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Ex vivo Vasoconstriction Effects of *Ephedra major* Host Hydroethanolic Extract on Wistar Rat Thoracic Aorta

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Abstract: Tendency to utilize Ephedra contain product are increasing due to its favorable properties such as energetic, fat burning, decongestant etc. Present of cardiovascular agonism α and β adrenergic compound and lack of systematic research on this issue, we conduct this study to determine the *Ephedra major* Host. The present work aimed to study the effect of hydroethanolic extract of Ephedra major plant on vascular system and its mechanism of action. Isolated aorta from 48 male Wistar rats (Body weight 220±30 g) were tested in organ bath. Rats were anesthetized and aorta isolated and placed in Krebs-Ringer bicarbonate solution continuously bubbled with 95% O_2 and %5 CO_2 Carbogene then cut into ring segment 4mm in width which then mounted in organ bath and contractive response recorded in 8 groups. Statistical compression made by student T test and ANOVA (SPSS Ver.19). A P value of =0.05 was considered as statically significant. Half Maximal Effective concentration calculated by Lab Chart Ver.7 software. The result were shown *E. major* extract have constriction effect on rat aorta.The Contractile mechanism, wasn't only through agonistic adrenergic mechanism of aorta induced by extract is releasing internal calcium sources mediated by metabotropic G protein. This constriction effects is due to a adrenergic agonistic mechanism accompanied with other possible mechanisms including depolarizing the calcium channels or by mediating the release of vasoconstrictive agent and or inhibition the release of endothelium relaxant.

Key words: *Ephedra* Herb · Vasoconstrictor · α-Blocker · Terazosin · Yohimbine

INTRODUCTION

One of the oldest medicinal herbs known to mankind is probably Ephedra, or ma huang which contains ephedrine alkaloids. A member of family Ephedraceae, *Ephedra sinica* is the primary species that has been used in China for more than 5000 years and is still being used in Ephedra preparations and extracts all around the world [1, 2]. Ephedra, which can also be synthesized in a laboratory, was available in many over-the-counter dietary supplements promoting weight loss, enhanced athletic performance and increased energy. Ephedra is the active ingredient in both over-the counter and prescription drug products for treating allergies, asthma and nasal congestion. Ephedrine is a potent sympathomimetic agent with direct and indirect effects on adrenergic receptors. It has α and β-adrenergic activity and enhances release of norepinephrine from sympathetic neurons [3]. Dietary supplements that contain ephedra alkaloids are widely promoted and used in the United States as a means of losing weight and increasing energy. In the light of recently reported adverse events related to use of these products, the Food and Drug Administration (FDA) has proposed limits on the dose and duration of use of such supplements. The FDA requested an independent review of reports of adverse events related to the use of supplements that contained ephedra alkaloids to assess causation and to estimate the level of risk the use of these supplements poses to consumers [4-6]. Ma huang has significant vasopressor activity in the pulmonary vascular bed of the cat meditated predominantly by α_1-adrenergic receptor activation [7]. In other findings suggest that Ephedra herb contracts the urethra via arachidonic acidmetabolites together with α_1-adrenoceptor stimulation[8].

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The aim of this study is to provide deliberation of the scientific facts and mechanism of action on vascular system about the Ephedra herb and to touch on the prospects of its future utilization in traditional medicine and in the herbals industry.

MATERIALS AND METHODS

Plant Material: Wild plant *Ephedra Major* was collected during May-June 2011 from Alborz Province, Iran (29°35.49′ N, 10° 51.02′ E) and identified at Department of Biology, Islamic Azad University (IAU) Karaj branch.

Extraction Method: Airial plant parts were aired and subsequently powdered using a mixer for the preparation of hydroethanolic extraction with 70% ethanolic alcohol. Air-dried, powdered plant material (40g) was macerated in 400ml, 70% ethanolic solvent at room temperature for 72 hrs on a rotary shaker. The extract was filtered through Whatman No.1 filter paper (Whatman International, Maidstone, UK) to remove the insoluble materials. After filtration, the extracts was concentrated under reduced pressure at 45°C by rotary vacuum evaporator (IKA RV05, Germany) approximately to 20-30ml and then dried in room temperature. The yield of dry matter from the extract was approximately 12%. The material was stored at 4°C until used. The EC_{50} value of 450µg/mL was used in which experiments that didn't use cumulative concentration of extract[9].

