http://www.herbmedpharmacol.com doi: 10.15171/jhp.2020.05

Journal of Herbmed Pharmacology

Effect of *Matricaria chamomilla* hydro-alcoholic and flavonoids rich extracts on rat isolated uterus

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ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 30 April 2019
Accepted: 1 October 2019

Keywords:
Matricaria chamomilla
Uterus
Spasmodic
Spasmolytic
Smooth muscle

ABSTRACT

Introduction: Pharmacological studies confirm antispasmodic activities of chamomile (*Matricaria chamomilla*) extract on intestinal smooth muscles and it has been suggested that chamomile increases uterus tone, but so far there is no scientific studies which support this assumption. Therefore, this study was designed to determine spasmodic and spasmolytic activities of *M. chamomilla* extracts on rat isolated uterus.

Methods: Hydro-alcoholic extract of *M. chamomilla* was prepared by maceration technique. Flavonoids rich extract was prepared by liquid in liquid extraction technique. Spasmodic effects of the extracts were assessed on spontaneously contracting rat uterus. The myorelaxant effect of *M. chamomilla* extracts was validated on isolated uterus contractions induced by KCl, acetylcholine (ACh), electrical field stimulation (EFS) and oxytocin.

Results: Hydro-alcoholic extract of *M. chamomilla* (0.8 and 1.6 mg/mL) enhanced spontaneous movement of rat isolated uterus smooth muscle suspended in organ bath. On the other hand, flavonoids rich fraction only diminished uterus contractile activities. Flavonoids rich extract of the plant at bath concentration ranges of 40 µg/mL to 400 µg/mL attenuated uterus response to ACh, KCl, EFS and oxytocin. The hydro-alcoholic extract of *M. chamomilla* at higher concentration ranges (250 µg/mL to 1.5 mg/mL) inhibited uterus contractions induced by the above spasmogens.

Conclusion: The present study confirms both spasmodic and spasmolytic activities *M. chamomilla* hydro-alcoholic extract. Therefore, medicinal use of the crude extract of *M. chamomilla* may initiate uterus contraction which could increase risk of spontaneous miscarriage or premature parturition.

Implication for health policy/practice/research/medical education:
This paper provides pharmacological evidence for spasmogenic and spasmolytic actions of *M. chamomilla* extract on isolated uterus. As *M. chamomilla* potentiated irregular periodic uterus contraction it should not be used during pregnancy.

Please cite this paper as: Sadraei H, Sajjadi SE, Asghari G, Khalili M. Effect of *Matricaria chamomilla* hydro-alcoholic and flavonoids rich extracts on rat isolated uterus. J Herbmed Pharmacol. 2020;9(1):35-41. doi: 10.15171/jhp.2020.05.

Introduction

Chamomile belongs to Asteraceae family and grows naturally in many places (1). Several species of chamomile are spread over the world, however, the most popular of all is known as German chamomile (*Matricaria chamomilla* L.) (2,3). *M. chamomilla* is cultivated in many parts of the world for its historical use in the food and cosmetic products as well as herb infusions for its medicinal benefits (4-7). In Iran, *M. chamomilla* is known as Babuna and traditionally is used for the treatment of a number of ailments such as gastrointestinal problems including indigestion, carminative, flatulence, colic and diarrhea (8). Other common traditional uses of *M. chamomilla* are for treatment of inflammatory diseases such as bronchitis, eczema and other skin problems (9). *M. chamomilla* is also traditionally used for treatment of common cold and fever (5). It is believed that *M. chamomilla* has analgesic effect and therefore it is used for treatment of painful conditions such as toothache, dysmenorrheal and rheumatism (4). Phytochemical studies identified presence of essential oils, flavonoids and coumarins constituents in the *M. chamomilla* (10-12). Antispasmodic and anti-

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inflammatory effects of *M. chamomilla* is attributed to the constituents of the essential oil and the flavonoids (13,14). Most abundant flavonoids which have been identified in *M. chamomilla* are apigenin, luteolin, quercetin, and isorhamnetin which often are found in the glycoside forms (15,16). As mentioned above *M. chamomilla* is famous for its antispasmodic activity especially on the gastrointestinal tract and pharmacological studies have shown that both aqueous and hydro-alcoholic extracts of *M. chamomilla* inhibit guinea pig ileum contractions induced by acetylcholine (ACh) and histamine (17-19). As a gastrointestinal smooth muscle relaxant, it is expected that *M. chamomilla* can also relax uterus smooth muscle. Nevertheless, so for there is no report on relaxant effect of *M. chamomilla* extract on isolated uterus. On the other hand, there are suggestions that *M. chamomilla* consumption is associated with increase in uterine tonicity (20). However, so far there is no pharmacological evidence which could confirm or reject this assumption. Therefore, the objective of this research was to investigate both excitatory and inhibitory effects of *M. chamomilla* hydro-alcoholic and its flavonoids rich extracts on uterus contractions.

