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Original Article

Pharmacological Effects of *Peganum harmala* Seeds Extract on Isolated Rat Uterus

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Abstract

The effects of hydroalcoholic extract of *Peganum harmala* seeds (EPS) on spontaneous rhythmic contractions of isolated rat uterine was investigated in this study. EPS was tested on the isolated uterus and endometrium free (i.e. stripped myometrium) preparations. EPS was found to exhibit significant spontaneous contractions of the uterus and stripped myometrium relative to the solvent control. After recording the pattern of uterus tissue spontaneous motility, in order to determine mechanism of EPS pharmacological effects, atropine, indomethacin, or prazocin was added into the organ baths. Pretreatment with atropine (70 nM) in both the whole uterus and in the stripped myometrium preparations had no effects on the response to cumulative dosage of EPS. Calcium-free solution decreased the uterus contractions. In calcium dose-response curves, EPS in some concentrations produced uterotonic effect in calcium-free solution in the presence of KCl. This finding showed that EPS may increase calcium influx through voltage-dependant calcium channels.

Keywords: Oxytocic; *Peganum harmala*; Stripped myometrium; Uterus contraction.
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1. Introduction

Peganum harmala L. (Zygophyllaceae) or Syrian Rue is a perennial herbaceous plant which is widely distributed in dry areas from Mediterranean east to northern India. *Peganum harmala* is widely known and used herb in its native areas. This plant is known as "Espand" in Iran, "Mexican Rue" or "Turkish Rue" in United States. *Peganum harmala* is a widespread species growing wild in Iran and has been used as antiseptic by burning its seeds [1, 2]. The seeds yield a dye ('Turkish red' or 'Syrian red') long used in "Persian" carpets. The plant is used traditionally as an emmenagogue and an abortifacient agent in the Middle East and North Africa [3, 4]. Abortion is frequent in animals that digested this plant in dry year. Seeds and roots contain β-carboline alkaloids, mostly harmine, as well as harmaline, harmalol, harman, peganine, isopeganine, dipeganine and quinazolin derivatives such as vasicine, vasicinone and deoxyvasicinone. Alkaloidal content of the

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unriped seeds is less than the ripe ones [4-7]. These alkaloids have a wide spectrum of pharmacological actions including alteration of uterine contractions induced by drugs or phytochemicals. It, therefore, is of great importance in obstetrics practice, as it could lead to disruption of normal course of parturition. This study investigated the potential mechanisms involved in its effect.

2. Materials and methods

2.1. Preparation of Peganum harmala seeds extract

Seeds of *Peganum harmala* were collected locally around East Azarbyjan province, Iran. The powdered seeds (100 g) were defatted with petroleum ether in a soxhlet apparatus and were then extracted by maceration in 70% MeOH (4 x 1l). The extract was evaporated under reduced pressure below 60°C to give a final yield of 18.62% hydroalcoholic extract of seeds. For standardization of the extract β-carboline alkaloids in the EPS were determined by HPLC [4]. Harmine, harmaline and harmalol were found as bright blue florescent zones in the RF=0.1, RF=0.25 and RF=0.75, respectively. Harmine, the active fraction of EPS was further separated by preparative TLC [8] and used as a pure sample. Then the EPS was analyzed by using HPLC on a Shimadzu LC-6A Chromatograph and a 4.6 mm i.dx250 mm Lichrosorb reverse-phase column [4]. The amount of harmine of the EPS was determined as 1.8%.

2.2. Chemicals and tissue bathing medium

Atropine sulphate as a nonspecific muscarinic antagonist was purchased from Sigma Company. Indomethacin (Sigma) was used as a prostaglandin synthesis inhibitor. Prazocin hydrochloride as an alpha receptor antagonist was purchased from S.A. Ajinomoto Ominichem N.V. Ominichem division. EDTA (Sigma) was used for chelating calcium. Tissue bathing medium: Krebs solution (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.6, $NaHCO_3$ 24.3, KH_2PO_4 1.18, glucose 5.6. Indomethacin was dissolved in ethanol and EPS was dissolved in water.

