ÖZGÜN ARAŞTIRMA / ORIGINAL RESEARCH

DOI: 10.17944/mkutfd.1007917
MKÜ Tıp Dergisi 2022;13(47):290-295

Effects of Achillea millefolium extract on spontaneous and oxytocin-induced isolated rat uterine contractions

Ali Eker[1], Faik Özdengül[1], Melda Pelin Yargıç[2], Aysu Şen[1]

[1] Necmettin Erbakan University, Faculty of Medicine, Department of Physiology, Konya, Türkiye
[2] Ankara Medipol University, Faculty of Medicine, Department of Physiology, Ankara, Turkey

Abstract

Effects of Achillea millefolium extract on spontaneous and oxytocin-induced isolated rat uterine contractions

Objective: *Achillea millefolium* (AM) is widely used in traditional medicine in a wide geography due to its effects on the female reproductive system. However, its effect on uterine smooth muscle contractions is unknown. Our study aims to investigate the effects of AM on spontaneous and oxytocin-induced isolated rat uterine contractions.
Method: Myometrial strips were obtained from 32 adult Wistar Albino rats. Contraction amplitudes and frequencies were recorded by isolated tissue bath system after either of the following: administration of only Krebs-Hanseleit (KBH) solution, administration of Achillea millefolium extract (AME); administration of KBH or AME after inducing contractions with oxytocin. The differences at the level of $p<0.05$ was considered significant.
Results: When AM extract was administered at a dose of 2mg/ml, it significantly reduced the spontaneous (non-induced) contraction frequency compared to the control group with only KBH addition to the medium (AME: 2.37±0.49, KBH: 9.25±1.69, $p=0.002$). AME significantly reduced the amplitude of spontaneous uterine contractions in the administrations of 0.5, 1, and 2mg/ml ($p<0.05$). On the other hand, AME significantly reduced the amplitude of oxytocin-induced contractions in each increasing dose ($p<0.05$).
Conclusion: It was revealed that AME significantly reduced the frequency and amplitude of both spontaneous and oxytocin-induced uterine contractions depending on the dose. AME should be used with caution in cases of preterm birth or miscarriage risk.
Keywords: *Achillea millefolium*, Oxytocin, Oxytocin-Induced Contraction, Uterine Contraction, Yarrow

Öz

Achillea millefolium ekstraktının spontan ve oksitosin ile indüklenen izole sıçan uterus kasılmaları üzerine etkileri

Amaç: *Achillea millefolium* (AM), kadın üreme sistemi üzerindeki etkileri nedeniyle geniş bir coğrafyada geleneksel tıpta yaygın olarak kullanılmaktadır. Ancak uterus düz kas kasılmaları üzerindeki etkisi bilinmemektedir. Çalışmamız AM'nin spontan ve oksitosin kaynaklı izole sıçan uterus kasılmaları üzerindeki etkilerini araştırmayı amaçlamaktadır.
Yöntem: Myometrial şeritler 32 yetişkin Wistar Albino sıçanından elde edildi. Kasılma genlikleri ve frekansları, belirtilen uygulamaların ardından izole organ banyosu sisteminde kaydedildi: Yalnızca Krebs-Hanseleit (KBH) çözeltisinin uygulanması, Achillea millefolium ekstraktının (AME) uygulanması; oksitosin ile kasılmaları indükledikten sonra KBH veya AME uygulaması. $p<0.05$ değeri anlamlı olarak kabul edildi.
Bulgular: AM ekstraktı 2mg/ml dozunda uygulandığında, ortama sadece KBH ilavesi ile spontan (uyarılmamış) kasılma sıklığını kontrol grubuna kıyasla önemli ölçüde azalttı (AME: 2.37±0.49, KBH: 9.25±1.69, $p=0.002$). AME, 0.5, 1 ve 2 mg/ml'lik uygulamalarda spontan uterus kasılmalarının amplitüdünü önemli ölçüde azalttı ($p<0.05$). Öte yandan AME, artan her dozda oksitosin kaynaklı kasılmaların amplitüdünü önemli ölçüde azalttı ($p<0.05$).
Sonuç: AME'nin doza bağlı olarak hem spontan hem de oksitosin kaynaklı uterus kasılmalarının sıklığını ve amplitüdünü önemli ölçüde azalttığı ortaya çıktı. AME, erken doğum veya düşük riski durumlarında dikkatli kullanılmalıdır.
Anahtar Kelimeler: *Achillea millefolium*, Oksitosin, Oksitosinle Indüklenmiş Kasılma, Uterin Kasılma, Civanperçemi

