ISSN 1819-1878

Asian Journal of Animal Sciences

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Asian Journal of Animal Sciences 6 (6): 300-308, 2012
ISSN 1819-1878 / DOI: 10.3923/ajas.2012.300.308

Cimicifuga racemosa Potentiates Antimuscarinic, Anti-adrenergic and Antihistaminic Mediated Tocolysis of Buffalo Myometrium

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ABSTRACT

Ethnoveterinary practices hold a promising scientific resource and can have far-reaching implications on economic development and enhancement of veterinary health care system. The present study was undertaken to evaluate the oxytocic/tocolytic activities of *Cimicifuga racemosa* (*C. racemosa*) roots extracts on isolated uterine strips of pregnant buffalo. Hot methanolic extraction of authenticated roots of *C. racemosa* was done using soxhlet extractor. Hot methanolic extract of *C. racemosa* roots was found to exert a myometrial relaxant effect which was potentiated after inhibition of excitatory muscarinic, alpha and beta adrenergic, H_1-histaminergic and 5-HT receptors. Further studies are required on mechanistic aspects of tocolytic effect of *C. racemosa* particularly to elucidate the involvement of Ca^{2+} and K^+ channels, NO and other signaling mechanisms including second messengers.

Key words: *Cimicifuga racemosa*, buffalo, myometrium, alcoholic, pharmacodynamic, tocolysis

INTRODUCTION

A wide range of herbal remedies are advocated in folklore medicine for female reproductive problems including hormonal imbalances like long postpartum estrus (Rahman and Rahman, 2006; Mota-Rojas *et al*., 2007), endometriosis and other fertility disorders (Attitalla, 2011; Risvanli, 2011). *Cimicifuga racemosa* (*C. racemosa*), known as black cohosh, has been used in Indian medicine for relieving pain during menstruation and childbirth (Little *et al*., 2003; Hutchens, 1991; Foster and Duke, 2000). It achieved special prominence as a "partus preparator," given to women in the last 4 weeks of pregnancy to aid in childbirth (McKenna *et al*., 2001). A phenolic compound, isoferulic acid present in *C. racemosa* has been found to exhibit anti-inflammatory activity (Hirabayashi *et al*., 1995) by significantly reducing the proinflammatory cytokines (Marotta *et al*., 2006) thus reducing the muscular spasms (Shibata *et al*., 1980). *In vitro* estrogenic activity of fukinolic acid present in *C. racemosa* has also been reported (Kruse *et al*., 1999). More recently, black cohosh has also been advocated for treatment of menopausal issues, including hot flashes, joint aches and neurovegetative symptoms (Jellin *et al*., 2004). In traditional Chinese medicine, it has also been used for uterine and rectal prolapse. The old age ethnoveterinary practices seem to possess a promising scientific resource and can have far-reaching implications on economic development (Preciado *et al*., 2011) and enhancement of veterinary health care system (Hashemi and Davoodi, 2012). Therefore, throughout the world, there is an urgent need to avoid abuse and misuse of ethno-medicine knowledge, as well as to validate and standardize such practices (Sarwar *et al*., 2011).

In view of the promising pharmacodynamic activity of *C. racemosa* cited in ancient literature (including those on uterus), the present study was undertaken to evaluate tocolytic activity of hot alcoholic extract of *C. racemosa*.

MATERIALS AND METHODS

Preparation of hot extract of raw plant material: Authenticated roots of *C. racemosa* finely chopped, shade dried and coarsely ground.

The coarsely ground roots of *C. racemosa* was extracted with methanol (1:5) in Soxhlet extractor (40±5°C continuously for 20-22 cycles). The extract was dried at moderate temperature (37°C) to obtain the residual material.

Collection of uterine tissues: Complete uterine along with the ovaries of adult nondescript buffaloes were collected from local abattoir, Mathura. Uterine tissue were collected from mid cornual region in case of early stage of pregnancy while in case of mid or late stage of pregnancy, complete corneal part of uterine tissue was collected. The gestation stage was determined by measuring Curved-crown Versus Rump (CVR) length of foetus by applying the formula (Soliman, 1970):

- When CVR length was below 20 cm, Y = 28.66+4.498X
- When CVR length was 20 cm or above, Y = 73.544+2.256X

where, Y is the days of gestation and X is curve crown rump length in centimeters.

