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RESEARCH ARTICLE

The vasorelaxant effect of *Adiantum capillus-veneries* extract in the vascular activity of goat's isolated renal artery

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ABSTRACT

The current study represents the first attempt to investigate the effect of *Adiantum capillus-veneris* extract (ACVE) on goat's isolated renal artery smooth muscle's cell. Therefore, this research aims to investigate the possible action of *Adiantum capillus-veneris* extract (ACVE) (1×10^{-4}–10^{-9} mg/ml) in the vascular activity of goat isolated renal artery by using the organ bath and PowerLab data acquisition system, and the phytochemical analysis for the ACVE extract was done by using gas chromatography-mass spectrometry (GC/MS).

The results of the current study showed that ACVE caused concentration-dependent relaxation of endothelium intact renal artery rings precontracted with a high level of KCl (60 mM) or phenylephrine (PE) (10^{-5} M), also they are exhibited potent inhibitory effects on PE, and less potent on KCl-induced contractions.

Renal artery rings preincubated with potassium (K^+) channel blocker (tetraethylammonium, TEA), barium chloride ($BaCl_2$), 4-aminopyridine (4-AP), (indomethacin) and (clotrimazole) showed a significant effect in renal artery smooth muscle relaxation induced by ACVE. While glibenclamide (Glib) and L-NAME did not exhibit any role in the relaxation effect of ACVE.

Furthermore, the role of nifedipine L-type calcium channels blocker in the effects of ACVE suggests that a Ca^{2+} channel blocking mechanism has a relaxant effect in the goat's renal artery smooth muscles. Renal artery rings preincubated with nifedipine (1×10^{-5} and 3×10^{-5}), and ACVE (1×10^{-5} and 3×10^{-5}), produced a potent inhibitory effect on $CaCl_2$ induced contraction as compared with the control group, and also reduced the maximum contraction.

Results of the current study, it can be concluded that ACVE have potent vasorelaxation effects on renal artery rings which are mediated, partly, by the enhancement of PGI_2, EET and modulating different K^+ channels and L-type Ca^{2+} channels activities. Additionally, the results of the current study provide the mechanism of action of some constituents of the medicinal plant *Adiantum capillus-veneris* which can be exploited to develop more specific drugs to be used for the treatment of various vascular diseases based on phytochemical analysis of the extract.

Keywords: Adiantum capillus, Smooth muscle relaxation, renal artery, K+ channels blocker, Ca2+channels blocker.

INTRODUCTION

Medicinal natural sources, especially the plants, have been used in the treatment of diseases since ancient times. Medicinal plants are an important source of synthetic and herbal drugs (Kheder, 2013). Plants might be an alternative source of drugs which are used in the treatment of many diseases because they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in integrated management programs (Cheng et al., 2009). Herbs act as a medicinal treatment because they contain antioxidant, vasorelaxant or diuretic effects (Anwar et al., 2016).

The*Adiantum capillus-veneris* is one of the most frequent species with potential importance for the medicinal and nutritive purposes (Janbaz *et al*., 2015). The genus *Adiantum* belongs to family Adiantaceae which comprises of 150 to 200 species which are universally distributed (Jain *et al*., 2014). Half of the species have been used in traditional Chinese medication for human and animal diseases, that includes relief of internal heat or fever, enhancement of urination, exclusion of urinary calculus, as well as treatment of urinary tract infection and calculus (Pan *et al*., 2011). *Adiantum capillus-veneris* has various chemical constituents such as tannins, terpenoids, flavonoids, alkaloids, phenylpropanoids, triterpenoids and steroids (Dehdari and Hajimehdipoor, 2018, Ishaq *et al*., 2014, Nazim *et al*., 2018). Phenyl ethanoids, lipids, and long-chain compounds have been isolated from different plants of *Adiantum* genus (Khodaie *et al*.,

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2015). The triterpenoids structures have Ca^{2+}channel antagonist propertie (Yuan, 2015). The natural triterpenoids isolated from plants have vasodilator effects via cyclooxygenase synthase pathway (Al-Habib *et al*., 2015). Flavonoids of plant leave inhibit certain mammalian enzyme systems such as protein kinase C (PKC) and phospholipase C (PLC) and then could reduce Ca^{2+} release in the cytoplasm and lead to muscle relaxation (Janbaz *et al*., 2015). Researchers found that tannins inhibit Ca^{2+} channels and induce muscle relaxation. Tannins may act with proteins involved in the regulation of ryanodine receptors (RYR) and partly block Ca^{2+} release from sarcoplasmic reticulum (SR) (Belemtougri *et al*., 2006).

The current study aims to investigate the effects of the Adiantum capillus-veneris extract (ACVE) for its action as alpha-blocker agent in the smooth muscles of renal artery rings isolated from goats. The roles of potassium and calcium channels are also examined in this study, which is mediating the action of Adiantum capillus-veneries in goat renal artery smooth muscles. In another part of our study Phytochemical analysis of ACVE was determined by using GC/MS.