Methods and Materials

Extract preparation

Chamomile was collected during flowering time and the flowers were separated and dried in shade. A voucher specimen was identified and deposited in the herbarium of School of Pharmacy and Pharmaceutical Sciences (NO:1391). Dried flowers were ground into fine powder using an electronic grinder (Keep, Korea). Hydro-alcoholic extract was prepared by maceration method (21). Four hundred grams of dried powder was placed in a large container and 2 liters of ethanol (70%) was added, well mixed and top of the container was sealed. After 24 hours, solute was separated by help of a Buchner funnel. The maceration process repeated twice by adding fresh ethanol and all the collected solutes were added together and concentrated by rotary apparatus. Following drying a sample of extract, the percentage of remaining liquid in the extract was determined and extract yield was calculated.

For preparation of flavonoids rich extract, solvent in solvent partitioning technique was employed (22). For this purpose, 50 g of concentrated hydro-alcoholic extract was added into decanter filled with one liter of chloroform and water (1:1). This mixture was shaken for 20 minutes and then left until two liquid phases were completely separated. The lower chloroform phase was decanted and 500 mL fresh chloroform was added into decanter and the process was repeated. The remaining chloroform in the aqueous phase was removed by rotary evacuation at 30°C. Then hydrochloric acid was drip into the aqueous solution and pH of the liquid was adjusted (pH=2). After that, 500 mL ethyl acetate was added into the aqueous solution and well shaken. Fifteen minutes later, the lower aqueous phase was collected and by adding equal volume of ethyl acetate the process was repeated thrice. Final ethyl acetate phase was collected and concentrated as flavonoids extract. All the fractions were concentrated on the rotary evaporator at 60˚C for pharmacological studies. Flavonoids contents of separated fractions were assessed by aluminum chloride colorimetric method as described before (22). Ethyl acetate fraction possessed highest contents of flavonoids and therefore considered as flavonoids rich fraction.

Tissue preparation

Pharmacological studies were performed on isolated rat uterus. The animals were handled in accordance to international principles for laboratory animal use and care (23). For this purpose a day before experiment, Wistar non-pregnant female rats (180-220 g) were given a subcutaneous injection of estradiol (100 µg/kg)). Estrogen pretreated rats were killed and uterine horns were dissected and placed in oxygenated physiological solution (Tyrode's solution). In the pharmacology laboratory, uterine horns were separated and two ends were tied up with separate pieces of cotton thread. One end of the tissue was attached to special hook and secured into Harvard organ both filled with Tyrode's solution. Other thread was tied up into lever of an isotonic Harvard transducer. The tissue was hold under 1 g tension and continuously gassed with oxygen through the experiment. Following calibration of Oscillograph, the tissue was washed several times and allowed to relax into a stable baseline. Uterine contractions were amplified and recorded on Harvard Universal Oscillograph. Effect of the extract or drug was examined on uterine spontaneous contraction, electrical field stimulation (EFS), ACh, oxytocin and KCl induced contractions. Initially a series of pilot experiments were carried out to establish effective concentration of the extracts and then full concentration-response ranges of the extract were constructed. Relaxant effect of the extract was compared with vehicle treated time-matched controls using other uterine horn. Nifedipine was used as positive standard drug for comparison.

Drug and solutions

Tyrode's solution was composed of following chemicals: NaCl=136.9 mM; $CaCl_2$=1.8 mM; $NaHCO_3$=11.9 mM; $MgCl_2$=1.05 mM; KCl=2.68 mM; glucose=5.55 mM; NaH_2PO_4=0.42 mM. Tyrode's solution was prepared daily in distilled water and saturated with oxygen. KCl was prepared as 2 M stock solution in distilled water. Acetylcholine (ACh, Sigma) was made up as 100 mM solution (acidified with 0.1 mL acetic acid) and diluted to 0.25 mM stock solution. Oxytocin (Aburaihan Pharm., Iran) stock solution was prepared from 10 IU/mL ampoule in distilled water. Estradiol valerate (10 mg/mL ampoule, Aburaihan Pharm., Iran) was diluted in vegetable liquid oil as 100 µg/mL stock solution for subcutaneous injection.

Nifedipine (Sigma) was initially prepared as 10 mM stock solution in dimethyl sulfoxide (DMSO), further dilution was made up in DMSO or distilled water. All the extracts were made up as 40 mg/mL stock solution in 50% DMSO. Further dilution was prepared in distilled water. Unless stated, all the chemicals were from Merck (Germany).

Measurements and data analysis

Uterine tonic contraction was measured as maximum amplitude of contraction from recorded tissue baseline. Uterine rhythmic contraction was measured as mean amplitude. The relaxation of isolated tissue preparations was expressed as percent of the control response mediated by added spasmogen. Drug concentration causing 50% of maximum inhibitory response (IC_{50}) was calculated by plotting full concentration-response curve for each tissue. All the results are presented as mean ± standard error of mean (ESM) for each group of results (n=6). Each test group was compared with its corresponding time-matched control group treated with equivalent amount of the vehicle. One-way analysis of variance (ANOVA) or Student's *t* test was used for statistical analysis. *P* values less than 0.05 was considered statistically significant. SigmaPlot program was used for statistical analysis and plotting the graphs.