2.3. Evaluation of pharmacological activity

Wistar female rats (250-300 g) were injected with estradiol valerate (0.1 $mgKg^{-1}$ s.c.) 24-48 h before experiments and were then euthanized by inhalation of ether. The uterine horns were dissected out and opened longitudinally. The tissues were placed in 20 ml organ baths filled with Krebs solution aerated with 5% CO_2 in oxygen. The bath temperature was maintained at 37 °C. Isotonic contractions against a load of 1 g were recorded mechanically. The tissue strips were allowed to equilibrate for a period of 1 h, and the spontaneous rhythmic contractions were observed. Following the equilibration period, effect of cumulative concentrations of EPS (6.25-400 μg/ml) on the spontaneous contractions of the uterus was recorded by successive increase in EPS concentration in the tissue bath with the contact time of 15 min. for each concentration [9-12]. The frequency of contractions was obtained per min. [13] and the strength of contractions was given in milligrams [9-12]. In another sets of experiments, strips were pretreated with atropine (70 nM) [9], indomethacin (20 μM) [9] or prazocin (0.5910^{-4} M) [11], 15 min. before the addition of cumulative

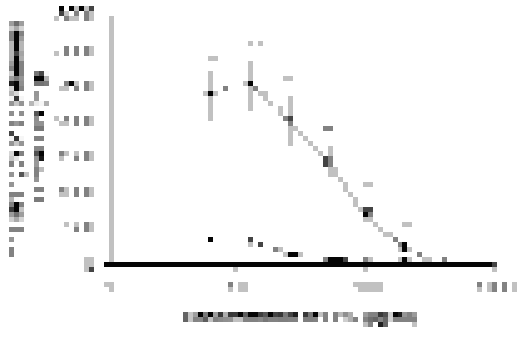

Figure 1. Effects of different concentrations of EPS on the contractions of the whole uterus. Each point represents mean S.E.M. (n=5; $^{**}p<0.05$).

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Figure 2. Effect of different concentrations of EPS on the contractions of stripped myometrium. Each point represents mean S.E.M. (n=5; $^{**}p<0.05$).

Figure 3. Effect of EPS on the frequency of contractions (n=5).

concentrations of EPS (6.25-400 µg/ml). In order to evaluate possible calcium independent mechanism, EDTA was added to calcium-free Krebs solution and in this condition the effect of EPS was evaluated. In another sets of experiments, the uterus strips were bathed for 2 h in nominally calcium-free Krebs solution, and then exposed for an additional 15 min. to a high-K^+ (60 mM) nominally calcium-free solution (depolarizing medium). Soon afterwards, cumulative concentration-response curves for $CaCl_2$ were obtained. Maximal response to $CaCl_2$ (0.0128 M) from control curve was taken as 100%, and all concentrations of EPS were calculated as a function of this value. EPS (12.5 and 50 µg/ml) was added to the preparations for 15 min., then the second and third cumulative concentration-response curve for $CaCl_2$ were obtained [14-16].

The results were summarized in the Microsoft Excel 2003 and analyzed statistically using the ANOVA, Post test, Tukey's and Unpaired t-test.

3. Results

The spontaneous rhythmic contractions of the whole uterus and stripped myometrium were increased by EPS related to the control (Figures 1 and 2). EPS increased the frequency of contractions per min. initially and then it was decreased compared to the control (Figure 3).

Pretreatment with atropine (70 nM) had no effect on the response of EPS in both whole uterus and stripped myometrium (Figure 4). Administration of indomethacin (20 µM) or prazocin (0.5910^{-4} M) also did not have any effect on the response to the EPS on the whole uterus (Figures 5 and 6). In calcium-free solution, EPS showed a decrease in the contractions of the whole uterus (Figure 7).