Materials and Methods

This study was conducted at (Health and Science Research Centre - Koya University). Renal artery of male goats (*Capra aegagrus hircus*) was used throughout this study, the kidneys of freshly slaughtered male goats, weighting from (15-20 Kg) are immediately collected from (Koya slaughterhouse). Then they were immersed in freshly prepared Kreb's solution with 7.4 PH and aerated with 95% O_2 and 5% CO_2 at 37 °C. The isolated renal artery was cleaned from adhering fat and blood. The dissected artery was cut into several rings (2-4 mm) in length and kept in the physiological saline prior to starting the experiments. The procedure which was described by Al-Habib and Shekha (2010) is followed with some changes to study the vascular reactivity in the isolated renal artery. Two stainless steel wire was carefully placed into lumen of the artery rings, one of them was anchored to a glass organ bath and the other wire was linked to force transducer, coupled to the trans bridge amplifier, and (AD Instrument Power Lab 26T Data Acquisition system) with computer running chart software (LabChart Version8) was used for measurement isometric tension of the isolated renal artery rings. Prior to the experiment, the organ bath was filled with double distilled water and the temperature was set at 37 °C for (60 – 90 min), followed by the addition of (10 ml) of Kreb's solution (in mM/L: 118 NaCl, 4.7 KCl, 25 NaHCO3, 1.2 KH2PO4, 1.2 $MgSO_4$, 2.4 $CaCl_2$, 11Glucose and 0.03 EDTA) or free Calcium Kreb's solution to each channel of the organ bath (Shin et al., 2005). The preparation was oxygenated continuously with (95% O_2 and 5% CO_2). The temperature of the solution inside the organ bath was maintained 37 °C by circulating water through water jacket from a circulating water bath set at 37 °C (Thermo circulator LabTech DAIHAN LABTECH CO., LTD.). The primary tension was set at (2 gm) weight. Renal artery rings were allowed to equilibrate (60-90 min) with buffer solution change every (15 min). For the integrity of functions**,** the prepared artery segments, KCl (60 mM) (Qu et al., 2014), was used and the maximum contraction developed was considered as standard percentage contractile response. After the maximum contraction by KCl was reached to plateau, the renal artery rings were washed and restabilized at the optimum tension for at least (30 min) before applying any vasoactive substances (Ahmed and Maulood, 2018). When tension had stabilized isometrically, concentration-response curves (CRCs) for PE (1× 10-5 M) and KCl (60 mM) were constructed against induced contraction and then the experiments started.

Plant material

In the current study *Adiantum capillus-veneris* plant was used, the dried aerial part of the *Adiantum capillus-veneris* was collected during September, 2018 from the (Warte) sub-district.

The collected samples cleaned from the debris by washing several times with tap water, then dried at room temperature for (2-3 weeks), cut into small pieces and then ground by using an electrical grinder. The product was stored in a capped container at nearly 5° C until its use for extraction.

The voucher specimen was preserved at the Health and Science Research center of Koya University. The given herbarium code for the species is 7600 (which means the voucher number). The plant material was kindly identified and authenticated by Assist. Prof. Dr. Abdullah Sh. Sardar (Herbarium of Education College- Salahaddin university).

Preparation of crude extracts

The *Adiantum capillus-veneris* were ground into coarse powder through an electrical grinder. 100g of powdered material was soaked in 1L of ethyl acetate for 24 hours and stirred overnight with a magnetic stirrer at room temperature (25 ± 2 C°), followed by rapid filtration through a gauze cloth and then filtered through Whatman No.1 filter paper. According to the method described by (Ishaq *et al*., 2014, Abdulazeez and Ponnusamy, 2016), the filtrates were collected in separate flasks.

The filtrates that obtained were completely concentrated in a rotary evaporator at 40°C under reduced pressure (-760 mmHg). These dry extracts were then preserved in separate dark glass containers and wrapped by aluminium foil to protect from sunlight and kept in the refrigerator. The desired serial dilutions were prepared with (DMSO for ethyl acetate extraction).

Phytochemical analysis by GC/MS

Gas chromatography -Mass spectrometry (GC-MS) analysis of the *A. capillus* ethyl acetate extracts was performed in the scientific research centre at Soran university by using a GC-MS model- Agilent 7890B with column type: J&W DB-5ms ultra inert GC column, 30 m, 0.25 mm, 0.25 µm. Helium was used as a carrier gas at a flow rate of (1 ml/min).

