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Original Article

Spasmodic versus spasmolytic activities of *Euphorbia spinidens* extract on rat isolated uterus

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Abstract

Preterm contraction of uterus is a main cause of miscarriages and preterm labour. *Euphorbia* known as Ferphion in Iranian traditional medicine texts like Al-Hawi, is reported for prevention of preterm labour. Therefore, the objective of this research was to investigate the effect of *Euphorbia spinidens* Bornm. Ex Prokh. on motility of rat uterus. Uterine horns were isolated form non-pregnant female rats pretreated with estrogen. *E. spinidens* hydroalcoholic extract was examined on KCl (80 mM) induced and spontaneous periodic contraction in isolate uterine strips suspended in an organ bath and compared with nifedipine and ritodrine. In isolated rat uterine strips, *E. spinidens* extract (1-500 µg/mL) showed mixed effects. At lower concentrations, firstly potentiated the spontaneous periodic contraction, while in concentrations above 256 µg/mL the spontaneous periodic contractions were completely attenuated. These findings demonstrated that although lower concentrations of hydroalcoholic extract potentiated the spontaneous periodic contraction of rat uterine smooth muscle, but at higher concentrations it had inhibitory effect on rat uterus contraction.

Keywords: *Euphorbia spinidens*; Uterus; Spasmodic; Spasmolytic; KCl

INTRODUCTION

Preterm labour is one of the major causes of newborn mortality (1). Despite significant progress in management of preterm labour, still the most suitable and economical approach is prevention of preterm labour by relaxing uterus contraction (2). β_2-Adrenoceptors agonists like ritodrine are a class of compounds widely used for relaxation of uterine smooth muscles (3). Calcium channel blockers like nifedipine are also employed, despite the fact that their cardiovascular effects often are not acceptable (4).

Cyclo-oxygenase (COX) inhibitors like indomethacin and oxytocin receptor antagonists like atosiban are another group of tocolytic drugs which are used to extend pregnancy period and prevent preterm labour (5,6).

Iranian traditional medical texts like Al-Hawi of Razes (864-930 AD) stated that "Ferphion (Euphorbia) closes uterine entrance and if given before abortion it will prevent spontaneous abortion" (7).

In Iranian traditional medicine, Ferphion is also suggested as diuretic, laxative, antispasmodic, antiasthmatic, emetic, relieving stomachache, and for menorrhagia treatment (7,8). But, it is generally regarded as a toxic plant and despite its relaxant effects, there are other reports in traditional medicine that if Ferphion is apply into uterine of pregnant women, it will kill the fetus (7). Therefore, its beneficial and / or harmful effects ought to be evaluated with the standard pharmacological techniques.

The objective of this research was to investigate relaxant effects of *Euphorbia spinidens* an endemic *Euphorbia* species of Iranian flora on isolated uterus smooth muscle contraction induced with KCl in comparison with nifedipine and ritodrine as standard tocolytic drugs.

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MATERIALS AND METHODS

Plant material

E. spinidens was collected from north of Mashad (Iran) in June 2011, at an altitude of 1700 meter above sea level. The plant was identified in the School of Pharmacy and Pharmaceutical Sciences, Isfahan, Iran, where a voucher specimen (No. 3533) was deposited. The air dried aerial parts of plant material were roughly cut and ground to a coarse powder. It was extracted with aqueous ethanol (70%) using maceration method for three days. Hydroalcoholic extract was concentrated at 40 °C in reduced pressure using rotary evaporator. Dried extract was kept frozen at 0 °C until use.

Drugs and Solutions

The following drugs were used in this research: *E. spinidens* extract, ritodrine (Reig Jofre Pharm. Co, Spain), nifedipine (Sigma, Germany), estradiol valerate (Aburaihan Pharm. Co, Iran). 17-β-estradiol prepared in cooking oil as 100 μg/mL stock solution.

Stock solution of the extract was prepared as 20 mg/mL solution in dimethyl sulfoxide (DMSO), and serially diluted with distilled water to obtain 2 mg/mL and 200 μg/mL solution. Ritodrine (1 mg/mL) stock and diluted solutions were prepared in distilled water. Nifedipine was prepared as 20 mg/mL stock solution in DMSO and diluted in distilled water. Different concentrations of plant extract and standard drugs were added into organ bath (Bath volume was 20 mL). Tyrode's solution composed of: NaCl, 136.9; KCl, 2.68; $CaCl_2$, 1.8; $MgCl_2$, 1.05; $NaHCO_3$, 11.9; NaH_2PO_4, 0.42; and glucose 5.55 (in mM) was prepared in distilled water. Unless stated, all chemicals and drugs were from Merck Company (Germany).