Uterine tissues were transported to laboratory in a thermos flask containing chilled (4°C±0.5°C) Ringer-Locke solution (RLS, pH 7.4) (Singh *et al*., 2008).

Solutions of chemicals/antagonist: Solutions of the chemicals [Atropine sulphate 10^{-3}-10^{-4} M (Merck); Ketanserin tartrate 10^{-3} M (Sigma Aldrich); Indomethacin 4 mg mL^{-1} (Sigma Aldrich); Prazosin 10^{-2} M (Sigma Aldrich); Propranolol 10^{-3} M) (Sigma Aldrich) used for evaluation of uterotonic activity in the present study were prepared as stock solutions in triple distilled water unless specified otherwise and stored at 4°C. Further dilutions of the required concentration were made in freshly prepared RLS on the day of use.

Uterotonic/oxytocic studies

Preparation and mounting of uterine tissue: Uterine strips were dissected out from the mid-cornual region and a perimetrial strip of about 3.0×0.5 cm was prepared by carefully removing the endometrial and myometrial tissues. Both the ends of strip were threaded and mounted in a thermostatically controlled (37.0±0.5°C) organ bath of 10 mL capacity containing continuously aerated RLS.

Calibration of physiograph and recording of responses: The change in tension of tissue was measured using a high sensitivity isometric force transducer and recorded in a PC using Chart V5.4.1 software programme (Powerlab, AD Instruments, Australia). Sensitivity of the instrument was set as required and the sampling rate was adjusted to 5 samples sec^{-1}. The bridge amplifier was calibrated at two points- Point 1:0 mV = 0 g and Point 2:1 mV = 1 g and signal range was kept at 10 mV.

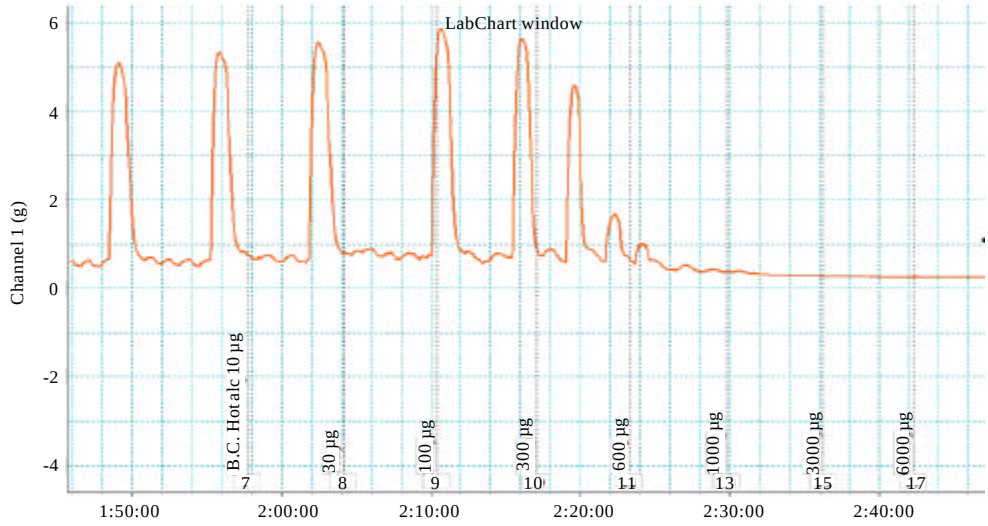

Fig. 1: Representative physiographic recording of the effect of cumulative concentrations of HMECRR (10-6000 µg) on perimetrial strip of pregnant buffalo

After calibration of the equipment, myometrial strip was set under an initial constant tension of 2 g and allowed to equilibrate for 90-120 min till the tissue developed regular phasic contractions (Fig. 1) before recording isometric muscle tension or response to plant extracts or drugs. During the equilibration period, the bath fluid was changed every 10-15 min.