Results

Isolated rat uterus primed with estrogen, exhibited irregular spontaneous periodic activities. These spontaneous contractions were not sustainable and gradually subsided down over the time. Repeated washing with fresh Tyrode's solution, facilitated uterine smooth muscle basal tension settlement. Addition of hydro-alcoholic extract of *M. chamomilla* into the organ bath had no effect the basal tension but significantly potentiated spontaneous uterus contractions in comparison to the control group. On the contrary, flavonoids rich extract of *M. chamomilla* attenuated uterine spontaneous activities (Figure 1).

Addition of KCl (2.5, 5, 10 and 20 mM) caused rhythmic contractile response imposed on a small tonic contraction. KCl at 40 mM bath contraction only induced tonic contraction in rat isolated uterus. Increasing KCl concentration to 80 mM produced a similar sustained tonic contraction with slightly higher amplitude. Effect of chloroform, flavonoids and aqueous fractions were initially screened on KCl induced tonic contraction. The aqueous fraction had no effect of KCl (80 mM) responses, while chloroform and flavonoids fractions concentration dependently inhibited KCl induced contraction (Figure 2). As the flavonoids rich fraction was four times more potent than the chloroform fraction, the flavonoids rich fraction was tested in the subsequent experiments.

Flavonoids rich fraction in a concentration manner inhibited uterus responses to above mentioned KCl concentrations. Inhibitory effect of flavonoids rich fraction was compared with the hydro-alcoholic extract

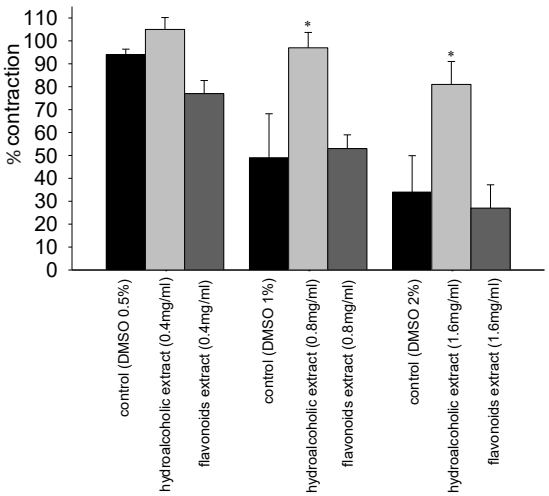

Figure 1. Potentiating effect of *Matricaria chamomilla* extract on spontaneous periodic contraction of rat isolated uterus preparation. Uterine strips of estrogenized rats were treated with hydro-alcoholic extract for comparison with flavonoids rich fraction of *M. chamomilla* and the control group. The values are presented as mean ± SEM (n=6). Stars show significant differences with the control group (*$P < 0.05$; Student's *t* test).

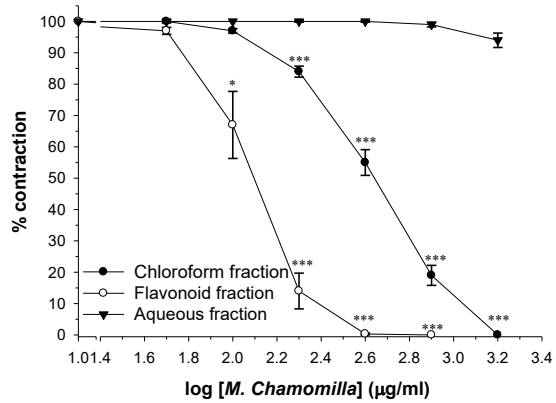

Figure 2. Antispasmodic effect of three fractions separated from hydro-alcoholic extract of *Matricaria chamomilla* on KCl (80 mM) induced contractions in rat isolated uterus preparations. Concentration-inhibitory response curves are plotted for chloroform, Ethyl acetate (flavonoids rich fraction) and aqueous fractions of *M. chamomilla*. The values are presented as mean ± SEM (n=6). Stars show significant differences with the corresponding control points (*$P < 0.05$, ***$P < 0.001$; Student's *t* test). Maximum concentration of DMSO was 1.9%.

of *M. chamomilla* in Figure 3. After washing the tissue with fresh Tyrode's solution, the response to KCl was restored. The inhibitory concentration causing 50% of maximum response (IC_{50} value) is presented in Table 1 for comparison. Time-matched control tissue treated with equivalent volume of vehicle (DMSO) showed no significant changes in the contraction induced by KCl (ANOVA) (Figure 3).