Experimental procedure

The experimental procedure of experiments includes, the recording of normal mechanical activity of renal artery smooth muscles, and studying the effects of potassium chloride (KCl)

and phenylephrine (PE) on normal mechanical activity of the goat renal artery smooth muscle within Kreb's solution, in addition to the effect of free Ca^{2+} Kreb's solution. Then the role of endothelial nitric oxide (NO), prostaglandin I2 (PGI2) and EET in the association with vasorelaxation induced by different doses of the *Adiantum capillus-veneries* extract (1×10^{-4} –10^{-9} M) were studied following after incubation intact renal artery rings for (10 min) separately with each of NO synthase inhibitor (L-Name (3×10-4 M)), PGI_2 inhibitor (Indomethacin (3× 10-5 M)) and EET (Clotrimazole (3 × 10-5 M)) and contracted with PE (1×10-5 M). Also the role of Potassium channels (K+ channel) and calcium channels (Ca^{2+} channel) in the development of vasorelaxation induced by different doses of *Adiantum capillus-veneris* extract (ACVE) (1×10^{-4}–10^{-9} mg/ml) were also studied by preincubation of the renal artery rings separately with each of the following potassium channel blockers, KCa channel blocker (TEA (1 mM)), KATP channel blocker (GLIB (1×10-5 M)), KIR channel blocker (BaCl2 (1mM)) and KV channel blocker (4-AP (1 mM)) and contracted with PE (1X10-5 M), and in the free Ca^{2+} solution either the L-type calcium channel blocker Nifedipine (Nif. 1×10^{-5} and 3×10^{-5} M) or the *Adiantum capillus-veneris* extract (ACVE) (1×10^{-5} and 3×10^{-5} mg/ml) were used for testing the role of the Ca^{2+} channels.

Statistical analysis

The data of this study were expressed as M ± SE and the effective mean concentrations (IC50 and EC50) were given as geometric mean with (95%) confidence intervals (CI) and the potency values were described as the negative logarithm (-log IC50 = pIC50 and -logEC50 = pEC50) of the mean of individual values for each tissue. For comparison between means of two groups two-way analysis of variance (Two-way ANOVA) was used supported by Sidak's multiple comparisons test, the concentration-response curve was analysed by non-linear regression.

Probability of less than 0.05 ($p<0.05$) was considered statistically significant. In all figures and tables, the symbols *, **, *** and **** indicate that the difference between means is significant at 0.05, 0.01, 0.001 and <0.0001 levels, respectively. All the graphs, calculations, and statistical analysis were done by GraphPad Prism software version 7.04 for windows, (GraphPad Software, USA). The maximum effect of relaxation ($E_{max.}$) was considered as a maximal amplitude response reached in concentration-effects for relaxant agent.

Results

Effect of Adiantum Capillus-veneries extract in renal artery precontracted with PE and KCl

The adiantum capillus-veneries extract ACVE at a concentration from (1×10^{-9}– 1×10^{-4} mg/ml) caused a statistically significant more relaxant ($P<0.05$) effect in the PE (10^{-5} M) compared to KCl (60 mM) precontracted renal artery rings. Dose-response curves for the effect of ACVE on PE- and KCl-induced contractions are shown in (Figure 1).

The pIC_{50} (-Log IC_{50}), (-Log IC_{50} of CI 95%) and E_{max} are shown in (Table 1). the ACVE produced a most potent inhibitory effect on PE- and KCl-induced contractions, with a pIC_{50}'s of 6.986 mg/mL, (-Log IC_{50} of CI 95% between 6.779 to 7.183) and 7.588 mg/ml (-LogIC_{50} of CI 95% between 7.27 to 7.884), respectively. The E_{max} (%) of renal artery rings precontracted with PE reduced to only 89.999 ±1.13 %, while in renal artery rings precontracted with KCl, the relaxation response was diminished as indicated by the increased contraction tone to 83.34 ± 2.539.

