

The effect of Zizyphus Spina-Christi leaf extract on the isolated rat aorta

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Abstract

Objective: To investigate the effect of Zizyphus spina christi leaf hydroalcoholic extract (ZLHE) on the isolated rat aorta.

Methods: The rings of the endothelium intact and denuded thoracic aorta of Male and female Sprague Dawley rats were placed in Krebs-Henseleit solution to measure isometric contractile force. To study the involvement of voltage dependent L type calcium channels, concentration of 10⁻⁷M verapamil was applied. Potassium chloride (50mM) was also added to the organ bath to compare the effect of extract and KCl. Potassium concentration of the extract at 2.5 and 5mg/ml was measured.

Results: ZLHE induced contraction in the endothelium intact and denuded aorta dose dependently and significantly ($P < 0.0001$). Also, the response to extract at 5 mg/ml was similar to that of KCl (50 mM). The application of verapamil reduced the contraction in the endothelium intact and denuded aorta by $66.7 \pm 3.1\%$ (mean \pm SEM.) and $71.6 \pm 3.8\%$ respectively.

Conclusion: The results showed the vasoconstrictive effect of ZLHE which was not endothelium-dependent and largely blocked by verapamil, suggesting that the voltage-dependent Calcium channels play a pivotal role in the mechanism of action (JPMA 59:537; 2009).

Introduction

Zizyphus spina Cristi (L.) Willd (Rhamnaceae) is a tropical evergreen tree grows in the East Africa and West Asia including Egypt, Nigeria, Saudi Arabia, Yemen, and South of Iran with edible, fresh and dried fruits.¹⁻⁴ It is used in traditional folk medicine for treatment of some diseases such as stomach pain and other gastrointestinal tract ailments, diabetes and diarrhoea.^{1,5} It is believed that its leaves have blood pressure reduction properties as well.⁶ It has been shown that the leaf extract has antimicrobial,^{2,4} antinociceptive,⁷ antidiabetic and antihyperglycaemic effects.^{5,8} Some pharmacological screening studies indicated that the aqueous extract of Zizyphus spina-christi root bark has an antinociceptive activity in mice and rats¹ and a central depressant effect in mice⁹ and the methanol extract of Zizyphus spina-christi stem bark has antidiarrhoeal effects in rats.¹⁰ From the butanol extract of the leaves of Zizyphus spina-christi (L.), Willd. (Rhamnaceae) growing in Egypt, four triterpenoidal saponin glycosides were isolated and named christinin-A, B, C and D, respectively. Christinin-A was the major saponin.¹¹ Hence aim of this study was to evaluate the effect of Zizyphus spina christi leaf hydro alcoholic extract (ZLHE) on isolated rat aorta.

Materials and Methods

Extract preparation:

The leaves of Zizyphus spina christi were collected in March from Ahwaz University of Medical Sciences, identified

and authenticated by the Tropical Plant Research Institute of Ahwaz. The leaves were dried under shadow and powdered to fine particles. The powder was left in 70% ethanol in room temperature for 72 hours and mixed occasionally. The extract was filtered using Whatman filter paper No 1 and dried by evaporating the solvent. A green powder was obtained (yield 11.45% w/w with respect to the dry leaf powder) and was stored in a closed container at 4°C until the use.

Chemicals and solutions:

The Krebs-Henseleit solution as a tissue media was prepared (in mM): NaCl, 118; KCl, 4.7; CaCl₂, 2.52; MgSO₄, 1.64; KH₂PO₄, 1.18; NaHCO₃, 7 and Glucose, 5.5. To avoid changing the organ bath electrolytes composition, the extract and drugs were dissolved in Kerbs-Henseleit solution. All chemicals were from Merck except, verapamil that was from Lek Ljubljana Company (Republic of Slovenia).

Animals and tissue preparation:

All animals used in the present study received humane care in compliance to institutional animal care guidelines. Male and female Sprague Dawley rats (209 \pm 5g) were maintained at 22 \pm 2°C with 12-h light/dark cycle. The animals were housed in the standard cages with free access to food (standard laboratory rodent's chow) and water. The animals were anaesthetized with urethane (1g/kg, I.P.), thoracic aorta was removed rapidly and carefully, placed in oxygenated cold Krebs-Henseleit solution. The aortas removed free of