Addition of oxytocin (0.001 IU/mL) into the bath produced strong and more regular rhythmic uterine contractions. Both hydro-alcoholic and flavonoids rich extract of *M. chamomilla* in a concentration dependent

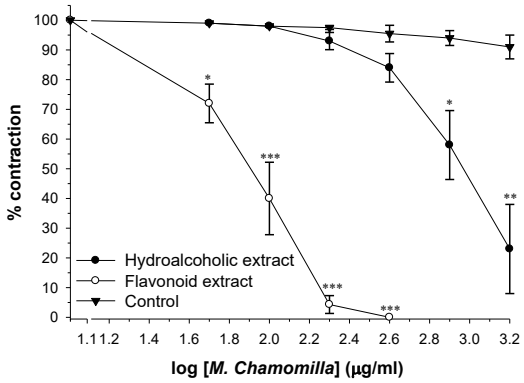

Figure 3. Antispasmodic effect of *Matricaria chamomilla* on KCl (40 mM) induced contractions in rat isolated uterus preparations. Concentration inhibitory response curves are plotted for hydro-alcoholic extract, flavonoids rich fraction of *M. chamomilla* and compared with the control group treated with equivolume amount of vehicle (DMSO). The values are presented as mean ± SEM (n=6). Stars show significant differences with the corresponding control group (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; Student's *t* test). Maximum concentration of DMSO in the bath was 1.9%. There are no statistically significant changes in the vehicle treated time-matched control responses over the course of experiment (ANOVA).

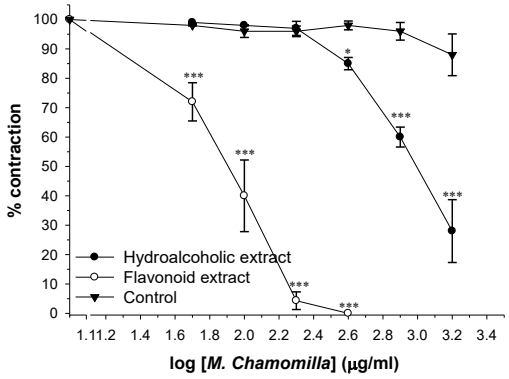

Figure 4. Antispasmodic effect of *Matricaria chamomilla* on oxytocin (0.001 IU/mL) induced contractions in rat isolated uterus preparations. Concentration inhibitory response curves are plotted for hydro-alcoholic extract, flavonoids rich fraction of *M. chamomilla* and compared with the control group treated with equivolume amount of vehicle (DMSO). The values are presented as mean ± SEM (n=6). Stars show significant differences with the corresponding control group (*$P < 0.05$, ***$P < 0.001$; Student's *t* test). Maximum concentration of DMSO in the bath was 1.9%. There are no statistically significant changes in the vehicle treated time-matched control responses over the course of experiment (ANOVA).

manner inhibited rat isolated uterine responses induced by oxytocin (Figure 4). The inhibitory effect of the extract was reversible following washing the tissue with fresh Tyrode's solution. No significant changes were observed in time-matched control tissue treated with vehicle (ANOVA). However, the flavonoids extract was at least 10 times more potent than the hydro-alcoholic extract. The IC_{50} values are compared in the Table 1.

Addition of ACh (1µM) into the organ bath solution induced a rapid phasic response in rat uterus smooth muscle within 30 seconds contact time. Uterus response to addition of ACh, concentration dependently was attenuated by addition of flavonoids rich fraction. Inhibition of uterus response was started with 40 µg/mL and complete inhibition was achieved with 400 µg/mL flavonoids extract in the bath (Figure 5). The hydro-alcoholic extract of *M. chamomilla* reversibly inhibited uterine smooth muscle contraction induced by ACh but at bath concentration above 400 µg/mL (Figure 5). For comparison of IC_{50} see Table 1. Equivalent volume of vehicle had no significant inhibitory effect on ACh responses (ANOVA).

EFS applied via a parallel platinum weirs caused a single sharp contraction in rat isolated smooth muscle suspended in organ bath. Hydro-alcoholic extract of *M. chamomilla* at similar ranges of concentration which inhibited oxytocin and ACh contractions, reduced uterine response to EFS. Inhibition of tissue response was reversible. Flavonoids rich extract also attenuated tissue response to EFS but at much lower concentrations (Figure 6). Concentration-response curve are shown in Figure 6 and IC_{50} values are compared in Table 1. Although at higher concentration, DMSO slightly affected the uterine response to EFS, but these changes were not statistically significant (ANOVA).

Nifedipine in a concentration dependent manner inhibited uterine contraction produced by KCl, ACh, oxytocin and EFS (Figure 7).

