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The effects of *Cassia italica* leaves aqueous extract on non-pregnant uterus contraction in rats

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Abstract

Background: Cassia italica or Senna is a wild plant distributed in south of Iran. It increased water consumption and has a laxative effect. In traditional medicine this plant has been used for the dysmenorrheal.

Objective: In this study we investigated the effect of aqueous extract of Cassia italica on uterus contraction.

Materials and Methods: Virgin Wistar rats 200-300g were purchased. After laparatomy, a piece of uterus (1.5cm) was excised and mounted in an organ bath (10ml) containing De Jalon (29°C) and isometric contractions were recorded under 1g tension. KCl (60mM) was used to produce contractions. Cassia italica extracts after washing were added at 0.5, 1, 2 and 4 mg/ml cumulatively to the organ bath and the contractions were recorded. Uterus was separately incubated with atropine sulfate (10μM), metoclopramide (10μM) and oxytocin (10mU/ml) and the tissue spasmodic effect of the extract were recorded.

Results: Cumulative concentrations of the extract (0.5-4 mg/ml) increased the basal, peak and frequency of uterus contractions, dose-dependently ($p<0.001$). Incubation of the tissue with atropine sulfate and metoclopramide did not reduce the spasmodic effect of the extract. Cassia italica extract was shown the oxytocic activity on the uterine smooth muscle which most concentrations of the extract (4mg/ml) were more potent than of oxytocin (10mU/ml).

Conclusion: Cassia italica stimulated the uterus contractions without involving dopaminergic (D2), and muscarinic receptors. This extract has oxytocin mimetic effects on uterus. Since the extract has uterus contraction, therefore we suggest that more study will be necessary about abortive or contraceptive effects of this plant on pregnant uterus.

Key words: *Cassia italica, Atropine, Oxytocin, Uterus.*

Introduction

Cassia italica is a wild plant distributed throughout the Arabian Peninsula (1) and has the scientific name of Senna italica Miller in Iran.
The form of this plant is sprig on the ground and its leaves have purgative effect in the humans and is a treatment for constipation (2).

The ethanolic extract of the whole plant parts of Cassia italica was investigated for bioactivities: namely anti-inflammatory, antipyretic, analgesic and prostaglandin (PG) release by rat peritoneal leucocytes, antineoplastic and antiviral (3). The crude ethanolic extract of Cassia italica has CNS depressant properties, manifested as antinociception and sedation (4). The laxative effects of the plant have been partly related to anthraquinone compounds. One of the side effects of anthraquinones is griping and the presence of

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antispasmodic flavonoid compounds in the plant may decrease griping effect. The percent of flavonoids in Iranian Senna determined 1.5 fold than Cassia angustifolia. These flavonoids may decrease these effects of Senna anthraquinones (5). In one study about the effects of Cassia italica leaves and pod extracts on intestinal motility in vitro, plant stimulates intestinal contractions with dose-dependent relation. Moreover, Cassia italica contractile activity was comparable to the acetylcholine and was inhibited by atropine (6). Since, in traditional medicine, one of the major uses of this herb is to relief dysmenorrheal, and since laxative and griping effects of the plant have been partly related to anthraquinone compounds (spasmodic), we decided to study the effect of aqueous extract of this plant in uterus contraction.

Materials and methods

Plant extraction

In this experimental study, dry Cassia italica leaves were purchased from Ahwaz green-grocery in summer 2009 and were powdered by grinder. After that, 50 gr powder of Cassia italica was mixed with 200 ml distilled boiled water in 30 min. The complex was filtered with Whatman No1 filter paper, then transmits from the strainer and centrifuge with 3500 rpm for 20 min. After that, solution dried at room temperature to obtain powder and Cassia italica extract powder was stored at 4ºC until being used (7).