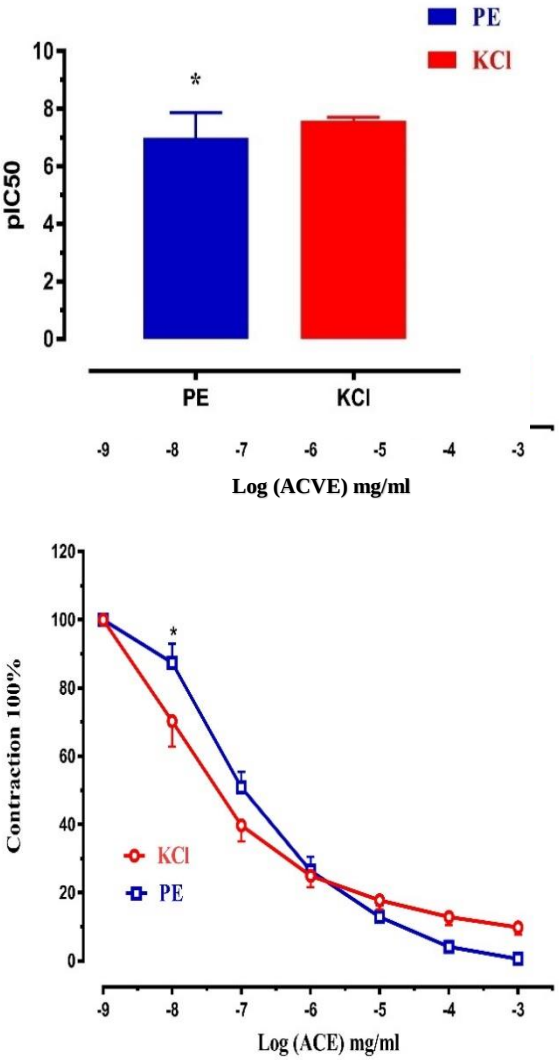

Figure 1 Cumulative dose-response curve for the effects of ACVE on PE (10^{-5}) and KCl (60mM) precontracted renal artery rings. Left panel: Histogram represent pIC_{50} and$E_{max.}$ of comparative vasorelaxation effects of KCl and PE

Table 1 The pIC_{50} (-Log IC_{50}), (-Log IC_{50} of CI 95%) and E_{max} (%) ± SEM for the effects of ACVE on PE and KCl precontracted renal artery rings

Treatment	ACVE	
Control	PE	KCl
pIC_{50}	6.986	7.588
-Log IC_{50} of CI 95%	6.779 to 7.183	7.270 to 7.884
E_{max} (%) ± SEM	89.999 ± 1.13*	83.34 ± 2.539

Role of Potassium channels in the vasorelaxant effect of *Adiantum capillus-veneries* extract

To investigate the role of potassium channels in vasorelaxation of renal artery rings were preincubated for 10 minutes with TEA (1mM), Glib. (10^{-5}), $BaCl_2$ (1mM) and 4-AP (1mM) individually, which are the blockers of K_{Ca}, K_{ATP}, K_{IR} and K_V channels respectively. Their relaxant effects were recorded.

(Table 2) shows the pIC_{50} (-LogIC_{50}), (-LogIC_{50} of CI 95%) and E_{max} (%) for the effect of K^+ channel inhibitors on the relaxant response to ACVE in goat's renal artery rings. Dose-response curves for the effect of ACVE against PE-induced contractions and preincubated with the K^+ channel blockers are shown in (Figures 2, 3, 4 and 5). The pre-treatment of renal artery rings with TEA, $BaCl_2$ and 4-AP showed a highly significant effect and shifted to the left, while GLIB remained unchanged. ACVE concentrations (1×10^{-9} to 10^{-4} M) caused a potent relaxation on PE (10^{-5}M) precontracted goat renal artery rings.

Pre-treatment of renal artery rings with TEA, $BaCl_2$ and 4-AP significantly ($P<0.001$) inhibited the dilation with pIC_{50} 6.962 mg/ml , (-LogIC_{50} of CI 95% between 6.384 to 7.462), 5.837 mg/ml (4.993 to 6.523) and 5.287 mg/ml (4.425 to 6.167) and also they reduce the percentage of relaxation to (57.55 ± 1.547% , 19.71 ± 0.109 % and 67.44 ± 2.521 %), respectively as compared to the control which was (89.999 ± 1.13 %). While Glib. pre-treatment did not alter the dilation induced by ACVE with pIC_{50} 7.566 mg/ml , (-LogIC_{50} of CI 95% between 7.213 to 7.899) and also the percentage of relaxation (87.690 ± 2.884%).

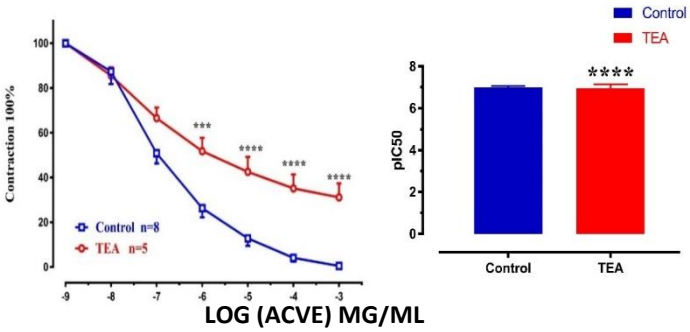

Figure 2 Cumulative dose-response curves for the vasorelaxant effects of ACVE on control and preincubated renal artery rings with TEA (1mM), precontracted with PE (1×10^{-5} M). Left panel: Histogram represent pIC_{50} and $E_{max.}$ of comparative vasorelaxation effects of PE and TEA