Discussion

Myometrium is an excitable tissue which implies that its contraction is accompanied by cell membrane excitation (24). Smooth muscle excitation can arise from membrane depolarization or receptor activation (25). Current understanding of the cellular basis of uterine contractility

Table 1. Comparison of IC_{50} values of hydro-alcoholic and flavonoids rich extracts of *Matricaria chamomilla* and nifedipine on rat isolated uterus

Matricaria chamomilla	IC_{50} Values			
	KCl (40 mM)	ACh (1 µM)	EFS	Oxytocin (0.001 IU/mL)
Hydro-alcoholic extract	1.1±0.22 mg/mL	2.03±0.26 mg/mL	1.9±0.18 mg/mL	1.02±0.12 mg/mL
Flavonoids rich extract	85±12 µg/mL	119±11.4 µg/mL	74±12.2 µg/mL	105±14.6 µg/mL
Nifedipine	22±9.5 nM	160±42 nM	90±27 nM	2.15±0.35 µM

ACh, acetylcholine; EFS, electrical field stimulation .

IC_{50} values (inhibitory concentration causing 50% of maximum response) were obtained by plotting full concentration response curve for each tissue. The data are presented as mean ± SEM (n=6).

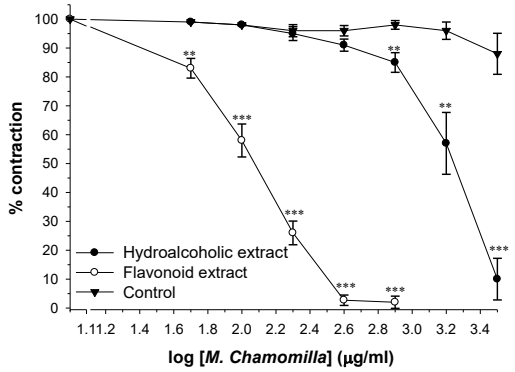

Figure 5. Antispasmodic effect of *Matricaria chamomilla* on acetylcholine (1µM) induced contractions in rat isolated uterus preparations. Concentration inhibitory response curves are plotted for hydro-alcoholic extract, flavonoids rich fraction of *M. chamomilla* and compared with the control group treated with equivolume amount of vehicle (DMSO). The values are presented as mean ± SEM (n=6). Stars show significant differences with the corresponding control group (***P* < 0.01, ****P* < 0.001; Student's *t* test). Maximum concentration of DMSO in the bath was 3.8%. There are no statistically significant changes in the vehicle treated time-matched control responses over the course of experiment (ANOVA).

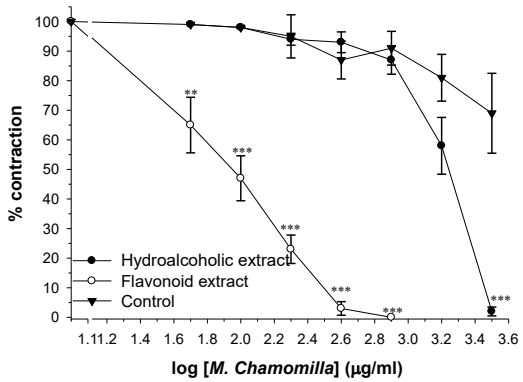

Figure 6. Antispasmodic effect of *Matricaria chamomilla* on electrical field stimulation (EFS, square pulse; 6 V, 1 s duration, 50 Hz) induced contractions in rat isolated uterus preparations. Concentration-inhibitory response curves are plotted for hydro-alcoholic extract, flavonoids rich fraction of *M. chamomilla* and compared with the control group treated with equivolume amount of vehicle (DMSO). The values are presented as mean ± SEM (n=6). Stars show significant differences with the corresponding control group (***P* < 0.01, ****P* < 0.001; Student's *t* test). Maximum concentration of DMSO in the bath was 3.8%. There are no statistically significant changes in the vehicle treated time-matched control responses over the course of experiment (ANOVA).

is that, increase in intracellular calcium ions is essential for normal uterine contractility. Calcium ions either come from extracellular fluid following arising of action potential or released from intracellular stores (26-33). Following depolarization of the myometrial cell membrane, Ca^{2+} influx occurs via voltage-gated Ca^{2+} channels (29). Rise in Ca^{2+} ions stimulate myosin light chain kinase via Ca^{2+}-calmodulin and force rises within the myometrium (34). Addition of KCl into the organ bath, causes myometrial cell membrane depolarization and opening of voltage-

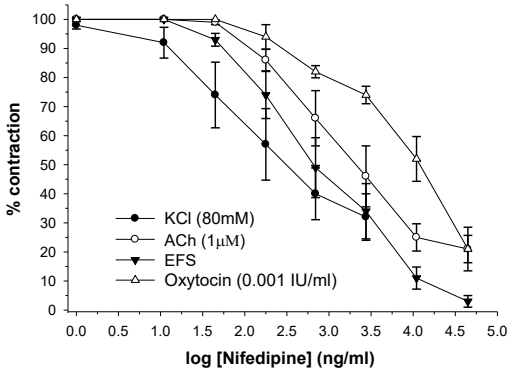

Figure 7. Antispasmodic effect of nifedipine on KCl (40 mM), acetylcholine (ACh), electrical field stimulation (EFS, square pulse; 6 V, 1 s duration, 50 Hz) and oxytocin induced contractions in rat isolated uterus preparations. Concentration inhibitory response curves are plotted for nifedipine. The values are presented as mean ± SEM (n=6). One-way analysis of variance (ANOVA) statistical test shows concentration-dependent inhibition of uterus responses to above spasmogens (*P* < 0.001).