Nasıl Atıf Yapmalı: Eker A, Özdengül F, Yargıç MP, Şen A.Effects of *Achillea millefolium* extract on spontaneous and oxytocin-induced isolated rat uterine contractions. MKÜ Tıp Dergisi. 2022;13(47): 290-295. https://doi.org/10.17944/mkutfd.1007917

Sorumlu Yazar/Corresponding Author: Aysu Şen
Email: aysusenmd@gmail.com
ORCID iD: 0000-0002-5271-7359

Geliş/Received: 12 Ekim 2021
Kabul/Accepted: 13 Mayıs 2022

INTRODUCTION

The *Achillea millefolium* (yarrow) plant, which is widely used in traditional medicine, is a member of the Asteraceae family. *Achillea millefolium* (AM), which grows in different parts of the world, blooms in June and September, particularly in the continents of Europe, Asia, and America (1,2,3). It is reported that it contains a lot of biologically active compounds (4). The studies on the bioactivity of this plant have revealed that AM extracts (AME) had hepatoprotective, anti-inflammatory, antinociceptive, antioxidant, antidiabetic, spasmolytic, antimicrobial, and Ca^{+2} antagonist activities (4,5).

Yarrow is known to be widely used in traditional medicine in Germany, Italy, Albania, Hungary, Serbia, Iraq, Iran, Israel, Turkey, Jordan, USA, Canada, and India (4,6,7). Besides its quite wide usage area in traditional medicine, the yarrow is mostly used in the treatment of digestive and female reproductive system diseases. Particularly in traditional eastern medicine, it is recommended to be used for female reproductive system diseases with indications such as menstrual irregularities, facilitation of birth, prevention of adhesions in the cervix, treatment of uterine infections (7).

A great number of studies have determined the effects of active ingredients in yarrow on smooth muscles in the digestive system. For example, an in vitro study determined that aqueous yarrow extract stimulated smooth muscle contractions in the mouse and human stomach antrum depending on the dose (8). Moreover, its flavonoid content was observed to have a relaxing effect on guinea-pig ileum (9). Although one of the most common uses of yarrow is female reproductive system diseases, there is not enough information on its effects on uterine smooth muscle. An article found that there was a significant reduction in "litter size and weight" when pregnant mice were regularly given AME orally and stated that AM consumption during pregnancy may be inconvenient (10). In a study conducted by university students, it was shown that regular consumption of AM during the first three days of menstruation significantly reduced pain intensity (11). These and similar studies provide important information about some clinical outcomes of AM use on the female reproductive system, however, it does not reveal the direct effects of yarrow on uterine smooth muscle.

Although yarrow is widely used in traditional medicine in a wide geography covering many continents due to its effects on the female reproductive system, its effect on uterine smooth muscle contractions is still unknown. This study aims to investigate the effects of *Achillea millefolium* on both spontaneous and oxytocin-induced isolated rat uterine contractions.

METHOD

Animals

In the study, 32 female Wistar Albino rats weighing 200-250 g were used. Rats were randomly distributed to four different groups, with 8 animals in each group. All animals were kept in plastic cages at stable room temperature (21 ± 2 °C) in a 12-hour light/12-hour dark environment, standard feeding was applied, and no restrictions were made. The uteruses of all animals in the experimental groups were removed between 09:00 and 10:00 in the morning.