Figure 4. Effect of EPS in the presence and absence of atropine (70 nM) in both whole uterus (A) and stripped myometrium (B), (n=5).

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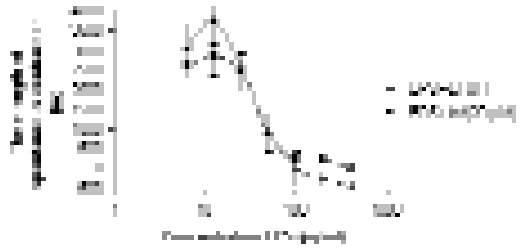

Figure 5. Effect of EPS alone or after pretreatment with Indomethacin (20 μM). Each point represents mean S.E.M. Indomethacin was administered 15 min. before addition of the EPS (n=5).

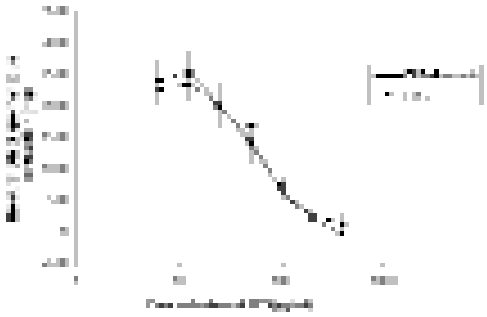

Figure 6. Effect of EPS alone or after pretreatment with prazocin (0.5910^{-4} M) on whole uterus. Each point represents mean S.E.M. Prazocin was administered 15 min. before the addition of EPS (n=5).

The mean cumulative concentration-response curve for $CaCl_2$ alone and in the presence of 12.5 and 50 μg/ml of EPS showed that EPS in some concentrations produced uterotonic effect in calcium-free solution.

4. Discussion

Under suitable physiological conditions, isolated mammalian uteri are capable of exhibiting spontaneous rhythmic contractions and are able to respond to oxytocic agents in a similar fashion to their *in vivo* activity.

The results obtained in this study have shown that EPS was able to directly initiate and maintain contractions in both whole uterus and stripped myometrium. Atropine (70 nM) pretreatment, as a non specific muscarinic antagonist [9], did not change the contractile response to EPS in both whole uterus and stripped myometrium. Indomethacin (20 μM), as a prostaglandin synthesis inhibitor had no significant effect on responses of the whole uterus to EPS. These findings suggest that unlike *Agapanthus africanus* and *Harpagophytum procumbens*, two oxytocic herbs which are used as a traditional medicine by South African women to induce or augment labor [9], muscarinic receptors and prostaglandins have no effect on contractions induced by EPS. The ability of EPS to modulate Ca^{2+} entry was assessed by obtaining concentration-response curve to $CaCl_2$ in the absence or in the presence of different concentration of EPS (12.5 and 50 μg/ml). In depolarizing medium (KCl 60 mM), EPS in some concentrations augmented uterotonic effect induced by cumulative addition of $CaCl_2$ in calcium-free solution in depolarizing medium. However, $CaCl_2$ did not induce any contraction in the absence of KCl, and EPS did not change these conditions. In other experiments in the absence of KCl in calcium-free solution plus EDTA, EPS did not have any contractive effects. Therefore, EPS did not have any calcium independent contractive effects. These findings indicate that the high concentration of KCl depolarized the membrane and increased calcium influx through voltage-dependant calcium channels and EPS in some concentrations facilitated this action. The extracellular (external) calcium is necessary for the uterotonic effect of EPS. In conclusion, the results of this study suggests that EPS-induced contractions of

Figure 7. Effect of EPS in calcium-free solution on the whole uterus (n=5).