Isolation of uterine strips

Wistar rats (180-220 g body weight) were given food and water *ad libitum*, and kept at animal house of School of Pharmacy and Pharmaceutical Sciences at Isfahan University of Medical Sciences. The rats were killed in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals, as recommended and confirmed by the Ethics Committee of Isfahan University of Medical Science with reference No. 194085 (9). They were injected subcutaneously 100 μg of 17β-estradiol to synchronize the estrous cycle and to obtain a spontaneous periodic contraction of myometrium (10). After 24 h, uterine horns were isolated and placed into oxygenated Tyrode's solution at room temperature. They were cut into four segments and placed in a chamber filled with 20 mL. Tyrode's solution bubbled continuously with oxygen at 37 °C.

Uterine contractile assessment

In each group six uterus strips were used to examine the effects of extract/drug on myometrial contractility. Each experiment was performed in parallel with vehicle-treated time-matched controls. The contractility of the uterine strip was monitored by an isotonic transducer (Harvard, England) with 1 g tension, and recorded on a Harvard Universal Oscillograph (England) pen recorder device. After calibrating the oscilograph, tissues were washed thrice and allowed to relax to a stable base line. After 30 min equilibration, the strip was preincubated with KCl (80 mM) for 15 min, and then exposed to the successive concentrations of extract at 1, 2, 4, 8, 16, 32, 64, 128, 256, and 500 μg/mL. In a separate set of experiments, effect of extracts or vehicle was examined on spontaneous uterine contractions *in vitro*. Effects of standard drugs ritodrine and nifedipine were also examined using similar protocol.

Measurements and statistical analysis

Contractions were assessed as amplitude of recorded response and presented as percentage of initial response. All the values are quoted as mean ± standard error of the mean (SEM). The significance of differences between the means was calculated by one-way analysis of variance (ANOVA) for repeated measures or two tailed Student's *t*-test. Differences were considered statistically significant for $P < 0.05$.

The IC_{50} value (drug concentration causing 50% of maximum response) of relaxant in isolated uterus was calculated for each tissue and stated as mean and SEM for each group of results. Sigma plot computer program (version 11) was used for statistical analysis and construction of the graphs for calculation of IC_{50} values.

RESULTS

Effect of extract on rat uterus

Uterine strips, isolated from pretreated rat with estrogen spontaneously contracted in a periodic manner in the organ bath (Fig. 1). In the isolated uterine strip, KCl (80 mM) caused a sustained tonic contraction. Hydroalcoholic extract at a concentration range of 1-500 µg/mL, caused a concentration dependent relaxation on KCl-induced uterus contraction (Fig. 2) with IC_{50} value of 36 ± 8.9 µg/mL (n = 6). The maximal inhibition was achieved with 500 µg/mL extract in the organ bath.

The inhibitory effect of the extract was reversible upon washout. Statistical analysis revealed no significant difference in contraction of vehicle- treated time matched control tissues over the course of study (Fig. 2). Ritodrine (5-320 µg/mL) in a concentration dependent manner relaxed KCl (80 mM) induced contractions of rat uterus with an IC_{50} value of 83 ± 9.2 µg/mL. Total inhibition was achieved with 320 µg/mL ritodrine in the bath (Fig. 3). Nifedipine (0.1-0.8 ng/mL) in a similar manner inhibited tonic contraction induced by KCl. Nifedipine at 0.8 ng/mL bath concentration completely blocked the KCl-induced contraction (Fig. 3).

Effect of the extract was also examined on the spontaneous periodic contraction of rat uterus (Figs. 1 and 4). Hydroalcoholic extract in concentration range of 1-256 µg/mL potentiated the spontaneous periodic contraction of rat uterus whereas in the control group the periodic spontaneous activity of the uterine strips were gradually attenuated over the time (Fig. 4).

Unlike the extract, both ritodrine and nifedipine abolished the spontaneously periodic contraction of rat uterus.