connective tissue and fat, and then cut transversely into rings of 5mm in length. The rings of aorta submerged in organ bath containing 10ml of Krebs-Henseleit solution (pH 7.4 and 37°C) gassed with oxygen. The rings were mounted by means of two parallel L-shaped stainless-steel holders inserted into the lumen. One holder served as an anchor, while the other was connected to a force-displacement transducer (Harvard UF1) to measure isometric contractile force recorded by a Harvard Universal Oscillograph. A basal tension of 2 g was applied. Each preparation was allowed to equilibrate for 90 min prior to the initiation of the experimental procedures, and during this period, incubation media were changed every 15 min. All dissection procedures were done with extreme care to protect the endothelium from inadvertent damage. In some experiments, the endothelium was rubbed intentionally on the internal surface with a rough stainless steel needle and lack of functional endothelium was tested by applying 10 μ M acetylcholine and absence of relaxation.^{12,13} Aorta contraction force was calculated as mg/mm² of cross-sectional area¹⁴ and presented as mean \pm SEM. The final extract concentrations were 0.25, 0.5, 1, 2.5 and 5mg/ml. Potassium chloride (50mM) was also added to the organ bath to compare the effect of extract and KCl as a known voltage dependent calcium channels opener.^{12,13} Each aorta received up to 2-3 concentrations of extract or KCl (50mM). The time course between applying extract was at least 15 min or returning the tissue tension to basal level. To study, the involvement of voltage dependent L type calcium channels, concentration of 10 μ M verapamil¹³ was applied 5min prior adding the extract (5mg/ml). Potassium concentration of the extract at 2.5 and 5mg/ml was measured by flame photometer SEAC model FP20 (Italy).

Statistical analysis:

Results of different experiments were compared using One Way Analysis of Variance (ANOVA) followed by LSD test and Unpaired t-test appropriately. $P < 0.05$ was considered statistically significant. The number of the animals (n) have been used in each protocol is presented in the figures.

Results

Figure-1 indicates that, ZLHE (0.25, 0.5, 1, 2.5 and 5 mg/ml) induced contraction in the endothelium intact aorta dose dependently and significantly ($P < 0.0001$). Washing the tissues by refreshing the organ bath solution caused the disappearance of the ZLHE vasoconstrictive effect. Also, the response to extract at 5 mg/ml was similar to that of KCl (50 mM). In the endothelium denuded aorta, ZLHE (0.25, 0.5, 1, 2.5 and 5 mg/ml) induced contraction significantly and dose dependently too ($P < 0.0001$) (Figure-2). The response to KCl (50 mM) was also similar to that of ZLHE (5mg/ml). Pretreatment of the aorta (endothelium

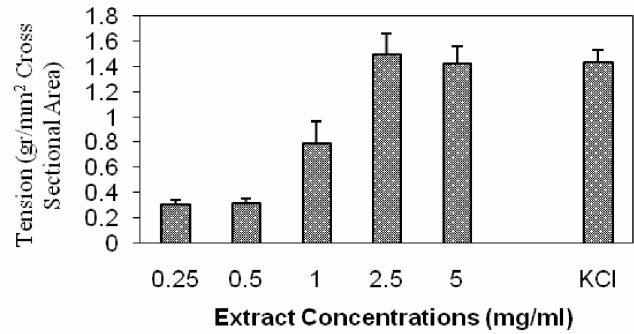


Figure-1: Effect of Zizyphus Spina-Christi leaves hydro alcoholic extract [0.25 (n=4), 0.5 (n=7), 1 (n=10), 2.5 (n=11) and 5 mg/ml (n=8)] and KCl [50 mM (n=3)] on the endothelium intact isolated rat aorta. Values are mean \pm SEM. Statistical significance (extract concentrations) ($P < 0.0001$), ANOVA.

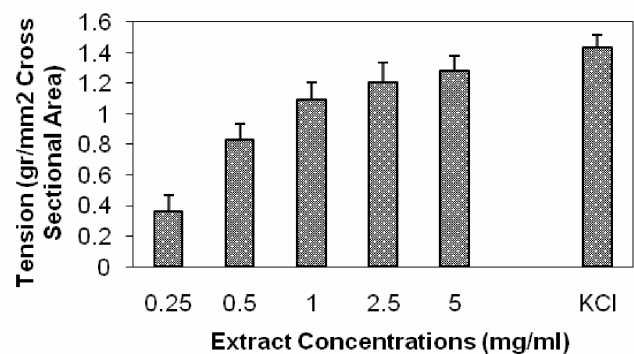


Figure-2: Effect of leaves hydro alcoholic extract of Zizyphus Spina-Christi [0.25 (n=8), 0.5 (n=15), 1 (n=19), 2.5 (n=21) and 5 mg/ml (n=21)] and KCl [50 mM (n=12)] on the endothelium denuded isolated rat aorta. Values are mean \pm SEM. Statistical significance (extract concentrations) ($P < 0.0001$), ANOVA.