Qualitative studies on the effect of *Cimicifuga racemosa* root extract on buffalo uterus: Working solutions of *C. racemosa* extracts (10, 30, 100, 300, 600, 1000, 3000, 6000 µg mL^{-1}) were prepared afresh on the day of experiment. Different concentrations of *C. racemosa* roots extracts were added in organ bath to record the responses of uterine tissues and also to record the minimum threshold concentration required for producing uterotonic effect.

Contact period of different plants extracts: Different concentrations of extracts of *C. racemosa* roots were added to the tissue bath in an increasing manner. Each concentration was allowed an initial contact period of 5-10 min and thereafter till the maximum effect of a particular concentration was evident. Once the maximal response was achieved the tissue was washed. A minimum washout period of 30 min was allowed between two successive recordings. Then, the uterine strips was incubated with different concentrations of the antagonist (Atropine sulphate, Ketanserin tartrate, Indomethacin, Prazosin,; Propranolol) in the organ bath for 10 min and again the different concentrations of the extract were added. After recording the tissue response(s), bathing fluid in organ bath was changed every 10 min till the base line was achieved.

RESULTS

Effect of crude hot methanolic extract of *Cimicifuga racemosa* roots (HMECRR) on myometrial contractility: *Cimicifuga* roots extracts produced an apparent increase in amplitude of spontaneous contractions at bath concentrations of 30 and 100 µg mL^{-1} (0% relaxation), however, with further increase in bath concentration up to 6000 µg mL^{-1} (100% relaxation), there

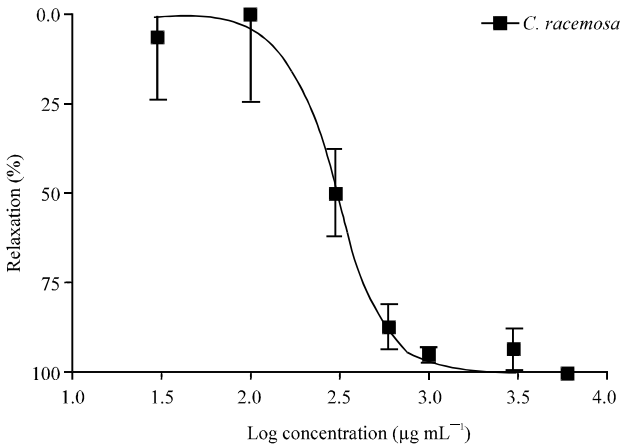

Fig. 2: Cumulative concentrations response curve of HMECRR on isolated myometrial strips of pregnant buffaloes, n = 6, Values are Mean±SE

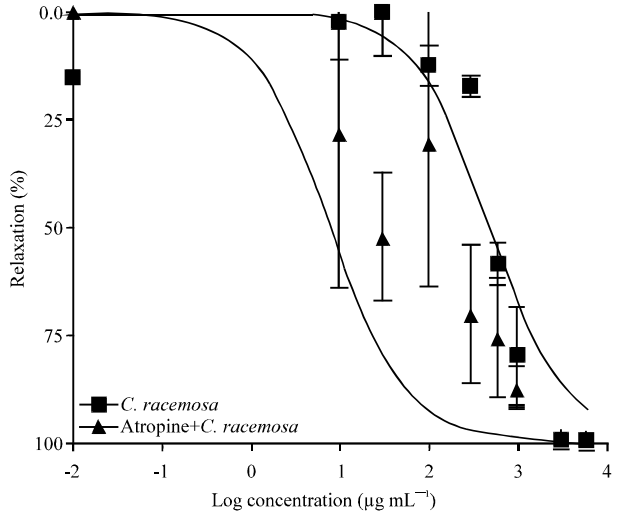

Fig. 3: Cumulative concentration response curves of HMECRR alone and in the presence of atropine sulphate (0.1 µM) on pregnant buffaloes isolated myometrial strips, n = 3, Values are Mean±SE

was marked decrease in amplitude of spontaneous contractions (Fig. 1, 2). After recording the maximal effect, bath fluid was drained off and repeated washings were given over a period of 45-60 min till the tissue regained its initial rhythmicity.