gated Ca^{2+} channels (27-30). Calcium ion entry in the rat uterus mostly occurs via L-type Ca^{2+} channels (33). In the presence of nifedipine, a blocker of L-type Ca^{2+} channels, Ca^{2+} ion influx is reduced and therefore contraction is inhibited.

Oxytocin is a powerful modulator of uterus contractions. It acts on its own specific receptors on the myometrium and increases activity of phospholipase-C on the cell membrane and in turn production of inositol triphosphate (IP_3) is increased (35). Rise in cytosolic Ca^{2+} ions results from interaction of IP_3 with specific receptors on the sarcoplasmic reticulum (SR). ACh interacts with the muscarinic M_3 receptors which exist on the myometrium and in a similar way increases uterine contraction by raising intracellular IP_3 production (32). It should be mentioned that some drugs might have more than one mechanism of action. Oxytocin, for example, also impairs Ca^{2+} efflux from the cell (27). As in the case of other smooth muscles, rat uterus is innervated by autonomic nerves system manipulating both excitation and inhibition responses (36). Application of EFS produced a rapid response indicating that excitatory neurotransmitters response is prominent. Inhibition of EFS by nifedipine indicates that L-type Ca^{2+} channels are involved in the process of excitation of uterus smooth muscle cells (37).

Physiological responses of uterus muscle vary at different stages of menstrual cycle (38). Therefore, to synchronize cycle time, all the rats used in these experiments were treated with estradiol. Rat uterus horns primed with estrogen exhibited spontaneous periodic contraction *in vitro*. This is because uterus is known as a myogenic organ, meaning that uterine smooth muscles contracts in absence of nervous or stimulating agents (27). It has been proposed that spontaneous contraction of rat uterus is associated with prostaglandin production (39).

Hydro-alcoholic extract of *M. chamomilla* did not affect uterus basal tension but potentiated both amplitude and frequency of spontaneous rhythmic contractions of rat isolated uterus. However, hydro-alcoholic extract at similar concentration ranges only inhibited uterine contractile responses to oxytocin, ACh, KCl and EFS. These results show that hydro-alcoholic extract *M. chamomilla* mainly affects myogenic induced contractions in rat uterus. This does not imply that *M. chamomilla* has no effect on uterus contractility induced by above spasmogens because first of all, both spasmogenic and spasmolytic effects of the extract are seen at similar concentration ranges. Therefore, it is likely that spasmogenic activity simply was not revealed. Secondly, *M. chamomilla* extract exhibited a relatively weak spasmogenic activity and in presence of a strong spasmogen such as oxytocin it would not make any significant difference to oxytocin response or other strong spasmogen.

Bioactivity assessment of fractions separated from hydro-alcoholic extract of *M. chamomilla* revealed that aqueous fraction had no effect on uterus contraction while both chloroform and flavonoids rich fractions in a concentration dependent manner inhibited uterus contraction. This indicates that the main bioactive compounds responsible for inhibitory action of *M. chamomilla* possessed non-polar properties. As the flavonoids rich fraction was 4 times more potent than the chloroform fraction, it is likely that the active substances may belong to flavonoids group. Apigenin and luteolin are two bioactive flavonoids with spasmolytic activities on smooth muscles (40). Both of these compounds have been identified in *M. chamomilla* hydro-alcoholic extract (15,16). Unlike hydro-alcoholic extract, flavonoids rich fraction did not potentiate spontaneous activity of rat isolated uterus. Therefore, it is likely that compounds responsible for stimulatory effect of *M. chamomilla* were not present in any significant amount in the flavonoids rich extract.

Conclusion

These finding indicate that *M. chamomilla* hydro-alcoholic extract contains mixtures of pharmacological active constituents. Some components possessed spasmodic activity on rat isolated uterus while others possessed spasmolytic activities. Active antispasmodic components are concentrated in the flavonoids rich fraction, suggesting that it is likely that they are a form of flavonoids. These results provide pharmacological evidence that crude extract of *M. chamomilla* has strong potential to enhance uterus spontaneous contractile activity and thus should be avoided in pregnancy.

Acknowledgements

The study was carried out during research project of Pharm.D student. The financial support was provided by Isfahan University of Medical Sciences, Iran.

Authors' contribution

HS was responsible for the project management, presentation and pharmacological studies. SES and GA supervised preparation of the extract. AG performed the experiments. All read and confirmed the final version of the article for publication.