***Ex vivo* Tension Studies in Rat Aortic Rings**

Animals: Experiments were performed on 12 weeks old (220±30g) male Wistar rats. Animals were housed under standard conditions of temperature (21-24ºC), humidity (40-60%) and 12 hrs light: dark cycle at the animal facilities of the Islamic Azad University of Science and research branch. All animals had free access to food and drinking water throughout the study. All animal experiments were conducted in accordance with the NIH Guide for the Care and Use Committee of the Islamic Azad University of Science and research branch[10].

Tissue Preparation: Under anesthesia, thoracic aortas were immediately excised and immersed in cold Krebs solution was blown with 95% O_2 and 5% CO_2, cleared of fat and connective tissue and cut into segments (rings of approximately 4 mm). Each ring was suspended on a isometric transducer (AD Instrument, Spain) with L type stainless steel hook in an organ bath (25 ml) with Krebs-Ringer bicarbonate solution (pH 7.4) which was kept at 37°C and continuously bubbled with 95% O_2 and 5% CO_2, aorta rings were equilibrated upstretched for 60 min. A load of 2g resting tension was applied to each ring by micromanipulator adjustment and the load was readjusted to this level for per 15 min and each readjusted Krebs solution was changed. Alterations in the tone were recorded by aisometric force transducer by use of Lab Chart ver.7 software and a Power Lab/16sp data acquisition system (AD Instruments, Castle Hill, Australia). Following a 60 min period of equilibration, the rings were exposed to 60mM KCl and after 5 min was washout KCl after equilibration of the aorta rings and confidence to viability of smooth muscle cells, arteries were constricted with KCl (60 mM)[11].

In the first and second series of experiments dose response curves to, pre-contraction response by KCl 20mM and 40mM(25 min) were obtained in the presence of Cumulative concentration of *Ephedra major* extract (EME), (20min per concentration). In the third and fourth series of experiments pre-contraction response by phenylephrine 0.01µM and 0.05µM (Phe), (15 min) were obtained in the presence of Cumulative concentration of EME, (20min per concentration). In the fifth and sixth series for the dissection of the role of α-adrenergic agonist mediated Of EME, pre-incubation of Terazosin 10^{-6}M (selective α_1-adrenergic inhibitors) and Yohimbine 10^{-6}M (non-selective α_2-adrenergic inhibitors) for 30 min and were obtained in the presence of effective concentration of EME (20min).

Data Analysis: The constriction response of EME is expressed as percentage (%)KCl (20mM and 40mM) and Phe (0.01µM and 0.05µM) induced pre-contraction. The value (EC_{50}) was determined from the which is the concentration required to produce a maximal half-effect in the concentration-response curves, calculated from log concentration-response curves of EME and pre-contraction by Phe and KCl by sigmoidal non-linear regression analysis within the 95% confidence intervals using Lab Chart ver.7.

Solutions and Drugs: All compounds for Krebs solution and Terazosin and Yohimbine α-adrenergic inhibitors were purchased from Sigma-Aldrich (Sigma-Aldrich Chemise, The Netherlands).

Statistical Analysis: All values are expressed as mean±SEM for n number of rats or separate experiments. Statistical differences were evaluated by analysis of variance (ANOVA) followed by Duncan's new multiple-range test and Student's t-test. A probability level of equal or less than 0.05 (p=0.05) was considered to be significantly different.

RESULT

EC_{50} Calculated: Half Maximal Effective concentration (EC_{50}) calculated from log concentration-response curves of EME and pre-contraction by Phe and KCl by sigmoidal non-linear regression analysis within the 95% confidence intervals using Lab Chart ver. 7.