Animals and tissue preparation

Virgin Wistar rats (200-250g) were purchased from Ahwaz Jundishapur University of Medical Sciences (AJUMS) animal facility. Animals used in this study were treated in accordance with principals and guidelines on animals care of AJUMS and were kept at 20-24ºC under 12hours light-12hours dark cycle and were allowed free access to tap water and commercial chow.

In this study, animals were anaesthetized by ether and after laparotomy, uterus was rapidly removed and cleaned or washed with cold and oxygenated De Jalon solution and cut into 1.5cm long pieces. Uterus was suspended between two stainless steel hocks; one of the hocks was fixed to the chamber wall while the other was attached to an isometric force transducer (UF1 Harvard Transducer, UK) and to an ink-writing curvilinear polygraph (Universal Harvard Oscillogragh, UK). The organ bath (10ml, 29ºC) contains De Jalon solution (with pH 7.4 before added to organ bath) with the following composition (mM): NaCl (154), KCl (5.6), CaCl2 (0.3), NaHCO3 (1.7), MgCl2 (1.4) and glucose (5.55) which continuously bubbled with air (8). Tissue was then maintained under 1g initial tension and allowed to equilibrate for 1h during which bath solution was refreshed every 15 min. All dissecting procedures were done with extreme care to protect the uterus from inadvertent damage. After all experiments, each uterus ring was weighted and compared with optimum resting force of 1 g initial tension then contraction forces (g/g tissue) were calculated for each tissue.

Drugs

Atropine sulfate (Daru-pakhsh, Iran), Metoclopramide and Oxytocin were purchased from Rasht-Iran Companies. Mineral solutions were purchased from Merck (Germany). Cassia italica extract powder and all chemicals were dissolved in De Jalon and volumes added to bath were less than 5% of the organ bath volume and degree of drugs solution was equivalent with environment of laboratories temperature (29ºC).

Experimental protocols

After equilibrium period, the uterus was contracted by KCL 60mM (9, 10) because the contractility and reproducibility of contraction was evaluated by introducing the tissue in KCl (60 mM) (7). Once the plateau achieved for KCL induced contraction, and after assurance of tissue aliveness, we washed KCL from bath and after 15min the extracts (0.5, 1, 2 and 4 mg/ml) were added cumulatively to the organ bath with 10 min intervals and no refreshing bath solution and the contraction was recorded. The new portions of the uterus were separately incubated with Atropine sulfate (10µM) and Metoclopramide (10µM) (11) for blocking muscarinic (12) and dopaminergic (D2) receptors (13) about 5min without refreshing, the extracts (0.5, 1, 2 and 4 mg/ml) were added cumulatively to the organ bath and the contraction was recorded. Oxytocin (10mU/ml) (14) was added to the bath for 3min and then the tissue was washed. In next stage, oxytocin (10mU/ml) was added to the bath for 3min, and then extract (0.5mg/ml) was added for 3min and other doses of extracts were used in this way not cumulatively, then tissue spasmodic effect of various doses of extract with oxytocin (10mU/ml) were recorded.

Statistical analysis

The mean±SEM of contraction forces (g/g tissue) were calculated for each group. The results were statistically analyzed by one way ANOVA

and post hoc LSD tests and p-values less than 0.05 were considered as significant.

Results

Effect of Cassia italica leaves aqueous extract on rat uterus contraction

After refreshing KCL, cumulative concentration of extract (0.5–4 mg/ml) increased the basal, peak and frequency of uterus contractions. Concentration of 4mg/ml was significantly different with 0.5mg/ml ($p<0.01$) and 1mg/ml ($p<0.05$) in basal uterus contractions. In addition, this concentration of extract had shown significant difference with 0.5mg/ml ($p<0.001$), 1mg/ml ($p<0.01$) and 2mg/ml ($p<0.05$) in peak uterus contractions. Finally, 4mg/ml of Cassia italica extract had shown significant difference in making frequency of uterus contractions with 0.5mg/ml ($p<0.001$) and 1mg/ml ($p<0.01$). Similarly, 2mg/ml of this extract had significant difference with 0.5mg/ml ($p<0.01$), and 1mg/ml ($p<0.05$) in frequency of uterus contractions (Table I).