Pharmacodynamics of the effect of HMECRR

Effect of atropine sulphate: HMECRR produced inhibitory effect on myometrial contractility. Atropine sulphate (0.1 µM) pretreatment resulted in a decrease in tissue tension and a leftward shift of the HMECRR-induced myometrial rhythmic activity indicating potentiation shown in Fig. 3. The tissue tension was markedly reduced in the presence of atropine sulphate.

Effect of prazosin hydrochloride: The amplitude of spontaneous rhythmic spikes of myometrium was markedly reduced by prazosin revealing the antagonistic effect of prazosin

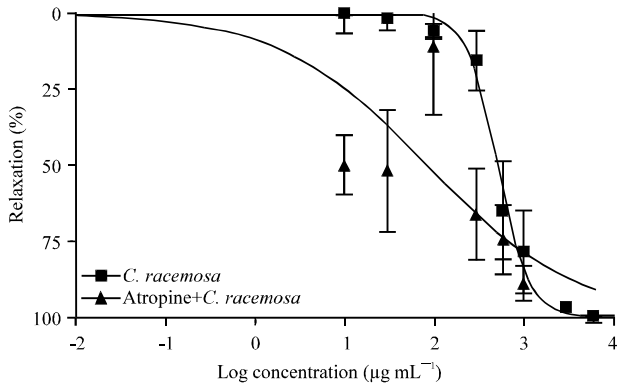

Fig. 4: Cumulative concentration response curves of HMECRR alone and in the presence of prazosin hydrochloride (10^{-5} M) on pregnant buffaloes isolated myometrial strips, n = 6, Values are Mean±SE

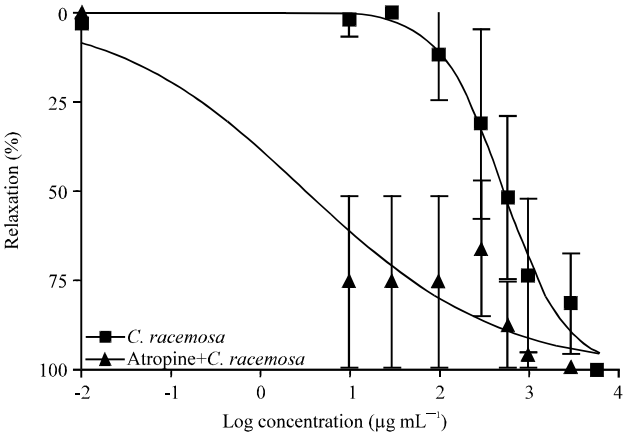

Fig. 5: Cumulative concentration response curves of HMECRR alone and in the presence of mepyramine (1 µM) on pregnant buffaloes isolated myometrial strips, n = 6, Values are Mean±SE

hydrochloride on HMECRR-induced uterotonic effect up to 600 µg mL^{-1} bath concentration. The dose-response curve of HMECRR was shifted leftward, thereby revealing pronounced inhibitory effect of HMECRR after inhibition of α-adrenergic receptors (Fig. 4).

Effect of mepyramine: Mepyramine (1 µM) produced complete inhibitory effect on tissue tension and rhythmic spontaneity of buffalo myometrium as shown in Fig. 5. HMECRR almost completely failed to produce any appreciable or apparent alteration in myometrial spontaneity or rhythmic contractions. The data reveals leftward shift of the dose-response curve of HMECRR in the presence of mepyramine i.e., HMECRR-induced tension in tissue was reduced.

Effect of ketanserin: In the presence of ketanserin (1 µM), not only normal spontaneity of the tissue was reduced but HMECRR also produced more marked inhibitory effect in rhythmic contractions of buffalo myometrium indicated by a dose dependent fall in tissue tension (Fig. 6). Concentration dependent inhibitory effect of HMECRR on myometrial contractions was further reduced in the presence of ketanserin and the DRC was shifted leftward.