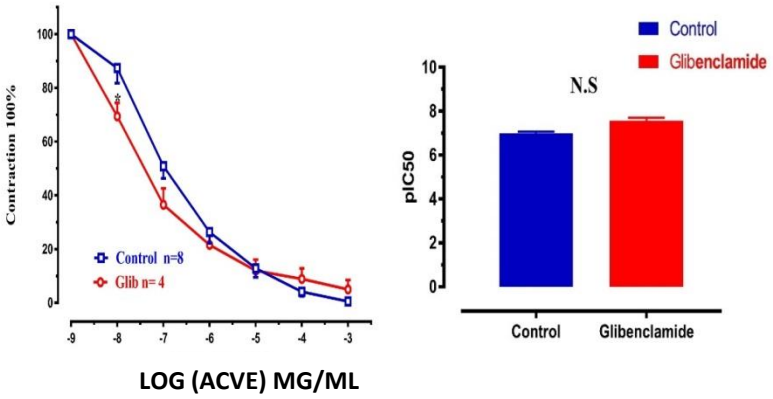

Figure 3 Cumulative dose-response curves for the vasorelaxant effects of ACVE on control and preincubated renal artery rings with Glib (1×10-5 M), precontracted with PE (1×10-5 M). Left panel: Histogram represent pIC50 and Emax. of comparative vasorelaxation effects of PE and Glib

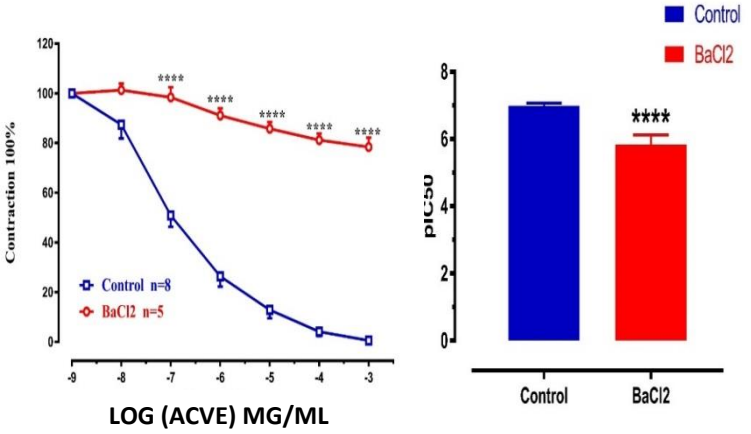

Figure 4 Cumulative dose-response curves for the vasorelaxant effects of ACVE on control and preincubated renal artery rings with BaCl2 (1mM), precontracted with PE (1×10-5 M). Left panel: Histogram represent pIC50 and Emax. of comparative vasorelaxation effects of PE and BaCl2

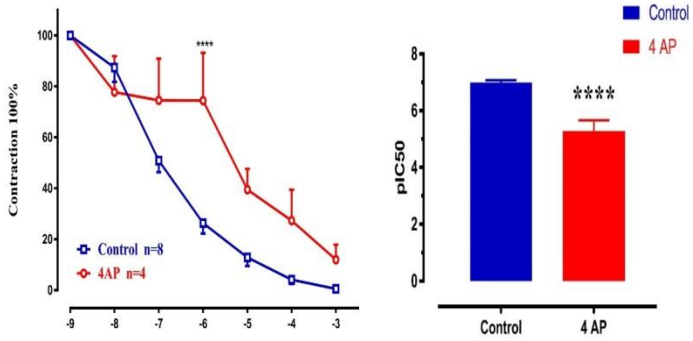

Figure 5 Cumulative dose-response curves for the vasorelaxant effects of ACVE on control and preincubated renal artery rings with 4AP (1mM), precontracted with PE (1×10^{-5} M). Left panel: Histogram represent pIC_{50} and $E_{max.}$ of comparative vasorelaxation effects of PE and 4AP

Table 2 The pIC_{50} (-Log IC_{50}), (-Log IC_{50} of CI 95%) and E_{max} (%) ± SEM for the effects of ACVE on preincubated renal artery rings with K^+ channel blockers.

Treatment / Statistical Measurements	Control	TEA (1mM)	Glib. (10-5 M)	BaCl2 (1mM)	4Ap (1mM)
pIC_{50}	6.986	6.962	7.566	5.837	5.287
-$LogIC_{50}$ of CI 95%	6.779 to 7.183	6.384 to 7.462	7.213 to 7.899	4.993 To 6.523	4.425 to 6.167
E_{max} (%) ± SEM	89.999 ± 1.13	57.55 ± 1.547*** *	87.69 ± 2.884	19.71 ± 0.109****	67.44 ± 2.521*** *

Role of endothelium/ NO, PGI_2 and EET in the vasorelaxant effect of the *Adiantum capillus-veneries* extract

The percentage of relaxation, pIC_{50} (-$LogIC_{50}$), and (-$LogIC_{50}$ of CI 95%) for the relaxant response to ACVE were highly significant in renal artery rings preincubated with clotrimazole and slightly with indomethacin compared to the control rings (Figures 6, 7 and 8) with pIC_{50} 7.096 mg/ml, and (-$LogIC_{50}$ of CI 95% between 6.547 to 7.68) and 7.873 mg/ml (-$LogIC_{50}$ of CI 95% between 7.251 to 8.36) and E_{max} were (61.37 ± 1.798 and 82.76 ± 1.921), respectively. While L-NAME pre-treatments did not alter the dilation induced by ACVE with pIC_{50} 6.732 mg/ml, and (-$LogIC_{50}$ of CI 95% between 5.576 to 7.586) and E_{max} was (71.600 ± 2.245) (Table3).