Cassia italica leaves aqueous extract activity after tissue incubation with Atropine sulfate

After refreshing KCL and incubation (10µM) with Atropine sulfates for 5min, cumulative concentrations of the extract (0.5–4 mg/ml) increased basal uterus contractions ($p>0.05$), in addition, peak and frequency of uterus contractions in some doses of extract were increased significantly, as 4mg/ml of this extract had shown significant difference with 0.5 and 1mg/ml ($p<0.01$) in peak uterus contractions. 4mg/ml concentration of the extract had significant difference with 0.5mg/ml ($p<0.001$), and 1mg/ml concentration ($p<0.01$). Similarly 2mg/ml of the extract had shown significant difference with 0.5mg/ml ($p<0.01$), and 1mg/ml ($p<0.05$) in frequency of uterus contractions (Table I).

Cassia italica leaves aqueous extract activity after tissue incubation with Metoclopramide

After refreshing KCL and incubation (10µM) with Metoclopramide for 5min, cumulative concentrations of the extract (0.5–4mg/ml) increased the basal and peak of uterus contractions. 4mg/ml of this extract had significant difference with 0.5mg/ml ($p<0.01$), and 1mg/ml ($p<0.05$) in basal uterus contractions. This concentration of the extract showed significant difference with 0.5 and 1mg/ml ($p<0.05$) in peak uterus contractions (Table I). There was no difference between groups in frequency of uterus contractions ($p>0.05$).

Comparison of spasmodic effects of Cassia italica leaves aqueous extract and this extract with incubation of Atropine sulfate and Metoclopramide.

There was no significant difference ($p>0.05$) between groups of various dose of extract in basal, peak and frequency of uterus contractions in presence of atropine sulfate and metoclopramide incubation in comparison with the same doses of extract alone (Table I).

Cassia italica leaves aqueous extract activity after tissue incubation with oxytocin

1, 2 and 4mg/ml of extract increased the spasmodic effect of oxytocin 10mU/ml significantly ($p<0.05$) and the results compared with extract group had shown significant difference between all concentration of extract and oxytocin ($p<0.001$) except 4mg/ml contraction (Figure 1).

Table I. Comparison of cumulative concentration of aqueous extract of Cassia italica and this extract with Metoclopramide (10µM) and Atropine sulfate (10µM) incubations on basal, peak and frequency of uterus contractions.

Extract concentrations		0.5mg/ml	1mg/ml	2mg/ml	4mg/ml
Basal contraction (g/g tissue)	extract	$0.43\pm0.16^{d**}$	$1.77\pm1^{d*}$	$3.36\pm1.97^{d*}$	$3.45\pm1.3^{a**b*c*}$
	extract + Atropine	1.32 ± 0.55	2.32 ± 0.96	3.5 ± 1.45	3.7 ± 1.59
	extract + Metoclopramide	$0.48\pm0.21^{d**}$	$1.12\pm0.44^{d*}$	1.7 ± 0.56	$3.27\pm0.97^{a**b*}$
Peak contraction (g/g tissue)	extract	$5.8\pm5.2^{d***}$	$12\pm8^{d***}$	$18.95\pm10.9^{d*}$	$29.94\pm11.6^{a***b***c*}$
	extract + Atropine	$7.74\pm4.5^{d**}$	$10.2\pm5^{d**}$	17.49 ± 7.3	$30.78\pm7.8^{a**b**}$
	extract + Metoclopramide	$3.84\pm2.1^{d*}$	$6.1\pm3.3^{d*}$	10.77 ± 4.1	$17.12\pm5^{a*b*}$
Frequency of contraction (pulse /min)	extract	$0.06\pm0.02^{c**d***}$	$0.15\pm0.03^{c*d**}$	$0.4\pm0.1^{a**b*}$	$0.37\pm0.04^{a***b**}$
	extract + Atropine	$0.08\pm0.03^{c**d***}$	$0.27\pm0.13^{c*d**}$	$0.31\pm0.06^{a**b*}$	$0.66\pm0.3^{a***b**}$
	extract+ Metoclopramide	0.09 ± 0.03	0.14 ± 0.05	0.21 ± 0.06	0.24 ± 0.06