Preparation of Myometrium Strips

In the study, 32 female 12 weeks old adult rats of Wistar Albino type between 200-250 g were used which were obtained from Necmettin Erbakan University KONÜDAM Experimental Medicine Application and Research Center. After cervical dislocation, abdominal areas of the non-pregnant rats, which were in the follicular phase of the cycle, were opened. By eliminating the intestines and other abdominal organs, two uterine horns were carefully cut between the ovaries and the uterine body and placed in a petri dish containing Krebs–Henseleit solution (KHS, mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.18, $CaCl_2$ 2.4, $NaHCO_3$ 15.8, Glucose 1.5, EDTA:0.016).

The antimesenteric edge of the uterine horn was properly opened in the longitudinal direction. The uterus was longitudinally divided into strips by taking 1.2x2x1 cm sections containing all uterine layers from the opened uterine horns. The strips were tied with silk threads at both ends; one end of it was fixed on the bottom of the chamber with KHS and the other end was fixed to the isometric power transducer also using a silk thread and hanged vertically in the organ bath.

Preparation of AME

The flower parts of the dried AM herb were ground and pulverized. 30 g of it was taken and 400 ml of ethanol was added. The mixture was stirred using a magnetic stirrer for 48 hours and then, it was filtered off to remove large particles. Then the alcohol ingredient of the obtained solution was evaporated at 84 °C to obtain the extract. The final concentration was prepared by dissolving the obtained extract in five different KHS doses with 0.125, 0.25, 0.5, 1, and 2 mg/ml.

Experiment Procedure

The prepared myometrial strips were hanged in an isolated organ bath which was constantly gassed with a gas containing 95% oxygen, 5% carbon dioxide at 38°C with a pH of 7.4 and 5 mL of KHS. At the end of the stress compliance period of ninety minutes, the contractions of regular self-contracting strips were recorded for 10 minutes using an isometric force transducer and these data were used as control data.

The contractions were recorded with a physiological power convertor (FDT05, Commat Ltd.) and with MP150WS Windows (Biopac Systems Inc).

Experimental Group 1 and Control Group 1

Five different doses of AME with 0.125, 0.25, 0.5, 1, and 2 mg/ml were administered in the experimental group, respectively, whereas, in the control group, KHS was administered cumulatively to the bath five times provided the same amount as AME.

Experimental Group 2 and Control Group 2

Contractions were induced by adding 0.0004 IU/mL oxytocin to the medium. Oxytocin-induced contraction was recorded as a control value. Immediately after this control period of 10 minutes, five different doses of AME with 0.125, 0.25, 0.5, 1, and 2 mg/ml were administered in the experimental group, respectively, whereas, in the control group, KHS was administered cumulatively to the bath five times provided the same amount as AME.

For all four groups, we waited for 10 minutes between each administration and then recorded the responses obtained.

Statistical Analysis

It was determined that the data showed normal distribution using the "Shapiro-Wilk" test. Arithmetic means and standard deviations of all parameters were calculated. "Independent Sample T-Test" was used to compare two groups. "Repeated Measures Analysis of Variance Test" was used to determine the difference between different administrations within the group. The differences at the level of $p<0.05$ was considered significant. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 21 (IBM Corp.).

RESULTS

Contraction frequencies and contraction amplitudes of the myometrium strips of animals in the experimental and control groups after administration of AME (or equal doses of KHS) in different doses are given in Table 1 and Table 2, respectively.

Contraction Frequencies

AM extract was found to have a significant reduction effect on the spontaneous (non-induced) contraction frequency compared to the control group only when a dose of 2mg/ml was administered (Experimental Group 1: 2.37 ± 0.49, Control Group 1: 9.25 ± 1.69, $p = 0.002$) Administration of AM at other doses did not affect the frequency of spontaneous uterine contractions (Table 1).