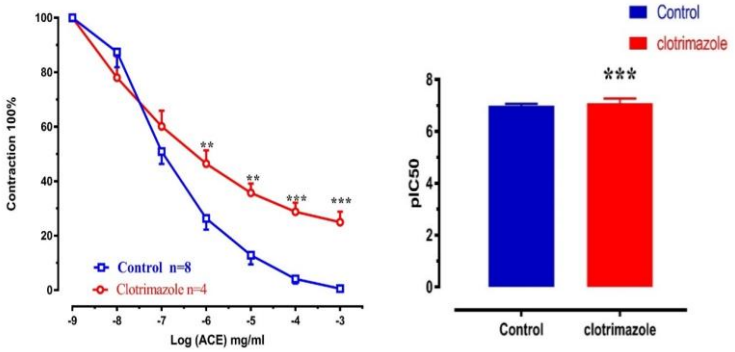

Figure 7 Cumulative dose-response curve for the vasorelaxant effects of ACVE on control and preincubated renal artery rings with clotrimazole (3×10^{-5} M), precontracted with PE (10^{-5} M). Left panel: Histogram represent pIC_{50} and $E_{max.}$ of comparative vasorelaxation effects of PE and clotrimazole

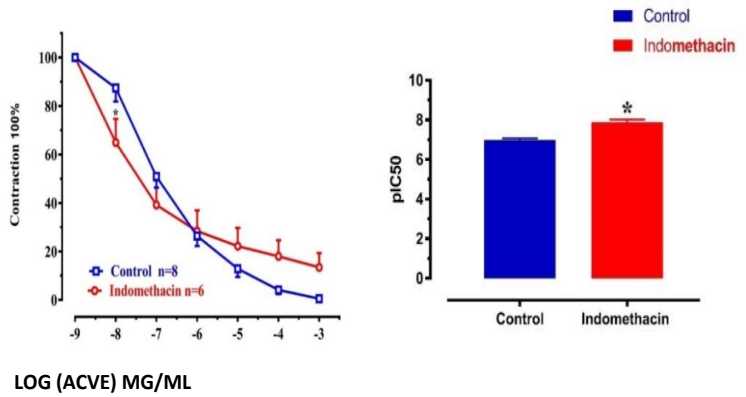

Figure 6 Cumulative dose-response curve for the vasorelaxant effects of ACVE on control and preincubated
renal artery rings with indomethacin (3×10^{-5} M), precontracted with PE (10^{-5} M). Left panel: Histogram represent pIC_{50} and $E_{max.}$ of comparative vasorelaxation effects of PE and indomethacin

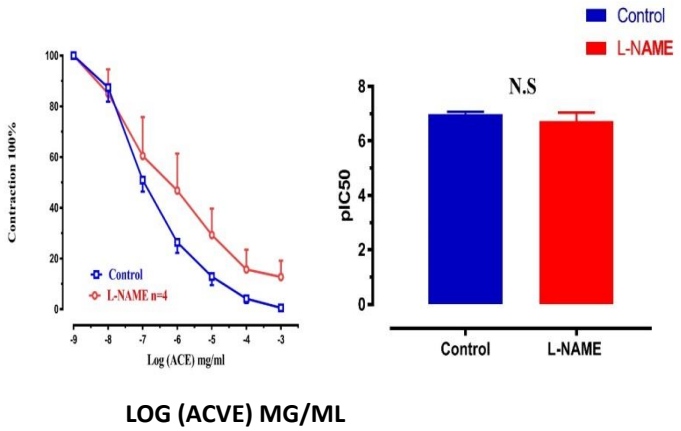

Figure8 Cumulative dose-response curve for the vasorelaxant effects of ACVE on control and preincubated renal artery rings with L- NAME (3×10^{-4} M), precontracted with PE (10^{-5} M). Left panel: Histogram represent pIC_{50} and $E_{max.}$ of comparative vasorelaxation effects of PE and L-NAME

Table 3 Table (3) pIC_{50} (-Log IC_{50}), (-Log IC_{50} of CI 95%) and E_{max} (%) ± SEM for the effects of ACVE on preincubated renal artery rings with clotrimazole, indomethacin, and L-NAME

Treatment / Statistical Measurement	Control	Clotrimazole (3×10^{-5} M)	Indomethacin (3×10^{-5} M)	L-NAME (3×10^{-4} M)
pIC_{50}	6.986	7.096	7.873	6.732
-$LogIC_{50}$ of CI 95%	6.779 to 7.183	6.547 To 7.68	7.251 to 8.36	5.576 to 7.586
E_{max} (%) ± SEM	89.999 ± 1.13	61.37 ± 1.798***	82.76 ± 1.921*	71.6 ± 2.245