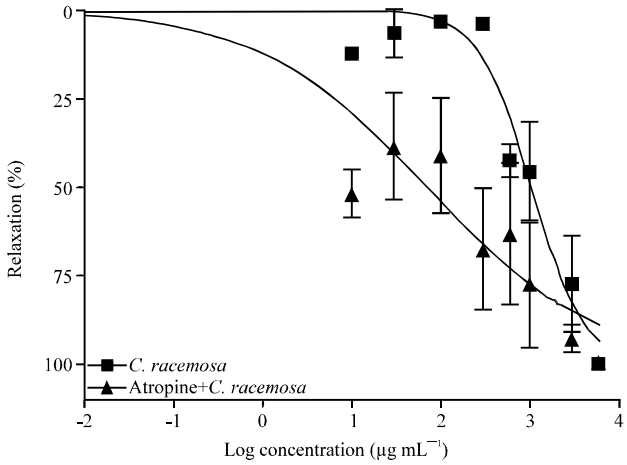

Fig. 6: Cumulative concentration response curves of HMECRR alone and in the presence of ketanserin (1 µM) on pregnant buffaloes isolated myometrial strips, n = 6, Values are Mean±SE

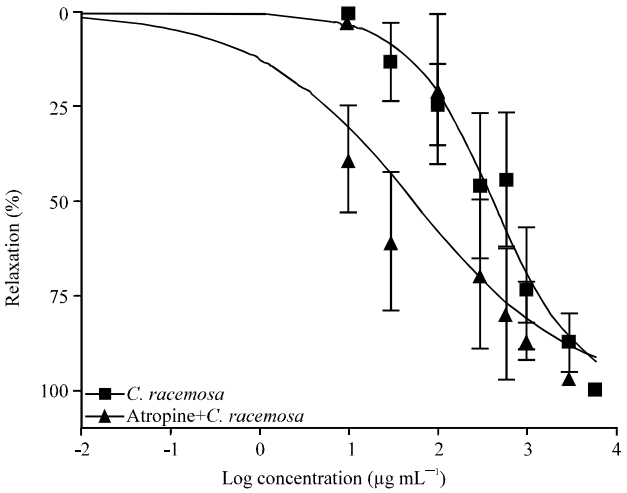

Fig. 7: Cumulative concentration response curves of HMECRR alone and in the presence of propranolol (10^{-6} M) on pregnant buffaloes isolated myometrial strips, n = 4, Values are Mean±SE

Effect of propranolol: The inhibitory effect of HMECRR on myometrial rhythmicity was further inhibited i.e., potentiated in the presence of propranolol as the dose response curve was shifted to left (Fig. 7).

DISCUSSION

Herbal remedies are becoming indispensable and constitute an integral part of primary health care systems (Bafor *et al*., 2009). *C. racemosa* is widely used to relieve premenstrual symptoms, dysmenorrheal and menopause symptoms and the effect is similar to hormones. In homeopathy, it is used for treating discomfort in late pregnancy, headache, depression as well as for labor pains. Therefore, in the present study, an attempt had been made to work out the possible mechanism(s) of uterotonic/tocolytic effect(s) of *C. racemosa* roots extract using isolated uterine strips of pregnant buffaloes.

Conventionally, for studies on oxytocic/abortifacients or/and tocolytics, rat uterus is used as an experimental model (Elvotiz and Mrinalini, 2004). The patterns of myogenic contractions in buffalo uterus are almost comparable to human myometrium(Bradley *et al*., 1998) Moreover, the isolated buffalo uterine strips are highly sensitive to different spasmogens, oxytocic and tocolytic agents and it exhibits very consistent responses (Narayan *et al*., 1993; Garg *et al*., 2004, 2005). Therefore, isolated uterine strips from pregnant buffaloes were used in the present study to validate the ethno-practices for human as well as veterinary use.