According to the results of Repeated Measurements ANOVA test for Experimental Group 1 in which just AME was administered and spontaneous contractions were observed, the mean values of contraction frequencies obtained with doses of 0.5mg/ml, 1 mg/ml, and 2mg/ml within the group were found to be significantly lower than the contraction frequency obtained with doses of 0.125 mg/ml and 0.25mg/ml in the control group ($p <0.05$). On the other hand, the results of the Repeated Measurements ANOVA test for Control Group 1 revealed that different amounts of KHS additions did not cause any significant difference in contraction frequency ($p> 0.05$).

In Experiment group 2, in which induced contractions were observed after the administrations of oxytocin and AME, the contraction frequency obtained as a result of each administration was found to be significantly different from the other ($p <0.05$). In Control Group 2, in which the contractions were induced using oxytocin, but AME administration was not made, no difference was observed between the 3rd and 4th administrations, however, the contraction frequency was observed to change significantly in all other administrations.

Contraction Amplitude

The initial contraction amplitude of the Experimental Group 1 was found to be significantly lower than the Control Group 1 (Table 2). This situation continued in all administrations. Therefore, it is not appropriate to evaluate the comparison of Experimental Group 1 and Control Group 1 for different administrations. While the results of the Repeated Measures ANOVA Test, which was conducted to evaluate the mean contraction amplitude change in Control Group 1 for different administrations did not show a statistically significant difference ($p> 0.05$), the 3rd, 4th, and 5th administrations in Experimental Group 1 were found to be significantly different from other administrations ($p<0.05$). In other words, AME significantly reduced the spontaneous uterine contractions amplitude in administrations of 0.5, 1, and 2mg/ml.

Comparing Experimental Group 2 with Control Group 2, there was a significant difference only for the 5th administration ($p = 0.011$). While the amplitude of oxytocin-induced contractions did not differ significantly for different administration (Control Group 2), the amplitude of oxytocin-induced contractions decreased significantly in each administration when increasing doses of AME were administered (Experimental Group 2) ($p <0.05$).

DISCUSSION

In the study, the effect of AME on spontaneous contractions and oxytocin-induced in vitro rat uterine smooth muscle contractility was investigated. Moreover, the effective doses of AME which are effective in in vitro rat uterine smooth muscle contractility have been tried to be determined. At the end of the research, it was revealed that AME significantly reduced the frequency and amplitude of both spontaneous and oxytocin-induced uterine contractions depending on the dose.

Table 1: Uterus contraction frequencies of the experimental groups (cycle/10 minutes)

	Doses	Experimental Group 1 (AME) (n=8)	Control Group 1 (KHS) (n=8)	p value*	Experimental Group 2 (Oxytocin+AME) (n=8)	Control Group 2 (Oxytocin) (n=8)	p value**
	Control	**10.87±1.69^{A}**	**8.87±3.09^{A}**	**0.156**	**19.37±5.02^{A}**	**20.75±2.53^{A}**	**0.528**
1st Administration:	0.125 mg/ml AME or the same amount of KHS	10.37±2.05^{A}	9.50±4.06^{A}	0.619	16.87±3.51^{B}	17.37±1.99^{B}	0.748
2nd Administration:	0.25 mg/ml AME or the same amount of KHS	10.00±2.06^{A}	9.25±4.05^{A}	0.669	15.37±3.23^{C}	15.25±1.56^{C}	0.928
3rd Administration:	0.5 mg/ml AME or the same amount of KHS	9.25±2.53^{B}	10.00±4.21^{A}	0.693	13.12±3.40^{D}	13.75±2.63^{D}	0.707
4th Administration:	0.5 mg/ml AME or the same amount of KHS	7.00±2.44^{C}	9.00±4.87^{A}	0.348	11.12±4.01^{E}	13.00±2.59^{D}	0.317
5th Administration:	0.5 mg/ml AME or the same amount of KHS	2.37±1.47^{D}	9.25±4.72^{A}	0.002	7.87±4.53^{F}	11.75±2.16^{E}	0.061

