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Comparative study on the vasorelaxant effects of three alkaloids-rich extracts from seeds of *Peganum harmala* L.

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Abstract

The effect of seed methanolic, ethyl-acetate and chloroformic extracts of *Peganum harmala* L. on KCl- and Nor-adrenaline-induced contractions of aortic strips was studied. In aortic strips with endothelium intact, contractions induced using 80 mM KCl and 10^{-6} M Nor-adrenaline were dose-dependently relaxed by the extracts, a more significant effect being seen with Nor-adrenaline-induced contractions. Following mechanical damage to the aortic endothelium, the results showed that the inhibition of contraction by the three extracts was not endothelium dependent. The rank order of relaxation potency was MeOH > EtoAc> $CHCl_3$-extracts of *Peganum harmala* L. The results suggest that the relaxation effect of the three alkaloids-rich extracts may be attributed to the inhibiting of the AMP cyclic nucleotide PDE (Phosphodiesterase).

Keywords: *Peganum harmala* L., extracts, aortic, vasorelaxation. Corresponding author:

1. Introduction

Peganum harmala L. (Zygophyllaceae), commonly named (harmal), grows spontaneously in uncultivated and steppes area from south of Spain and south-east Morocco (semiarid and predesertic regions) [1]. *Peganum Harmala* L. is used in traditional medicine as an antihelmintic, lactogogue, antispasmodic, antipyretic, abortifacient, emetic [2,3]. anticancerous [4] antiviral [5]. and antihypertensif plant[1]. The principal compounds of this plant are alkaloids and are known for their vasorelaxant [6] antimicrobial [7,8]. hypothermic [9,10]. and hallucinogenic properties [11,12]. There were also some report concerning the cardiovascular

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actions of harmala alkaloids such as harmine which reduces systemic arterial blood pressure and total peripheral vascular resistance and the effect of harmalol on these two parameters are inconsistent [13]. In addition, these compound also have antioxidative [14]. platelet aggregation inhibitory and immunomodulatory effects [15]. In the present study the comparative effect of methanolic (MeOH), chloroformic ($CHCl_3$) and ethyl acetate (EtOAc) extracts from the seeds of *Peganum Harmala* on vascular smooth muscle were investigated with the purpose of validate its ethno-medicinal uses.

2. Materials and Methods

2.1. Plant

Peganum harmala L. (Zygophyllaceae) fresh seeds were collected from the medium of atlas region (Morocco) and identified in our laboratory where a voucher specimen has been deposited in the medicinal plant unity.

2.2. Preparation of extract

Fresh and powdered seeds were successively extracted in a soxhlet apparatus with Petrol-ether (60-80°C), $CHCl_3$ (Trichlormethan), EtOAc (Ethyl acetate) and MeOH (Methanol). The obtained extracts were concentrated under reduced pressure to yield dry residues: 0.67, 1.42, 2.8, 31% (w/w), respectively.

2.3. Animals

Female and male Wistar rats (12 –14 weeks old) and weighing 250-300 g were used. Animals were maintained in a temperature-controlled room (22 ± 2°C) under a light-dark cycle of 12h, with standard rat chow with free access to drinking water. The animals were killed by a blow on the head and the aorta was rapidly dissected.