(n=12, *=p<0.05, **=p<0.01, ***=p<0.001). a= difference between 0.5mg/ml group with other groups, b= difference between 1mg/ml group with other groups, c= difference between 2mg/ml group with other groups, d= difference between 4mg/ml group with other groups.

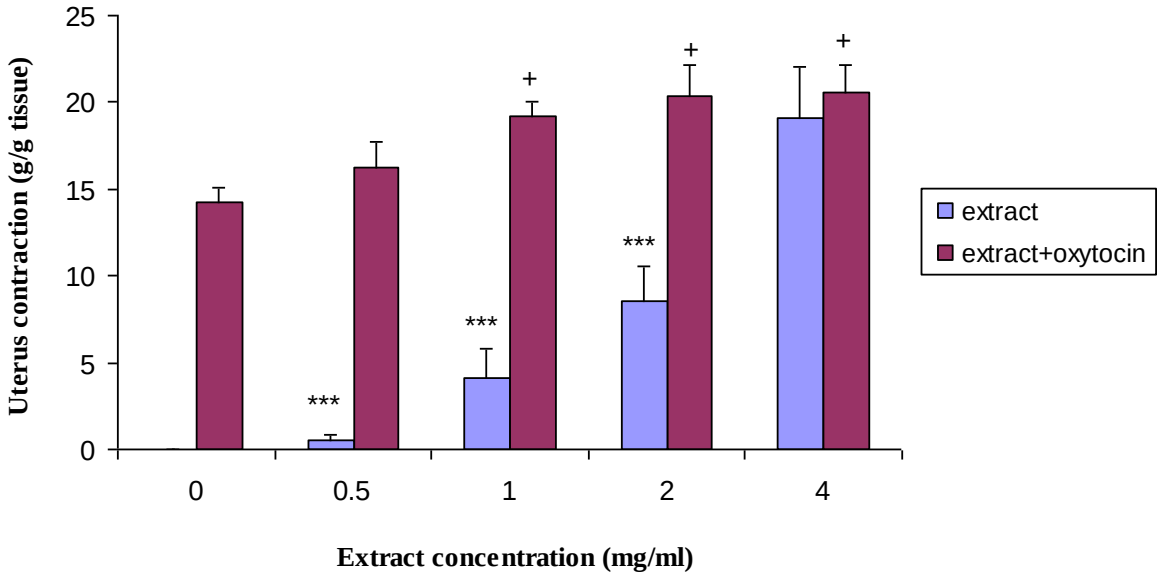


Figure 1. Effect of noncumulative concentration of aqueous extract of Cassia italica on peak of uterus contractions in presence of oxytocin (10mU/ml) (n=12, ***=p<0.001 difference between extract in comparison with extract + oxytocin group), (+=p<0.05 difference between oxytocin group with extract + oxytocin group).

Discussion

Present results showed that aqueous extract of Cassia italica leaves induce spasmodic effect on the rat uterine tissue. Also highest dose of the aqueous extract had most contractile effect on the basal, peak and frequency of uterus contraction.
Similarly, Cassia italica leave extract increased the contractile activity of the uterine specimens incubated with Atropine Sulfate. In this case, highest concentration of the extract (4mg/ml) increased the baseline, peak and frequency of contractions that was only significant for peak and frequency ($p<0.01$). Acetylcholine has spontaneous contraction in uterus that makes hyper tonicity contractions and this contraction is dose-dependent (15).