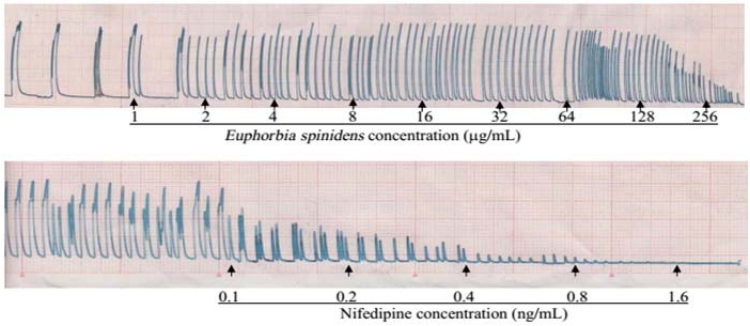

Fig. 1. Typical recording of spontaneous periodic contractions of isolated rat uterus pretreated with estrogen. Uterine strips were treated with either hydroalcoholic extract of *E. spinidens* (top trace) or nifedipine (bottom trace).

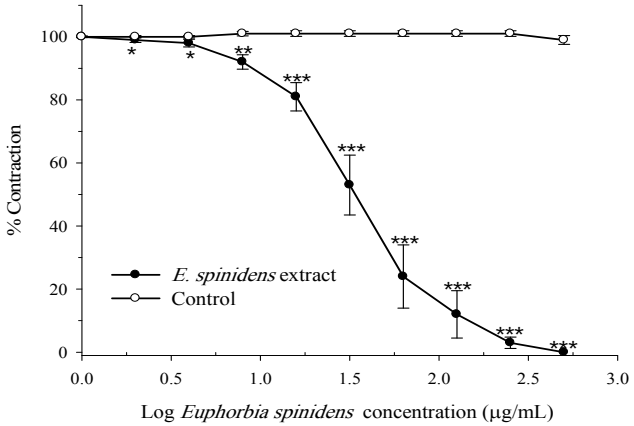

Fig. 2. Inhibitory effect of *E. spinidens* extract on tension developments in the isolated uterus of non-pregnant rat treated with KCl at 80 mM. Contractile response was measured relative to the baseline. Ordinate scales: spasm remaining as percent of the contraction prior to compounds addition. Abscissa scales: $\log_{10}$ concentration of compounds. Each point is mean of six experiments and the vertical lines show the SEM. The asterisks show significant differences for each concentration with its corresponding control group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (*Student's t-test*). Maximum concentration of DMSO in the bath was 2.5%.

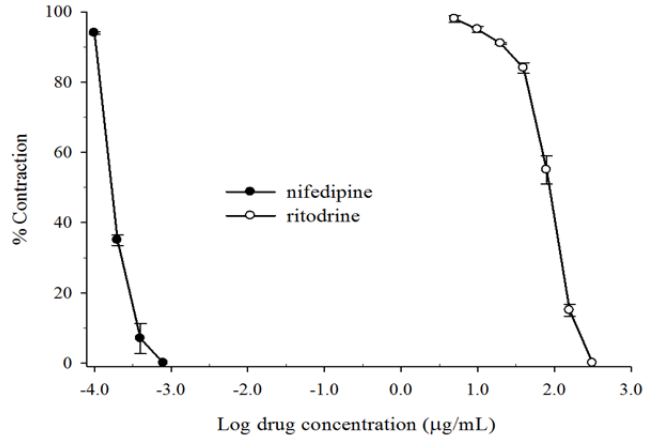

Fig. 3. Inhibitory concentration response curve of nifedipine and ritodrine on tension developments in the isolated uterus of non-pregnant rat treated with KCl at 80 mM. Contractile response was measured relative to the baseline. Ordinate scales: spasm remaining as percent of the contraction prior to compounds addition. Abscissa scales: $\log_{10}$ concentration of compounds. Each point is the mean of six experiments and the vertical lines show the SEM. Maximum concentration of DMSO in the bath was 0.08%.