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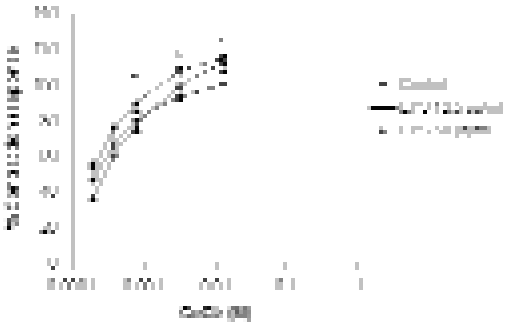

Figure 8. Effect of different concentrations of EPS on the cumulative concentrations of $CaCl_2$ in nominally calcium-free depolarizing medium (KCl 60 mM) in isolated rat uterus. The concentrations of EPS were 12.5 and 50 µg/ml. Data are reported as mean S.E.M for 5 preparations.

uterus are not dependent on prostaglandins, muscarinic and alpha receptors. These contractions are related to external calcium.

References

[1] Rechinger KH. *Flora Iranica*, Graz: Akademische Druck – u.Verlagsanstalt, 1982; pp.18-20.

[2] Amin G. *Popular medicinal plants of Iran*. Tehran: Iranian research institute of medicinal plants, 1991; p. 85 .

[3] Mahmoudian M, Jalilpour H, Salehian P. Toxicity of *Peganum harmala*: Review and a case report. *Iranian J Pharmacol Therap* 2002; 1: 1-4.

[4] Kartal M, Altun ML, Kurucu S. HPLC method for the analysis of harmol, harmalol, harmin and harmaline in the seeds of *Peganum harmala* L. *J Pharmaceut Biomed Anal* 2003; 31:263-9.

[5] Al-Shamma A, Drake S, Flynn D, Mitscher A, Bark YH, Rao GSR, Simpson A, Swayze K, Veysolu T, Wu TS. Antimicrobial agents from higher plants. *J Nat Prod* 1981; 14: 745-7.

[6] Manske RHF, Holmes HL. *The alkaloids chemistry and physiology*. Volume II, 1st ed., New York: Academic Press INC, 1952; pp. 393-4.

[7] Swan GA. *An introduction to the alkaloids*. 1st ed., New York: Joun Wiley & Sons INC, 1961; pp. 205-6.

[8] Wagner H, Bladt S. *Plant drug analysis*. 2nd ed., England: Springer, 1995; p. 30.

[9] Veale DJM, Oliver DW, Havlic I. The effects of herbal oxytocics on the isolated stripped myometrium model. *Life Sci* 2000; 61: pp. 1381-8.

[10] Adebiyi A, Ganesan A, Prasad RNV. Tocolytic and toxic activity of papaya seeds extract on isolated rat uterus. *Life Sci* 2003; 74: pp. 581-92.

[11] Veale DJH, Havlik I, Oliver DW, Dekker TG. Pharmacological effects of *Agapanthus africanus* on the isolated rat uterus. *J Enthnopharmacol* 1999; 66: 251-62.

[12] Perry WLM. *Pharmacological experiments on isolated preparations.* 1st ed. London: Churchill Livingstone, 1970; pp. 92-5.

[13] Czerski A, Zawadzki W, Zawadzki M, Czerska Z. Influence of dopamine on rat uterine motility *in vitro*. *Acta Vet Brno* 2005; 74: 9-15.

[14] Lee HA, Seong Y, Lee WJ. 11-β-Estradiol inhibits calcium-dependant but not calcium independent contractions in isolated rat aorta. *Naunyh Schmiedebergs Arch Pharmacol* 2005; 371: 152-7.

[15] Guedes DN, Silva DF, Barbosa-Filho JM, Medeivos IA. Calcium antagonism and the vasorelaxation of the rat aorta induced by rotudifolone. *Brazilian J Biol Res* 2004; 37: 1881-7.

[16] Shi CC, Chen SY, Wang GJ, Liao JF, Chen CF. Vasorelaxation effect of harman. *European J Pharmacol* 2000; 390: 319-25.

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