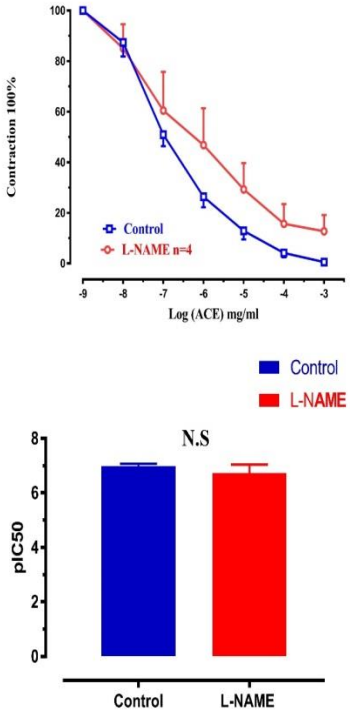

Figure9 Cumulative dose-response curve for the vasorelaxant effects of ACVE on control and preincubated renal artery rings with L- NAME (3×10-4 M), precontracted with PE (10–5 M). Left panel: Histogram represent pIC50 and Emax. of comparative vasorelaxation effects of PE and L-NAME

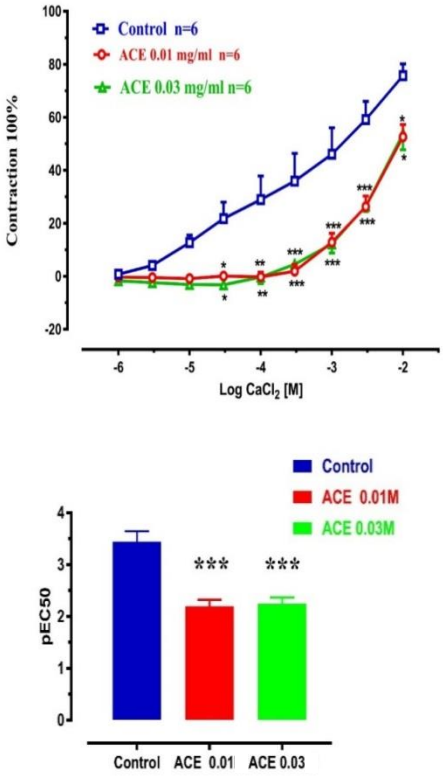

Figure 10 Cumulative dose-response curves of CaCl2 in renal artery rings pre-incubated with different doses of ACVE (1×10-5 mg/ml and 3×10-5 mg/ml). Left panel: Histogram represent pEC50 and Emax. of comparative vasorelaxation effects of ACVE and CaCl2

Effect of *Adiantum capillus-veneries* extract in renal artery contraction induced by $CaCl_2$

Dose-response curves of ACVE induced by $CaCl_2$ in renal artery rings. Both doses of ACVE (1×10^{-5} mg/ml and 3×10^{-5} mg/ml) produced highly significant (P<0.001) vasoconstriction effects on $CaCl_2$ induced dose-dependent contraction in renal artery rings pre-incubated with ACVE as compared to the control (Figure 9). The pEC_{50} (-$LogEC_{50}$), (-$LogEC_{50}$ of CI 95%) and the maximum contraction are shown in (Table 4). Both ACVE doses (1×10^{-5} and 3×10^{-5} mg/ml) showed highly significant effects on $CaCl_2$ contracted goat artery rings with pEC_{50} (2.195) mg/ml (-$LogEC_{50}$ of CI 95% between 1.878 to 2.44) and (2.243) mg/ml (-$LogEC_{50}$ of CI 95% between 1.93 to 2.493), and the maximum contraction (57.231 ± 4.194) and (57.423 ± 3.424) respectively.

Effect of Nifedipine in renal artery contraction induced by $CaCl_2$

Dose-response curves of Nifedipine induced by $CaCl_2$ in renal artery rings. Both doses of Nifedipine (1×10^{-5} M and 3×10^{-5} M) produced highly significant (P<0.0001) vasoconstriction effects on $CaCl_2$ induced dose-dependent contraction in renal artery rings pre-incubated with Nifedipine as compared to the control (Figure 10). The pEC_{50}, (-$LogEC_{50}$ of CI 95%) and the maximum contraction are shown in (Table 4). Both Nifedipine doses (1×10^{-5} and 3×10^{-5} M) showed highly significant effects on $CaCl_2$ contracted goat artery rings with pEC_{50} 2.567 mg/ml (-$LogEC_{50}$ of CI 95% between 2.325 to 2.808) and 2.628 mg/ml (-$LogEC_{50}$ of CI 95% between 2.214 to 2.986), and the maximum contraction (46.123 ± 3.185) and (46.371 ± 3.705) respectively $CaCl_2$

Dose-response curves of Nifedipine induced by $CaCl_2$ in renal artery rings. Both doses of Nifedipine (1×10^{-5} M and 3×10^{-5} M) produced highly significant (P<0.0001) vasoconstriction effects

