion, *M. vulgare* represent an ideal source for obtaining lead compounds for designing new therapeutic agents with potential vasorelaxant and antihypertensive effects.

KEYWORDS: Aortic Ring; *Marrubium vulgare*; Mexican Medicinal Plant; Vasorelaxant.

INTRODUCTION

In Mexico, *M. vulgare* is used to avoid the stomach pain, treating diarrhea, hypertension, and diabetes [1], also, to date been reported to have been isolated a large number of secondary metabolites with a large structural diversity from other *Marrubium* species [2-5]. Consequently, the objective of this study was carried out in order to investigate vasorelaxant effect of *M. vulgare* with the purpose of offering it as an ideal source for obtaining lead compounds for designing new therapeutic agents with potential vasorelaxant and antihypertensive effects, since, actually the hypertension is a cardiovascular disease with the most epidemiological impact in the world and also represents a major risk factor for

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developing other diseases as endothelial dysfunction, metabolic syndrome, diabetes, renal dysfunction, congestive heart failure, coronary artery disease and stroke.

MATERIALS & METHODS

Chemicals

All reagents used were grade analytical and purchased from Sigma-Aldrich™. For *in vitro* experiments, extracts were dissolved in distilled water and dimethylsulfoxide (DMSO, 1% v/v) and other reagents were dissolved in distilled water and sonicated just before use.

Plant material and extraction

Marrubium vulgare was collected and identified by Dr. Patricia Castillo-España in Yautepec, Morelos, Mexico. A voucher specimen was deposited at the Herbarium of Morelos State University. Briefly, the plant material was separated (roots, flowers, stems) and subjected to successive maceration with ethanol (3 times for 72 h at room temperature). After filtration, extracts were concentrated at 40 °C.

Animals

Male Wistar rats (250–350 g) were used. They were maintained under standard laboratory conditions with free access to food and water. The study was reviewed and approved by the local institutional review board.

Preparation of rat aortic rings and effect of extracts on the contraction induced by NE

The experimental design was performed according to described by Ibarra [6]. The aortic rings with and without endothelium were precontracted with NE (1×10^{-7}M). Once the plateau was attained, concentration–response curves of extracts-induced relaxation (0.15 µg/mL to 50 µg/mL) were obtained by adding cumulative concentrations to the bath.

Effect of Ethanol Extract from Root of Marrubium vulgare (EERMv) on the cumulative contraction induced by NE

Endothelium-denuded aortic rings were incubated with 40 and 72 µg/mL of EERMv during 15 min, and then NE was added at different concentrations ($1x10^{-11}$M to $3.16x10^{-6}$ M). Finally, the contractile effect induced by NE was compared in the absence (control group) and presence of the extract.

Effect of EERMv on extracellular Ca^{2+}-induced contraction activated by KCl

To determine whether the inhibition of extracellular Ca^{2+} influx was involved in EERMv-induced relaxation, the experiments were carried out in Ca^{2+}-free Krebs solution. Endothelium-denuded aortic rings were washed with Ca^{2+}-free solution (approximately 20 min) and then rinsed with Ca2+-free solution containing KCl (80 mM). The cumulative concentration-response curves for $CaCl_2$ ($3x10^{-5}$ M to 0.02 M) were obtained in the absence of EERMv (control group) or after a 15 min incubation with the extract (72 and 120, µg/mL). Finally, the contractile effect induced by $CaCl_2$ was compared in the absence (control group) and presence of EERMv.

Data analysis

Data were analysed using ANOVA with repeated measures. Statistical significance was set a priori at $P<0.05$ for all comparison. Data were expressed as means ± standard error of the mean.

RESULTS & DISCUSSION

The current investigation represents the first effort to describe the vasodilator effect of ethanol extracts from different parts of *M. vulgare*. Roots, flower and stem ethanol extracts relaxed NE (1×10^{-7} M) precontracted aortic rings with and without endothelium, in a dose-dependent manner (Table 1), suggesting that vasodilatation is motivated by the interference on a common pathway which several receptor agonists exert, such as the augment of free cytosolic Ca^{2+} levels [7,8]. In this regard, in smooth muscle cells there are two kinds of Ca^{2+}