* p value shows the result of independent t-test between Experiment Group 1 and Control Group 1 for each administration.
** p value shows the result of independent t-test between Experiment Group 2 and Control Group 2 for each administration.
A, B, C, D, E, F: It refers to the result of comparing the contraction frequencies obtained in different administrations in a single group using repeated measures analysis of variance method. Different capital letters in the same column indicate a statistically significant difference between the means ($p < 0.05$)

Studies investigating the effects of AME on smooth muscle were usually carried out on ileum and vascular smooth muscle. Yaeesh et al administered 70% methanol extract of AM with doses ranging between 0.3-10 mg/kg to isolated jejunum preparations and they determined that it inhibited spontaneous and potassium-induced smooth muscle contractions (12). In another study, Babaei et al. reported that different doses of hydroalcoholic extract of AM suppress in vitro guinea-pig ileum smooth muscle contractions depending on the dose (13). Again, Moradi et al. found that AME ethanol extract reduced ACh-stimulated isolated rat ileum smooth muscle contractions (14). The reports of the above researchers are similar to the results we obtained in the study. According to Yaeesh et al., this important similarity might be because the antispasmodic activity of flavonoid-derived compounds in AME exhibits an antagonistic effect on calcium release (12). According to Yadegari et al., the inhibitory effect of AME on smooth muscle is due to the vasodilating effect of apigenin, luteolin, quercetin, and lignan; According to Grossini et al., it is because of the relaxing effect on vascular smooth muscles by increasing the nitric oxide release due to the so-called artemetin compound in AME (15,16).

Oxytocin increases the strength, time, and frequency of contractions (17). Oxytocin increases intracellular Ca^{+2} concentration. In myometrial cells, rapid Ca^{+2} entry into the cell occurs through L-type Ca^{+2} channels and receptor sensitive Ca^{+2} channels due to the effect of Oxytocin. Also, oxytocin inhibits Ca-ATPase and prevents Ca^{+2} from leaving the cell. Oxytocin interacts with a number of G-protein-coupled receptors in myometrial cells and this activates the phospholipase C enzyme, hydrolyzes the phosphoinositides, increases the intracellular Ca^{+2}, and induces the contraction. In their study, Lemmens-Gruber et al. also stated that AM flavonoid compounds inhibited contraction in the isolated guinea-pig ileum by blocking the flow of Ca^{+2} into the cell (9). Yaeesh et al. stated that it might be the result of the antagonist effect on calcium release due to the antispasmodic activity of the flavonoid derivative compounds in AME (12). The effect of different doses of AME on reducing oxytocin-induced uterine smooth muscle contraction responses is thought to be realized through voltage-sensitive L type Ca^{+2} channels.

The significant difference observed in contraction amplitudes in the baseline measurements of Experimental Group 1, in which the effect of AME on spontaneous contractions was investigated, and Control Group 1 made the comparison between the two groups indisputable in the following administrations: this situation is the weakness of our study. On the other hand, investigating the effect of AME in different doses and revealing the dose-dependent relationship, as well as being the first study to reveal the effect of AME on decreasing both spontaneous and oxytocin-induced contractions are also strong aspects of our study.

Table 2: Uterus contraction amplitudes of the experimental groups (mg)