Atropine sulfate (muscarinic receptor antagonist) inhibits the contractions caused by Acetylcholine. Since using of Atropine sulfate (10µM) didn't show any prohibition to the contraction effect of Cassia italica extract, therefore we conclude that say the aqueous extract of this plant didn't have any function by the way of moscarinic receptors.

Spasmodic effect of Cassia italica leaves aqueous extract with metoclopramide (10µM) incubation in uterus, increased significantly ($p<0.05$). In the other hand, incubation of the tissue with metoclopramide did not reduce the spasmodic effect of the extract. Also concentration (4mg/ml) of this extract increased more spasmodic effect on basal and peak uterus contraction with presence of metoclopramide significantly ($p<0.05$) but this concentration showed more tendency to increase frequency of uterus contraction. Metoclopramide (dopaminergic (D2) receptor antagonist) inhibit the contractions by dopamine with central inhibition of dopamine receptor (16). In contrast, Estañ *et al* have shown that dopamine produced a concentration-dependent relaxation in the K+-depolarized rat uterus. They concluded that dopamine produced a concentration-dependent relaxation of the uterus from diethylstilboestrol-treated rats by direct activation of beta-adrenoceptors (17). In another study, Lechner and Bergant measured the changes of spontaneous activity after the application of metoclopramide on uteri and showed that metoclopramide made a highly significant decrease of uterine activity (18).

Gomes *et al* showed that metoclopramide lead to endometrial proliferation and interferes with the ovarian function (19). Liu *et al* showed that erythromycin dose-dependently increased contractile frequency and tension in non-pregnant uterine smooth muscle strips in rats and that these actions were not affected by pretreatment with atropine and metoclopramide (11).

We study dopaminergic (D2) receptors and showed that there was no change from the extract effect with metoclopramide incubation. We can say aqueous extract of Cassia italica leaves didn't

act from the dopaminergic (D2) receptors and/or there was no evidence for indirect action (dopamine release and neuronal uptake mechanisms) and specific dopamine receptor mediated relaxation have not been found in this preparation.

In this study, aqueous extract of Cassia italica increased the oxytocin contractions and these contractions were dose-dependent. According to present results, 1, 2 and 4mg/ml of the extract in combination with oxytocin 10mU/ml increased the contraction significantly in comparison with oxytocin 10mU/ml; therefore we can say this extract has mimetic effects with oxytocin on uterus that is approximately similar to Ie and Zam study of the aqueous extract of Globimetula braunii (20). At the end, according to oxytocin mimetic effect of this extract with and without oxytocin on peak of uterus contraction, we can say that 4mg/ml of Cassia italica extract have more spasmodic effect on uterus contraction in comparison with oxytocin 10mU/ml.

Shmygol *et al* (21) have used mag-fluo-4 and confocal microscopy to obtain 3D reconstructions of the SR in uterine myocytes tonic contraction seen at the beginning of oxytocin application and showed that changes in frequency of contractions are mediated by the SR Ca^{2+}, and potentiation of contraction amplitude is achieved by sensitization of contractile machinery to Ca^{2+}.

In Conclusion, our result indicated that Cassia italica leaves aqueous extract induces spasmodic effect on isolated rat uterus and this extract stimulated the uterus contractions without involving dopaminergic (D2), and muscarinic receptors. We suggest that probably changes in contractions frequency of this extract are mediated by the SR Ca^{2+} (21) or indirectly by activation of ATP-dependent potassium channels partially (22). Since this extract increased the effect of oxytocin 10mU/ml, we can suggest that this extract has oxytocin mimetic effects on isolated uterus contraction (20).

According to this research, more study will be necessary about abortive or contraceptive effects of Cassia italica on pregnant uterus. Probably this plant increase prostaglandin (PG) release in uterus (3) and it seems necessary to examine the effect of the extract on pregnant uterus and effective contraceptive of this plant can study into developing potential of a female contraceptive. This process appears to be most relevant physiologically and should be concentrated on in the future research.

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