eISSN: 2249 1023

channels: voltage-dependent Ca^{2+} channels (high KCl induced contraction due to membrane depolarization, leading to increased Ca^{2+} influx through voltage dependent channels) and receptor operated Ca^{2+} channels (contraction induced by NE in Ca^{2+}-free medium is due to intracellular Ca^{2+} release, through sarcoplasmic reticulum Ca^{2+} channels activated by IP_3) [9, 10]. Therefore, agents acting directly on the vascular smooth muscle cell may alter tone by three mechanisms: altering intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$), varying the sensitivity of the contractile regulatory apparatus to $[Ca^{2+}]_i$ or modulating the sensitivity to other vasoactive inputs [11]. In this context, EERMv (72 and 120 µg/mL) inhibited the concentration–response contraction of NE in a nonparallel manner and depressed the maximal response (Fig. 1a), suggesting that extract might block voltage-dependent and receptor operated Ca^{2+} channels [9]. Moreover, we found that 72 and 120 µg/mL of the extract significantly inhibited $CaCl_2$-induced contraction of control group in a parallel manner and depressed their maximal responses (Fig. 1b), supporting the idea that EERMv possesses a Ca^{2+} entry blocking activity [9, 12]. It is important to mention that the relaxant effect showed by EERMv is in accord with previous relaxant effects produced by aqueous extracts obtained from *M. vulgare* and other Marrubium species where the presence of terpenes derivatives was confirmed, which are presumably responsible of the relaxant effect [2–5]. Therefore, it is necessary to direct the attention to compounds present in organic extracts. In conclusion, the present results provide pharmacological support for the use of *M. vulgare* in ethnomedical practices as antihypertensive in Mexico. Moreover, present efforts are directed to isolate the active constituents from extracts of this species to allow us understanding its mechanism(s) of action and to design new therapeutic agents with potential antihypertensive effects.

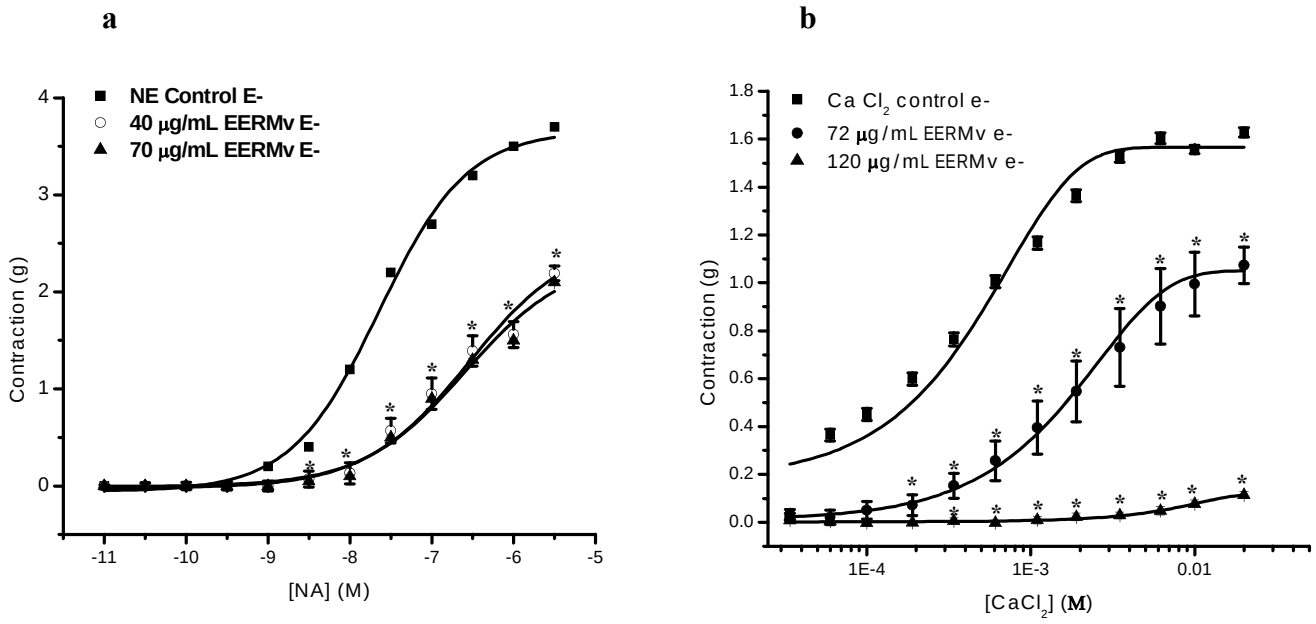

Figure 1. a) Inhibitory effects of EERMv on the contraction induced by NE (1×10^{-11}M to 3.16×10^{-6}M) in endothelium-denuded aortic rings, b) inhibitory effect of EERMv on the cumulative–contraction curve dependent on extracellular Ca^{2+} influx induced by 80 mM KCl in Ca^{2+}-free solution.

eISSN: 2249 1023

Vasorelaxant agent	With endothelium (E+)		With out endothelium (E+)	
	EC_{50} (µg/mL)	E_{max} (%)	EC_{50} (µg/mL)	E_{max} (%)
Carbachol	0.002	100.00±1.01	ND	ND
SNP	ND	ND	0.044	72.8±9.24
EERMv	24.32	83.80±3.28	47.72	100.48±3.45*
EEFMv	34.90	70.08±5.58	72.42	99.72±5.45*
EESMv	55.99	99.90±11.26	62.15	68.44±6.90*

Table 1 Relaxatory effects induced by ethanol extracts obtained from M. vulgare on the contraction induced by NE 1×10−7M. Results are presented as mean ± S.E.M., n=6. P*<0.05 compared with aortic rings with endothelium. EERMv: Ethanol extract from roots of *Marrubium vulgare*. EEFMv: Ethanol extract from flowers of *Marrubium vulgare*. EESMv: Ethanol extract from stem of *Marrubium vulgare*. ND: Non determinate.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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