Contraction Effect of EME on Aorta: To investigate the contraction effect of EME on the aortic rings cumulative dose-response curves were obtained EME pre-contracted with the Kcl 20mM (Figure1. A) and 40 mM (Figure1. B). In the series of KCl 20mM pre-contraction, after pre-contraction, the 10 and 30µg/ml initial adding concentration of EME causes relaxation of aorta and the further concentration 50-400µg/ml causes contraction and then adding 600-1000 again causes relaxation of aorta. In the series of KCl 40mM pre-contraction, after adding concentration10µg/ml of EME causes was somewhat constricted and since adding concentration 30µg/ml of EME causes was somewhat relaxation and the next concentration was same as Kcl 20mM pre-contraction.

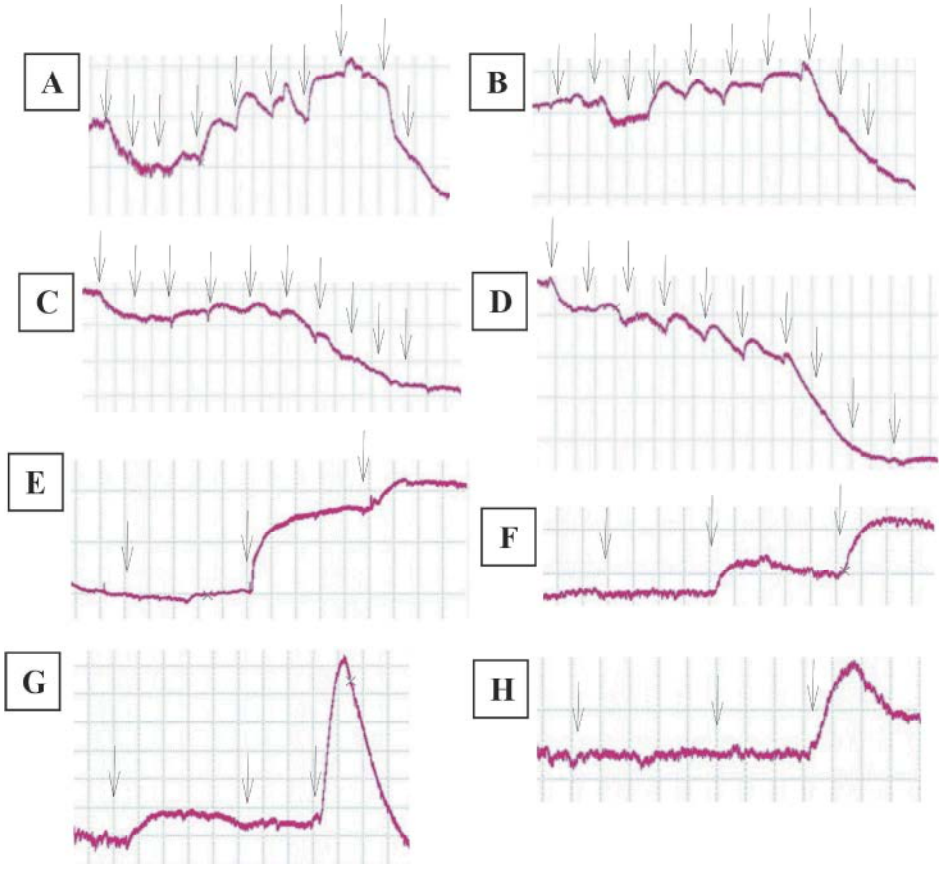

Fig. 1: Real diagram of the contractile responses to *Ephedra* herb in male rat aorta rings. Contractile responses to cumulative concentration (10^{-5} – 10^{-3} mg/ml) of *Ephedra* herb,KCl 20mM pre-contraction (A), KCl 40mM pre-contraction (B), Phenylephrine 0.01µM pre-contraction (C), Phenylephrine 0.05µM pre-contraction (D), 10^{-6} M terazosin incubation, contractile responses to effective concentration of *Ephedra* herb extract (450µg/ml) pre-contracted with KCl 20mM (E) and contractile responses to effective concentration of *Ephedra* herb extract pre-contracted with phenylephrine0.01µM (F), in the presence of 10^{-6} M Yohimbine, contractile responses to effective concentration of *Ephedra* herb extract pre-contracted with KCl 20mM (G) and contractile responses to effective concentration of *Ephedra* herb extract pre-contracted with phenylephrine0.01µM (H).