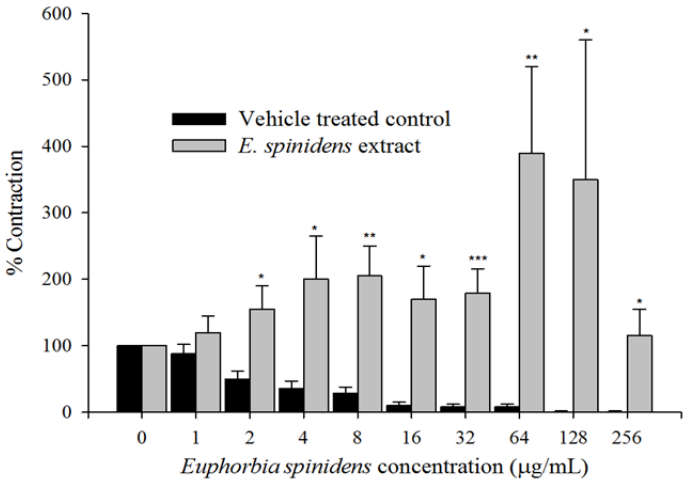

Fig. 4. Stimulatory effect of *E. spinidens* extract on spontaneous periodic contractions of isolated rat uterus pretreated with estrogen. Contractile responses are product of frequency and amplitude of the contraction over 5 min periods. Ordinate scales: percent spasm of the contraction prior to compounds addition. Abscissa scales: concentration of *E. spinidens*. Each bar is the mean of six experiments and the vertical lines show the SEM. **P*< 0.05, ***P* < 0.01, ****P* < 0.001 in comparison with initial concentration (*Student's t-test*). Maximum concentration of DMSO in the bath was 2.5%.

DISCUSSION

Preterm labour increase subsequent serious chronic health problems for infants and even mortality in the first year (1). The uterine contraction originates in the myometrial cells as pacemakers and give rise to a conducted action potential which could be regulated by both hormonal and neuron activities (11,12). Traditional medical books like Al-Hawi of Razes, stated that "Ferphion closes uterine entrance and if given before abortion, it will prevent spontaneous abortion" (7). In this paper, we studied the antispasmodic effects of *E. spinidens* extract on isolated rats uterus pretreated with estrogen. *E. spinidens* extract (1-500 µg/mL) concentration-dependently relaxed KCl-induced contractions in isolated strips of non-pregnant female rats. The standard drugs ritodrine and nifedipine also concentration-dependently inhibited uterine contraction. Ritodrine acts through the activating β_2-adrenoceptor which increases adenylyl cyclase activity and production of

intracellular cAMP, which inhibits contractile proteins like myosin and actin in smooth muscles (13). Inhibition of KCl induced contraction of rat uterus by hydroalcoholic extract and nifedipine might be due to the inhibition of voltage gated calcium channels. There are two sources for intracellular Ca^{2+} ions. One is the influx of Ca^{2+} from the outside cells through voltage-dependent calcium channels, and the other is the release of Ca^{2+} from intracellular storage sites (14). Addition of high concentration of KCl into the organ bath fluid caused cell depolarization. It consequently activates voltage dependent L-type calcium channels which are responsible for calcium ion entering into the myometrium cells (15). A rise in intracellular Ca^{2+} concentrations causes myosin heads attach to the actin. Once they attached, the actin spirals and pulls the myosin which activate smooth muscle contractions (15).

The spasmodic effect of the extract is in consistence with the report which suggests that Ferphion is toxic and may kill the fetus (7). The mechanism of action of stimulant substances in smooth muscle include activation of different excitation-contraction coupling mechanisms: (i) an acceleration of action potential discharge or an increase in spike amplitude may increase the cytoplasmic Ca^{2+} concentration and inward Ca^{2+} current responsible for the upstroke (16); (ii) a direct increase in Ca^{2+} permeability may be produced through receptor-operated channel activation (13,16).

Observation of both spasmolytic and spasmodic effects of the extract indicates the presence of different bioactive compounds. In order to get a selective response these components ought to be separated from each other, otherwise the raw extract is not suitable for treatment of preterm labour. Phytochemically, triterpenes, macrocyclic diterpenes, and tannins, are the major components in *Euphorbia* species which could be responsible for the observed effects (17,18). It is in agreement with a macrocyclic diterpene with myrsinane skeleton isolated from *Euphorbia connata* and *Pycnocycla spinosa* which showed potent relaxant effects on KCl-induced contraction in rat ileum (19,20).

CONCLUSION

In summary, we demonstrated that *E. spinidens* extract possess both inhibitory and excitatory properties on isolated rat uterus. It explains why some people suggested Ferphion for prevention of preterm labour, while other considered it as a toxic remedy for fetus. Therefore, the total extract is not a suitable remedy for treatment of preterm labour. Bioactivity guided isolation of *E. spinidens* extract is required to isolate the inhibitory components with pure antispasmodic effects.

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