Conflict of interests

The authors report no conflicts of interest. The authors are responsible for the content and writing of this article.

Ethical considerations

Animal care and experiments were performed in accordance with the guidelines for the care and use of laboratory animals of the Isfahan University of Medicine Sciences with the approval code number of IR.MUI.RESEARCH.REC.1397.157.

Finding/Support

This research project financially was supported by Research Department of Isfahan University of Isfahan University of Medical Sciences (Project No. 397083).

References

1. Ody P. Complete Guide to Medicinal Herbs. 2nd ed. London: Dorling Kindersley; 2000. p. 88.
2. Barnes J, Anderson LA, Phillipson JD. Herbal Medicine. 3rd ed. London: Pharmaceutical Press; 2007. p. 152-5.
3. Duke JA, Bogenschutz-Godwin MJ, duCellier J, Duke PAK. Handbook of Medicinal Herbs. 2nd ed. London: CRC Press; 2002. p. 174-6.
4. Murti K, Panchal MA, Gajera V, Solanki J. Pharmacological properties of *Matricaria recutita*: a review. Pharmacologia. 2012;3(8):348-51. doi: 10.5567/pharmacologia.2012.348.351.
5. Singh O, Khanam Z, Misra N, Srivastava MK. Chamomile (*Matricaria chamomilla* L.): an overview. Pharmacogn Rev. 2011;5(9):82-95. doi: 10.4103/0973-7847.79103.
6. Ahmad S, Koukab S, Razzaq N, Islam M, Rose A, Aslam M. Cultivation of *Matricaria recutita* L. in highlands of Balochistan, Pakistan. Pak J Agric Res. 2011;24(1-4):35-41.
7. Moghaddasi Mohammad S. Study on Cammomile (*Matricaria chamomilla* L.) usage and Farming. Adv Environ Biol. 2011;5(7):1446-53.
8. Gafari F, Moein A. Babunah in traditional medicine texts of Iran and Islam. J Trad Med Islam Iran. 2013;4(1):79-85. [Persian].
9. Tubaro A, Zilli C, Redaelli C, Loggia RD. Evaluation of antiinflammatory activity of a chamomile extract after topical application. Planta Med. 1984;50(4):359. doi: 10.1055/s-2007-969734.
10. Petruľová-Poracká V, Repčák M, Vilková M, Imrich J. Coumarins of *Matricaria chamomilla* L.: aglycones and glycosides. Food Chem. 2013;141(1):54-9. doi: 10.1016/j.foodchem.2013.03.004.
11. Hameed IH, Mohammed GJ, Kamal SA. A review: uses and pharmacological activity of *Matricaria chamomilla*. Ind J