2.4. Aortic rings preparation

The descending thoracic aorta was placed in a modified Krebs-Henseleit solution (K+1), containing (mM): NaCl 118, KCl 4.75, $NaHCO_3$ 25, $MgSO_4$ 1.2, $CaCl_2$ 1.8, KH_2PO_4 1.2 and glucose 11. After excess fat and connective tissue were removed, the aorta was cut into 2-3mm rings. Aortic rings were mounted under the basal tension of 2g in 20ml organ baths containing (K+1) and attached to an isometric transducer (Pioden UF-1) and the signal was recorded by a Powerlab[å] data acquisition system (AD-Instruments). The tissue bath was maintained at 37°C and bubbled with a 95% O_2-5% CO_2 gas mixture. In some experiments, the endothelium was mechanically removed by delicately rubbing the intimal surface. Removal of the endothelium was then verified by addition of acetylcholine chloride (ACh 10^{-6}M. Sigma chemical Co, USA) in aortic rings previously contracted by phenylephrine hydrochloride (Phe 10^{-6}M. Sigma chemical Co, USA). Each preparation was allowed to equilibrate for at least 60min prior to initiation of experimental procedures, and during this period the incubation media was changed every 20 min [16]. After equilibration, the following experiments were performed: Aortic rings were contracted by single sub-maximal concentration of Nor-adrenaline bitartrate salt (Nor-adrenaline 10^{-6}M. Sigma chemical Co, USA) or KCl (80mM). When the contractile response to either agonist was stable, the different extracts of *Peganum harmala* were added in progressively increasing cumulative concentration (1, 2, 5, 10 and 25 mg/ml) at 30 min intervals. The results were expressed as a percentage of the maximal control agonist-induced responses.

2.5. Statistical analysis

Results were expressed as a percentage from the initial pre-contraction level and as mean ± SEM for the number *n* determinations obtained from different animals. Analysis of variance

(ANOVA) followed by Tukey´s Multiple Comparisons test were used for statistical analysis. P<0.05 values were considered to present a significant difference. Doses-responses slopes were analyzed to give the concentration of compound producing a 50% inhibition of the maximal contractile response (IC_{50}) using a linear regression analysis. Statistical analyses were performed using Prism 4.0 version software.

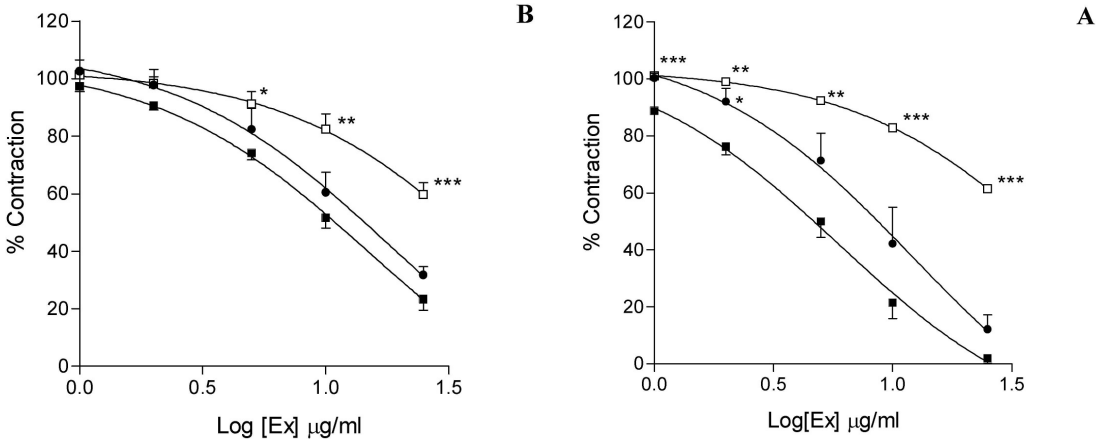

Fig 1. Relaxation response-curves of methanolic, ethyl-acetate and chloroformic extracts on NA 10^{-6}M (A) and KCl 80mM (B)-induced contractions in isolated aortic rat. EX: Extract. (¨) $CHCl_3$-extract, (l) EtoAc-extract, (<) MeOH-extract. *P<0.05, ** P<0.01, *** P<0.001 vs MeOH-extract.

Table 1

	IC_{50} (µg/ml)	
Extract	**NA(10^{-6})**	**KCl(80mM)**
MeOH	5,93 ± 1,02	14,94 ± 1,14
EtoAc	12,13 ± 1,23 **	17,87 ± 1,2
$CHCl_3$	55,32 ± 1,05 ***,###	55,45 ± 1,28 ***,##

IC_{50} values of MeOH, EtoAc and $CHCl_3$-extract to inhibit the contractions induced by Nor-adrenaline and KCl in the aortic rings. **,## P<0.01, ***, ### P<0.001. * vs MeOH, # vs EtoAC.