	Doses	Experimental Group 1 (AME) (n = 8)	Control Group 1 (KHS) (n=8)	p value*	Experimental Group 2 (Oxytocin+AME) (n=8)	Control Group 2 (Oxytocin) (n=8)	p value**
	Control	**2369.34 ±403.48^{A}**	**4284.10 ±1315.92^{A}**	**0.002**	**5855.47 ±1049.99^{A}**	**4893.10 ±1004.84^{A}**	**0.102**
1st Administration:	0.125 mg/ml AME or the same amount of KHS	2305.17 ±421.96^{A}	4208.68 ±1512.03^{A}	0.006	5855.22 ±1057.27^{B}	4917.29 ±954.97^{A}	0.103
2nd Administration:	0.25 mg/ml AME or the same amount of KHS	2241.14 ±422.58^{A}	4314.55 ±1385.26^{A}	0.002	5693.17 ±1101.79^{C}	4836.71 ±1083.49^{A}	0.165
3rd Administration:	0.5 mg/ml AME or the same amount of KHS	2190.23 ±441.98^{B}	4435.91 ±1422.32^{A}	0.001	5266.24 ±1243.17^{D}	4962.88 ±982.52^{A}	0.620
4th Administration:	0.5 mg/ml AME or the same amount of KHS	1956.49 ±489.45^{C}	4397.09 ±1332.91^{A}	0.000	4694.33 ±1324.83^{E}	4750.69 ±1023.33^{A}	0.930
5th Administration:	0.5 mg/ml AME or the same amount of KHS	1519.87 ±433.85^{D}	4311.92 ±1903.45^{A}	0.000	3461.78 ±1187.62^{F}	4843.23 ±1007.59^{A}	0.011

* p value shows the result of independent t-test between Experiment Group 1 and Control Group 1 for each administration.
** p value shows the result of independent t-test between Experiment Group 2 and Control Group 2 for each administration.
A, B, C, D, E, F: It refers to the result of comparing the contraction amplitudes obtained in different dose administrations in a single group using repeated measures analysis of variance method. Different capital letters in the same column indicate a statistically significant difference between the means ($p < 0.05$)

CONCLUSION

AME significantly reduced uterine spontaneous contraction amplitude values at all doses while it significantly reduced the frequency at the dose of 2 mg/ml. In oxytocin-induced contractions, the maximum dose decreased the contraction amplitude without affecting the frequency. It is necessary to take into consideration the AME's feature of weakening the uterine contractions in cases such as preterm birth and miscarriage risk.

ACKNOWLEDGEMENT

Peer-Review

Both externally and internally peer reviewed.

Conflict of Interest

The authors declare that they have no conflict of interests regarding content of this article.

Support Resources

This study was funded by Selçuk University Scientific Research Projects Office with the project number 181318016.

Ethical Declaration

This study was approved by the Necmettin Erbakan University Animal Experiments Local Ethics Committee with the date 19.10.18 and the decision no. 2018-035. All experiments complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving animals.

Authorship Contributions:

Concept: FÖ, Design: AE, FÖ, Supervising: FÖ, Financing and equipment: FÖ, AE, Data collection and entry: AE, Analysis and interpretation: AE, MPY, Literature search: MPY, FÖ, AE, AŞ, Writing: FÖ, AE, MPY, AŞ

REFERENCES

1. Pieroni A, Quave CL. Traditional pharmacopoeias and medicines among Albanians and Italians in southern Italy: A comparison. J Ethnopharmacol. 2005 Oct 3;101(1-3):258-70. https://doi.org/10.1016/j.jep.2005.04.028
2. Radušiene J, Gudaityte O. Distribution of proazulenes in Achillea millefolium s.l. wild populations in relation to phytosociological dependence and morphological characters. Plant Genet Resour. 2005 Aug;3(2):136-43. https://doi.org/10.1079/PGR200568
3. Passalacqua NG, Guarrera PM, De Fine G. Contribution to the knowledge of the folk plant medicine in Calabria region (Southern Italy). Fitoterapia. 2007 Jan;78(1):52-68. https://doi.org/10.1016/j.fitote.2006.07.005
4. Nemeth E, Bernath J. Biological Activities of Yarrow Species (Achillea spp.). Curr Pharm Des. 2008 Nov 12;14(29):3151-67.

https://doi.org/10.2174/138161208786404281

5. Stojanović G, Radulović N, Hashimoto T, Palić R. In vitro antimicrobial activity of extracts of four Achillea species: The composition of Achillea clavennae L. (Asteraceae) extract. J Ethnopharmacol. 2005 Oct 3;101(1-3):185-90. https://doi.org/10.1016/j.jep.2005.04.026

6. Lakshmi T, Geetha R, Roy A, Aravind Kumar S. Yarrow (Achillea millefolium linn). A herbal medicinal plant with broad therapeutic use-A review. Int J Pharm Sci Rev Res. 2011;9(2):136-41.