In the present study, hot methanolic extracts of *Cimicifuga racemosa* produced consistent, rapid and sharp dose-dependent inhibitory effect on myometrial spontaneity at concentration of >100 μg mL^{-1} as the spontaneous rhythmic contractions completely inhibited. On washing the tissue, most of the tissues exhibited almost complete return of spontaneity indicating the reversible nature of inhibition.

For probing mechanism of HMECRR-induced myometrial effects, atropine, prazosin, propanolol, ketanserin and mepyramine were employed. Atropine (0.1 μM) potentiated the HMECRR-induced inhibitory effect, thus evidently suggesting the existence of excitatory muscarinic cholinergic receptors in buffalo myometrium and blockade of these receptors resulted in pronounced inhibitory effect of HMECRR. Presence of M_4 receptors has been reported in myometrium of rat (Pennefather *et al*., 1994) as well as their role in the contractile response of uterine circular muscles (Kitazawa *et al*., 1999) is well documented. The effect of estrogen on hippocampal M_4 muscarinic receptors has also been reported in earlier studies which may have functional significance especially in relation to postmenopausal memory problems (Bafor *et al*., 2009; Patil *et al*., 2009; El-Bakri *et al*., 2002).

Buffalo myometrium is known to possess α-excitatory and β-inhibitory adrenergic receptors (Narayan *et al*., 1993). Prazosin (10^{-5} M), an α-adrenergic receptor antagonist, markedly potentiated HMECRR-induced buffalo myometrial relaxation as there was marked decrease in tissue tension and the cumulative concentration response curve was shifted leftward. Based on these observations, existence of α1 adrenergic receptors in buffalo myometrium is substantiated and following blockade of these excitatory receptors, inhibitory effect of HMECRR was potentiated.

Uterine strips of buffaloes have been found to be extremely sensitive to histamine. Mepyramine (1 μM) pretreatment almost inhibited the spontaneity of the myometrium. The inhibitory effect of HMECRR was potentiated by mepyramine as there was conspicuous decrease in myometrial tissue tension and dose-response curve was shifted leftward, thus suggesting that because of inhibition of excitatory H_1 receptors, HMECRR produced more pronounced inhibitory effect on buffalo myometrium. Further, the possibility of mediation of HMECRR effect through the inhibitory H_2 receptors can also be not ruled out as H_2 receptors have been reported to be present in myometrium of different species of animals.

Propranolol (10^{-6} M), beta adrenergic receptor blocker, produced a concentration-dependent potentiating effect on HMECRR-induced myometrial relaxant effect. Involvement of selective β_2 adrenoceptors in induction of tocolysis in buffaloes has already been reported (Garg *et al*., 2004). Blockade of β adrenergic receptors resulting into augmentation of HMECRR-inhibitory effect similar to that of inhibition of α_1 receptors also suggests involvement of certain obscure inhibitory ion-channels-pathways or signaling mechanism which need elucidation using specific chemicals.

In the presence of ketanserin (1 μM), the cumulative dose response curve of HMECRR was significantly shifted towards left. Thus possibility of existence of excitatory 5-HT receptors and their involvement in mediating myometrial rhythmicity cannot be ruled out. The exact pathophysiology

of hot flashes seen due to estrogen deficiency remains obscure but clearly estrogen receptive neurons in the hypothalamus utilizing monoamines serotonin, dopamine and norepinephrine are involved (Smith and Jennes, 2001). Inhibition of 5HT receptors with resultant potentiation of HMECRR inhibitory effect on buffalo myometrium was similar as obscured with the blockade of cholinergic, adrenergic (α,β) and histaminergic receptors, therefore, further studies are required to be undertaken.

From the results of the present study, it may be inferred that *Cimicifuga* roots methanolic extract exerts myometrial relaxant effect which is potentiated after inhibition of excitatory muscarinic, alpha adrenergic, H_1-histaminergic and 5-HT receptors and β-receptors. Further studies are warranted on mechanistic aspects of tocolytic effect of the extracts particularly to elucidate the involvement of ion channels, NO and other signaling mechanisms including involvement of second messengers.

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