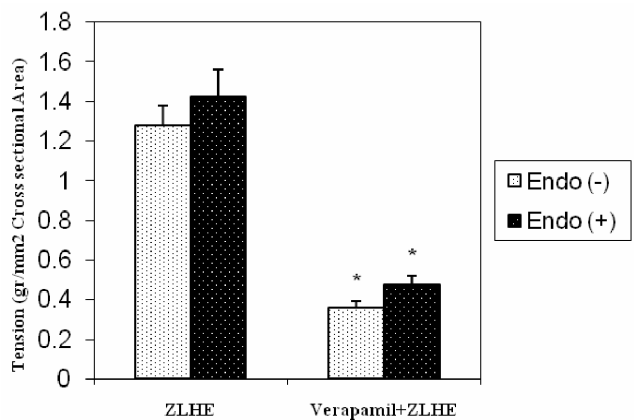


Figure-3: The effect of Pretreatment of the endothelium intact (+E), (n=11) and denuded (-E), (n=9) aorta with 10 μ M verapamil on the response to 5 mg/ml Zizyphus Spina-Christi leaves hydro alcoholic extract (ZLHE). Values are mean \pm SEM. * ($P < 0.05$) versus 5 mg/ml ZLHE in equivalent group, (Unpaired t-test).

intact and denuded) with 10 μ M verapamil (as a calcium channel blocker) for 5 minutes prior to the application of ZLHE (5mg/ml), reduced the contraction in the endothelium intact aorta (n=11) by 66.7 \pm 3.1% and in the

endothelium denuded aorta (n=9) by $71.6 \pm 3.8\%$ which are not significantly different from each other (Figure-3).

Discussion

The obtained results in this study indicate that, the *Zizyphus spina christi* leaf extract induces contraction in the rat aorta. The vasoconstrictive effect was significant and dose dependent. Washing the tissue by refreshing the organ bath solution caused the disappearance of the ZLHE vasoconstrictive effect. Therefore, it may be postulated that the effective component(s) of the extract should be dissociated from the postulated target(s) easily and the extract exert a reversible and not poisonous effect. This vasoconstrictive effect is in contrast with the responses obtained from the same extract on rat ileum¹⁵ and uterus¹⁶ in which, the ZLHE has induced relaxation in these two organs, indicating that there is a difference in tissue responsiveness to the same extract. The presence or absence of the endothelium had no effect on the aorta contraction induced by the extract indicating that the vasoconstrictive effect of the ZLHE is not endothelium dependent and possibly the extract acts on the aorta smooth muscle directly. It is not related to cholinergic activity of extract too because extract caused contraction in both endothelium intact and denuded aortas. In order to characterization the mechanism of antidiabetic and insulintropic action of the botanol extract of *Zizyphus Spina-Chisti* leaves, it has been shown that major saponin glycoside of *Zizyphus Spina-Chisti* leaves, christinin A dose dependently inhibit diazoxide induced relaxation in the norepinephrine contracted isolated rabbit aorta strips.⁵ Therefore, it has been postulated that christinin A and so botanol extract of *Zizyphus Spina-Chisti* leaves act via KATP channels to increase membrane potential and Ca^{2+} influx and consequently insulin secretion to exert antidiabetic activity.⁵ In this study verapamil (L-type Ca^{2+} channel blocker) reduced the vasoconstrictive effect of ZLHE, significantly showing that also this effect largely is dependen on the Ca^{2+} entry through the voltage-dependent Ca^{2+} channels too. It is consistent with previous mentioned mechanism of the effect of christinin A and so the botanol extract of *Zizyphus Spina-Chisti* leaves. On the other hand, one of the possible explanations was the high potassium content of the extract. The analysis of potassium content of the extract however, revealed that, the extract potassium concentration at 2.5 and 5 mg/ml in organ bath are one fortieth and one twentieth of 50 mM of KCl, respectively. The vasoconstrictive effect, therefore, are not induced by potassium content of the extract itself. Taken together, the above results showed that the vasoconstrictive effect of the Hydro alcoholic leaves extract of *Zizyphus Spina-Chisti* is not endothelium-dependent. In fact, this activity was largely blocked by

verapamil, suggesting that the vasoconstriction mainly is dependent on the flux of Ca^{++} into the smooth muscle cells through voltage-dependent Calcium channels directly, or on the basis of the other studies probably indirectly via KATP channel blockade and consequent depolarization. These vascular effects provide an explanation of its potential hypertensive effect of the extract rather than blood pressure lowering action of that which is believed in folkloric medicine. Also, we may postulate that the present extract induces different activities on the different smooth muscles. Further work is required to clarify the mechanism of the action of the extract in different tissues.

Acknowledgment

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