Public Health Res Dev. 2018;9(3):200-5. doi: 10.5958/0976-5506.2018.00209.7.
12. Ashnagar A, Gharib Naseri N, Alavi SY. Isolation and identification of the major chemical components in the capitula of *Matricaria chamomilla* grown in Khuzestan province of Iran. Asian J Chem. 2009;21(7):4981-86.
13. Achterrath-Tuckermann U, Kunde R, Flaskamp E, Isaac O, Thiemer K. [Pharmacological investigations with compounds of chamomile. V. Investigations on the spasmolytic effect of compounds of chamomile and Kamillosan on the isolated guinea pig ileum]. Planta Med. 1980;39(1):38-50. doi: 10.1055/s-2008-1074901.
14. Szelenyi I, Isaac O, Thiemer K. [Pharmacological experiments with compounds of chamomile. III. Experimental studies of the ulcerprotective effect of chamomile (author's transl)]. Planta Med. 1979;35(3):218-27. doi: 10.1055/s-0028-1097208.
15. Mehmood MH, Munir S, Khalid UA, Asrar M, Gilani AH. Antidiarrhoeal, antisecretory and antispasmodic activities of *Matricaria chamomilla* are mediated predominantly through K(+)-channels activation. BMC Complement Altern Med. 2015;15:75. doi: 10.1186/s12906-015-0595-6.
16. Srivastava JK, Gupta S. Extraction, characterization, stability and biological activity of flavonoids isolated from chamomile flowers. Mol Cell Pharmacol. 2009;1(3):138.
17. Srivastava JK, Shankar E, Gupta S. Chamomile: A herbal medicine of the past with bright future. Mol Med Rep. 2010;3(6):895-901. doi: 10.3892/mmr.2010.377.
18. Forster HB, Niklas H, Lutz S. Antispasmodic effects of some medicinal plants. Planta Med. 1980;40(4):309-19. doi: 10.1055/s-2008-1074977.
19. Vafaei A, Emami-Abargoei M, Taherian A, Sadeghi H. Effect of *Matricaria chamomilla* extract on the electrically stimulation of ileum of guinea-pig in in vitro model. Iran J Pharm Res. 2010;3(suppl 2):52. doi: 10.22037/ijpr.2010.449.
20. Shipochliev T. [Uterotonic action of extracts from a group of medicinal plants]. Vet Med Nauki. 1981;18(4):94-8.
21. Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. 2nd ed. London: Chapman & Hall; 1984. p. 4-7.
22. Sadraei H, Ghanadian SM, Moazeni S. Inhibitory effect of hydroalcoholic and flavonoids extracts of *Dracocephalum kotschyi*, and its components luteolin, apigenin and apigenin-4'-galactoside on intestinal transit in mice. J Herbmed Pharmacol. 2019;8(1):8-13. doi: 10.15171/jhp.2019.02.
23. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. Washington DC: The National Academies Press; 2010. p. 11-37.
24. Wray S, Kupittayanant S, Shmygol A, Smith RD, Burdyga T. The physiological basis of uterine contractility: a short review. Exp Physiol. 2001;86(2):239-46.
25. Horowitz A, Menice CB, Laporte R, Morgan KG. Mechanisms of smooth muscle contraction. Physiol Rev. 1996;76(4):967-1003. doi: 10.1152/physrev.1996.76.4.967.
26. Lee YH, Hwang MK, Morgan KG, Taggart MJ. Receptor-coupled contractility of uterine smooth muscle: from membrane to myofilaments. Exp Physiol. 2001;86(2):283-8.
27. Wray S. Uterine contraction and physiological mechanisms of modulation. Am J Physiol. 1993;264(1 Pt 1):C1-18. doi: 10.1152/ajpcell.1993.264.1.C1.
28. Martin C, Ashley RH. Reconstitution of single ryanodine-sensitive cation channels from rat myometrial microsomal membranes. J Physiol. 1995;487:90.
29. Parkington HC, Coleman HA. Ionic mechanisms underlying action potentials in myometrium. Clin Exp Pharmacol Physiol. 1988;15(9):657-65. doi: 10.1111/j.1440-1681.1988.tb01125.x.
30. Shmigol AV, Eisner DA, Wray S. Properties of voltage-activated [Ca2+]i transients in single smooth muscle cells isolated from pregnant rat uterus. J Physiol. 1998;511 (Pt 3):803-11. doi: 10.1111/j.1469-7793.1998.803bg.x.
31. Shmigol AV, Eisner DA, Wray S. The role of the sarcoplasmic reticulum as a Ca2+ sink in rat uterine smooth muscle cells. J Physiol. 1999;520 Pt 1:153-63. doi: 10.1111/j.1469-7793.1999.00153.x.
32. Taggart MJ, Wray S. Contribution of sarcoplasmic reticular calcium to smooth muscle contractile activation: gestational dependence in isolated rat uterus. J Physiol. 1998;511(Pt 1):133-44. doi: 10.1111/j.1469-7793.1998.133bi.x.
33. Young RC, Smith LH, McLaren MD. T-type and L-type calcium currents in freshly dispersed human uterine smooth muscle cells. Am J Obstet Gynecol. 1993;169(4):785-92. doi: 10.1016/0002-9378(93)90006-5.
34. Longbottom ER, Luckas MJ, Kupittayanant S, Badrick E, Shmigol T, Wray S. The effects of inhibiting myosin light chain kinase on contraction and calcium signalling in human and rat myometrium. Pflugers Arch. 2000;440(2):315-21. doi: 10.1007/s004240000305.
35. Holda JR, Oberti C, Perez-Reyes E, Blatter LA. Characterization of an oxytocin-induced rise in [Ca^{2+}] i in single human myometrium smooth muscle cells. Cell Calcium. 1996;20(1):43-51. doi: 10.1016/s0143-4160(96)90049-4.
36. Gnanamanickam GJ, Llewellyn-Smith IJ. Innervation of the rat uterus at estrus: a study in full-thickness, immunoperoxidase-stained whole-mount preparations. J Comp Neurol. 2011;519(4):621-43. doi: 10.1002/cne.22515.
37. Farre AJ, Colombo M, Fort M, Gutierrez B. Differential effects of various Ca^{2+} antagonists. Gen Pharmacol. 1991;22(1):177-81. doi: 10.1016/0306-3623(91)90331-y.
38. Coleman HA, Hart JD, Tonta MA, Parkington HC. Changes in the mechanisms involved in uterine contractions during pregnancy in guinea-pigs. J Physiol. 2000;523 Pt 3:785-98. doi: 10.1111/j.1469-7793.2000.00785.x.
39. Vane JR, Williams KI. The contribution of prostaglandin production to contractions of the isolated uterus of the rat. Br J Pharmacol. 1973;48(4):629-39. doi: 10.1111/j.1476-5381.1973.tb08250.x.
40. Sadraei H, Ghanadian M, Asghari G, Sekhavati N. Antispasmodic activity of apigenin and luteolin, two components of Dracocephalum kotschyi extract, on rat ileum contractions. J Herbmed Pharmacol. 2018;7(2):100-5. doi: 10.15171/jhp.2018.17.