3. Results

Addition of high KCl (80mM) or Nor-adrenaline (10^{-6}M), produced a contractile response which averaged (1.98 ± 0.10g and 2.42 ± 0.16, n = 6, respectively). At concentrations > 1µg/ml, all extracts of *Peganum harmala* L. inhibited in a concentration dependent manner the contractile response induced by both stimulatory agents (Fig 1). Cumulative increases in the concentrations of all extracts (1-25 µg/ml) in endothelium-intact aortic rings previously contracted with 80mM KCl and 10^{-6}M Nor-adrenaline also resulted in a concentration-dependent relaxation.

High concentrations of MeOH, EtoAC and $CHCl_3$-extracts (25µg/ml) produced respectively (76.62%, 68.30% and 40.36%) in aortic rings previously pre-contracted by 80 mM of KCl, whereas acetylcholine at concentration of (O.55 mM) produced 50% of relaxation [17].

The relaxation obtained in NA pre-contracted aorta, was positively correlated with *Peganum harmala* extracts concentrations [23.79% with MeOH, 8% with EtoAc and 1% with $CHCl_3$-extracts, at the dose of 2µg/ml to 98%, 87.6%

and 38.47% respectively at the dose of 25µg/m]. The relaxation effects on KCl and noradrenaline-induced contractions were not altered in the absence of a functional endothelium (data not shown).

It was observed (data not shown) that the extracts alone have no effect on the non-precontracted aortic rings. The IC50 values for Nor-adrenaline and KCl-induced contractions showed that the rank order of relaxation potency was MeOH> EtoAc>$CHCl_3$-extracts of *P. harmala*. (Table-1)

4. Discussion

The aim of this work was to study the comparative effects of the methanolic (MeOH), chloroformic ($CHCl_3$) and ethyl acetate (EtOAc) extracts from the seeds of *Peganum Harmala* on vascular smooth muscle and to investigate the purpose of validate its ethno-medicinal uses.

The contractile responses induced by Nor-adrenaline are associated with several mechanisms; the phasic component seems to depend on calcium release from the intracellular stores while the tonic component depends on calcium flux and activation of several proteins such as protein kinase C (PKC) [18, 19].

The three extracts inhibited this contraction in a dose-dependent manner, and the relaxation induced in aorta pre-contracted with Nor-adrenaline was not affected by endothelial removal which indicated that the vasodilator effects of these extracts were unrelated to the release of endothelium derived factor and acts directly on smooth muscle cells. This effect was not distinct from that of certain alkaloids present in *P. harmala* such as harman, which act on both endothelial and vascular smooth muscles cells [20].

On the other hand, the contractile responses induced by high KCl (80mM) are due to the influx of extracellular Ca^{2+} through L-type voltage-sensitive channels (VOCs) [21, 22]. The extracts inhibited these contractile responses in a concentration-dependent manner. As shown in the first case with Nor-adrenaline, the relaxant effects of extracts were not endothelium-dependent in KCl-induced contraction. Vasodilator effect of alkaloids was already been reported in the literature. Recently we demonstrated that the methanolic extract of peganum (MeOH) exert a vasodilatory effect by inhibiting the AMP cyclic nucleotide PDE (Phosphodiesterase) and have a non-competitive antagonism effects on a_1-adrenoseptors [6]. In the other study, Shi et al. [23] have reported that the vasorelaxant effect of harmine and harmaline are attributed to their actions on the endothelial cells to release NO (nitric oxide) and the vascular smooth muscles to inhibit the contraction induced by the activation of receptor-linked and voltage-dependent Ca^{2+} channels.

In conclusion, the present finding suggest that the vasorelaxant effect of the three Alkaloids-rich extracts can be attributed to the b-carboline-alkaloids and that the methanolic extract of *Peganum harmala* present the potent vasorelaxant effect compared to the Ethyl Acetate and chloroformic extract. These results open a large window to more investigate the mechanism of action of this extract and his use as a new drug in the treatment of some related cardiovascular disease such as hypertension.

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