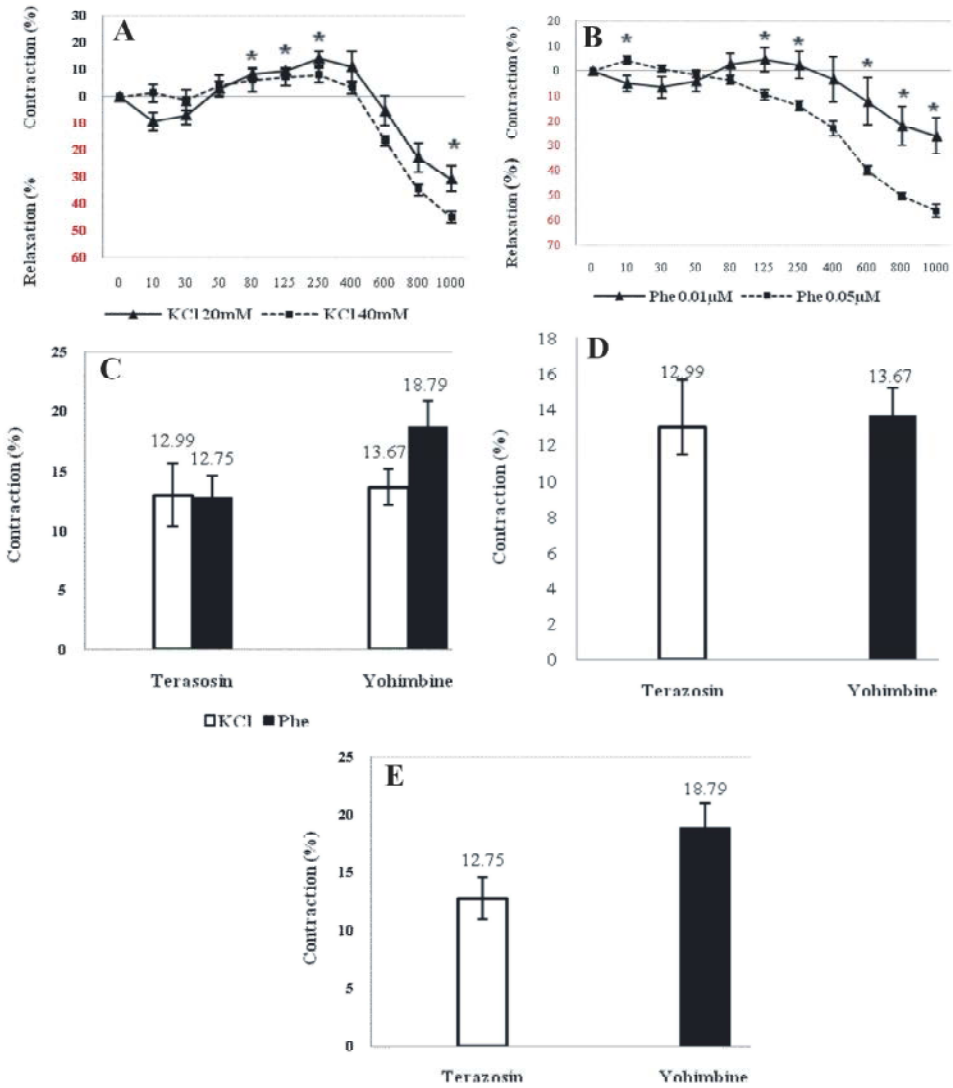

Fig. 2: Comparison between contractile responses to *Ephedra* herb extract in male rat aorta rings KCl 20mM(triangle) and KCl 40mM (Square) pre-contraction (A), comparison between contractile responses to *Ephedra* herb extract in male rat aorta rings phenylephrine 0.01µM and phenylephrine 0.05µM pre-contraction (B),comparison between contractile responses to effective concentration of *Ephedra* herb (450µg/ml) after 10^{-6} M terazosin incubation and Yohimbine incubation, pre-contracted with KCl 20mM and phenylephrine 0.01µM (C), comparison between 10^{-6} M terazosin incubation and 10^{-6} M Yohimbine incubation in pre-contracted with KCl 20mM (D) and comparison between 10^{-6} M terazosin incubation and 10^{-6} M Yohimbine incubation in pre-contracted with phenylephrine 0.01µM (E). Contractions induced by KCl 20mM and Phenylephrine 0.01µM were taken as 100%. n=6, number of aorta rings were used in per groups. Comparisons were made using the paired t-test and ANOVA. Bars mean= S.E.M. *P=0.05.