7. Zakeri S, Gorji N, Moeini R, Memariani Z. Therapeutic Application of Achillea millefolium L. in Female Reproductive Diseases from the Viewpoint of Persian Medicine and Current Medicine. J Med Plants [Internet]. 2019;4(72):107-21.

8. Borrelli F, Romano B, Fasolino I, Tagliatatela-Scafati O,Aprea G,Capasso R et al. Prokinetic effect of a standardized yarrow (Achillea millefolium) extract and its constituent choline: Studies in the mouse and human stomach. Neurogastroenterol Motil. 2012 Feb 1;24(2). https://doi.org/10.1111/j.1365-2982.2011.01827.x

9. Lemmens-Gruber R, Marchart E, Rawnduzi P, Engel N, Benedek B, Kopp B. Investigation of the spasmolytic activity of the flavonoid fraction of Achillea millefolium s.l. on isolated guinea-pig ilea. Arzneimittel-Forschung/Drug Res. 2006;56(8):582-6. https://doi.org/10.1055/s-0031-1296755

10. Ali M, Al-Imari J. Effects of Achillea Millifolium extract consumption by pregnant mice on pregnancy outcome and reproductive system of their female off spring. Kufa Journal For Veterinary Medical Sciences. 2012.

11. Radfar S, Shahoie R, Noori B, Jalilian F, Hashemi Nasab L. Comparative Study on the Effect of Matricaria chamomile and Achillea millefolium Capsules on Primary Dysmenorrhea Intensity of Dormitory Students of Kurdistan University of Medical Sciences, 2018. J Pharm Res Int. 2018;1-7. https://doi.org/10.9734/jpri/2018/v25i330101

12. Yaeesh S, Jamal Q, Khan AU, Gilani AH. Studies on hepatoprotective, antispasmodic and calcium antagonist activities of the aqueous-methanol extract of Achillea millefolium. Phyther Res. 2006 Jul;20(7):546-51. https://doi.org/10.1002/ptr.1897

13. Babaei M, Abarghoei ME, Akhavan MM, Ansari R, Vafaei AA, Taherian AA et al. Antimotility effect of hydroalcoholic extract of yarrow (Achillea millefolium) on the guinea-pig ileum. Pakistan J Biol Sci. 2007 Oct 15;10(20):3673-7. https://doi.org/10.3923/pjbs.2007.3673.3677

14. Moradi M-T, Rafieian-Koupaei M, Imani-Rastabi R, Nasiri J, Shahrani M, Rabiei Z et al. Antispasmodic effects of yarrow (Achillea millefolium L.) extract in the isolated ileum of rat. Afr J Tradit Complement Altern Med [Internet]. 2013 [cited 2020 Apr 8];10(6):499-503. https://doi.org/10.4314/ajtcam.v10i6.19

15. Yadegari M, Khamesipour F, Talebiyan R, Katsande S. Echocardiography Findings After Intravenous Injection of Achillea millefolium (Yarrow) Extract in The Dog. Malays Appl Biol. 2015;44(2):85-91.

16. Grossini E, Marotta P, Farruggio S, Siguado L, Qoqaiche F, Raina G et al. Effects of Artemetin on Nitric Oxide Release and Protection against Peroxidative Injuries in Porcine Coronary Artery Endothelial Cells. Phyther Res. 2015 Sep 1;29(9):1339-48. https://doi.org/10.1002/ptr.5386

17. Petrocelli T, Lye SJ. Regulation of transcripts encoding the myometrial gap junction protein, connexin-43, by estrogen andprogesterone. Endocrinology. 1993;133(1):284-90. https://doi.org/10.1210/endo.133.1.8391423