Comparison between KCl 20mM and 40mM pre-contraction showed the only significant difference exists between the 80, 125, 250 and 1000µg/ml concentration (Figure 2.A).In the third series pre-contracted with Phe 0.01µM (Figure 1.C), after pre-contraction, the 10, 30 and 50µg/ml initial adding concentration of EME causes relaxation of aorta and the further concentration 80-250µg/ml causes contraction and then adding 400-1000 again causes relaxation of aorta. In the fourth series pre-contracted with Phe 0.05µM (Figure 1 D), initial concentration (10µg/ml) of EME causes was somewhat constricted but since adding

concentration of EME causes gradually contraction was decreased. Comparison between Phe 0.01µM and Phe 0.05µM pre-contraction showed the only significant difference exists between the 10, 125, 250,600,800 and 1000µg/ml concentration (Figure 2 B). Terazosin as a selective antagonism ofα_1-adrenergic receptors were used in the Krebs, aortas were incubated 30 min with terazosin, after adding effective concentration an EME the contractile response curves was observed significant difference compared with KCl 20mM (Figure 1 E) and Phe 0.01µM (Figure1. F) pre-contraction but between two pre-contracted groups (KCl and Phe) contractile response curves wasn't observed significant difference (Figure 2. C). Yohimbine as a non-selective antagonism of α_2-adrenergic receptors were used in the Krebs, aortas were incubated 30 min with Yohimbine, after adding effective concentration an EME the contractile response curves was observed significant difference compared with KCl 20mM (Figure1. G) and Phe 0.01µM (Figure 1 H) pre-contraction, but between two pre-contracted groups (KCl and Phe) contractile response curves wasn't observed significant difference (Figure 2 C). Although contraction effect of effective concentration of the extract in Yohimbine incubation group somewhat was higher than the response contraction of the terazosin incubation, in both groups pre-contracted with KCl and Phe, but there was no significant difference between them.

DISCUSSION

The results were shown *Ephedra major* extract have constriction effect on isolated rat aorta rings. But initial concentration of EME causes relaxation of aorta. Although the relaxation response in the KCl 20mM group was less than KCl 40mM group, but there wasn't seen significant difference compared between them. Comparison between phe 0.01µM and 0.05µM groups used as a pre-contraction comparable statistical significant difference was observed in 10µg/ml of EME. EME in lower concentrations with KCl 20mM and Phe 0.01µM pre-contraction causes relaxation on isolated aorta rings, that properties was consistent with other studies onpartial antagonistic effect [12]. For identify and confirm the alpha-adrenergic mediation of alpha agonistic mechanism of extract terazosin (selective alpha 1 antagonism) and Yohimbine (non-selective alpha 2 antagonism) was used as inhibitor. After pre-contraction by KCl 20mM and Phe 0.01µM, adding the EME caused a contractile response in the aorta. Although the contraction observed in the Yohimbine group was more than Terazosin group but there wasn't found statistically significant difference compared between two pre-contracted (KCl and Phe) and between Terazosin and Yohimbine. Flow the present investigation consistent with other research, contractile mechanism wasn't only through α_1-agonistic adrenergic mechanism that induced by extract is releasing internal calcium sources mediated by metabotropic G protein [8]. This constriction effects is due to a adrenergic agonistic mechanism [7] accompanied with other possible mechanism including depolarizing the calcium channels or by mediating the release of vasoconstrictive agent and or inhibition the release of endothelium relaxant.

CONCLUSION

The results were shown *Ephedra major* extract have constriction effect on isolated rat aorta rings. Products containing ephedrine arenot to be used by people with heart disease,hypertension.

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