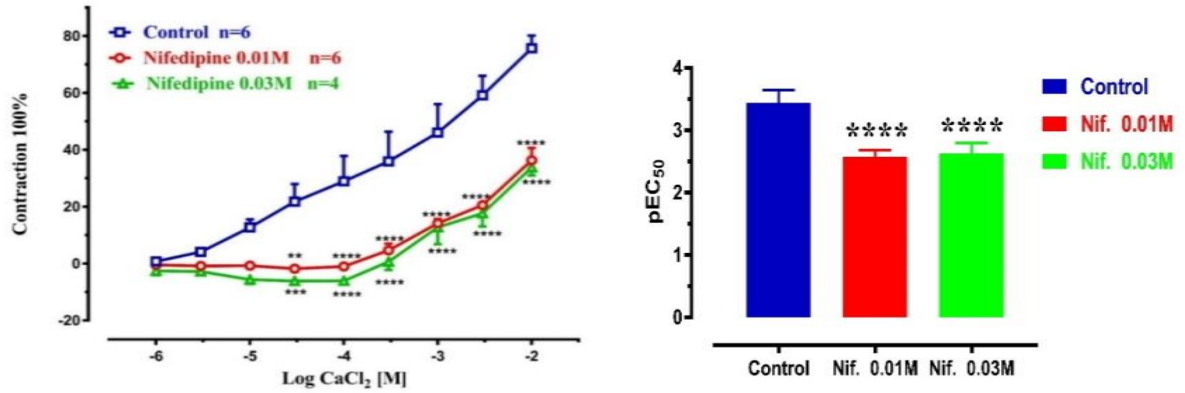

Figure 11 Cumulative dose-response curves of $CaCl_2$ in renal artery rings pre-incubated with different doses of Nifedipine (1×10^{-5} M and 3×10^{-5} M). Left panel: Histogram represent pEC_{50} and $E_{max.}$ of comparative vasorelaxation effects of Nifedipine and $CaCl_2$

Table 4 The pEC_{50} (-Log EC_{50}), (-Log EC_{50} of CI 95%) and E_{max} (%) ± SEM for the effects of Tam, ACVE, and Nifedipine on preincubated renal artery rings with $CaCl_2$

Treatment / Statistical measurement	Control	ACVE		Nifedipine	
		0.01M	0.03M	0.01 M	0.03M
pEC_{50}	3.442	2.195	2.243	2.576	2.628
-LogEC_{50} of CI 95%	2.827 to 4.074	1.878 to 2.44	1.93 to 2.493	2.325 to 2.808	2.214 to 2.986
E_{max} (%) ± SEM	61.402 ± 2.318	57.231 ± 4.194***	57.423 ± 3.424***	46.123 ± 3.185*** *	46.371 ± 3.705****

Phytochemical results

(Table 5) and (Figures 11 and 12) showed that the ACVE contain a number of chemical ingredients. The most important constituents are monoterpenoids as (trans-. beta. -Ionone (1.6%)), sesquiterpenoids as (Neophytadiene (0.44%)), terpenoids as (Ambrox (1.16%)), fatty acids as (2-chloro-Butanoic acid (1.22%) and n-Hexadecanoic acid (0.96%)), alkaloid as (indole (2.28%)), tannin as (gallic acid (0.87%)), isoflavone as (4H-1-Benzopyran-4-one (1.13%)), phenol (4.25%), phenolic acid as (2,5-Dihydroxybenzoic acid (0.37%)), 2-chloro-Propane (2.74%) and 2,4-Hexadiyne (1.76%). The considered priority of this part of the work was the analysis of the aerial parts of this plant to explore its bioactive components.

Table 5 Chemical compounds identified from ethyl acetate extract of *A. capillus* through GC/MS analysis

Name	RT	Area (%)	Formula
2-chloro-Propane	5.276	2.74	C_3H_7Cl
2,4-Hexadiyne	5.323	1.76	C_6H_6
2-chloro- Butanoic acid	7.772	1.22	$C_4H_7ClO_2$
Indole	14.479	2.28	C_8H_7N
trans-. beta. -Ionone	14.513	1.60	$C_{13}H_{20}O$
Gallic acid, 4TMS derivative	17.298	0.87	$C_{19}H_{38}O_5Si_4$
Phenol	19.784	4.25	C_6H_5OH
Neophytadiene	22.067	0.44	$C_{20}H_{38}$
n-Hexadecanoic acid	23.613	0.96	$C_{16}H_{32}O_2$
2,5-Dihydroxybenzoicacid, 3TMS	25.652	0.37	$C_{16}H_{30}O_4Si_3$
Ambrox	30.145	1.16	$C_{13}H_{19}Br_2ClN_2O$
4H-1-Benzopyran-4-one	31.458	1.13	$C_9H_5BrO_2$

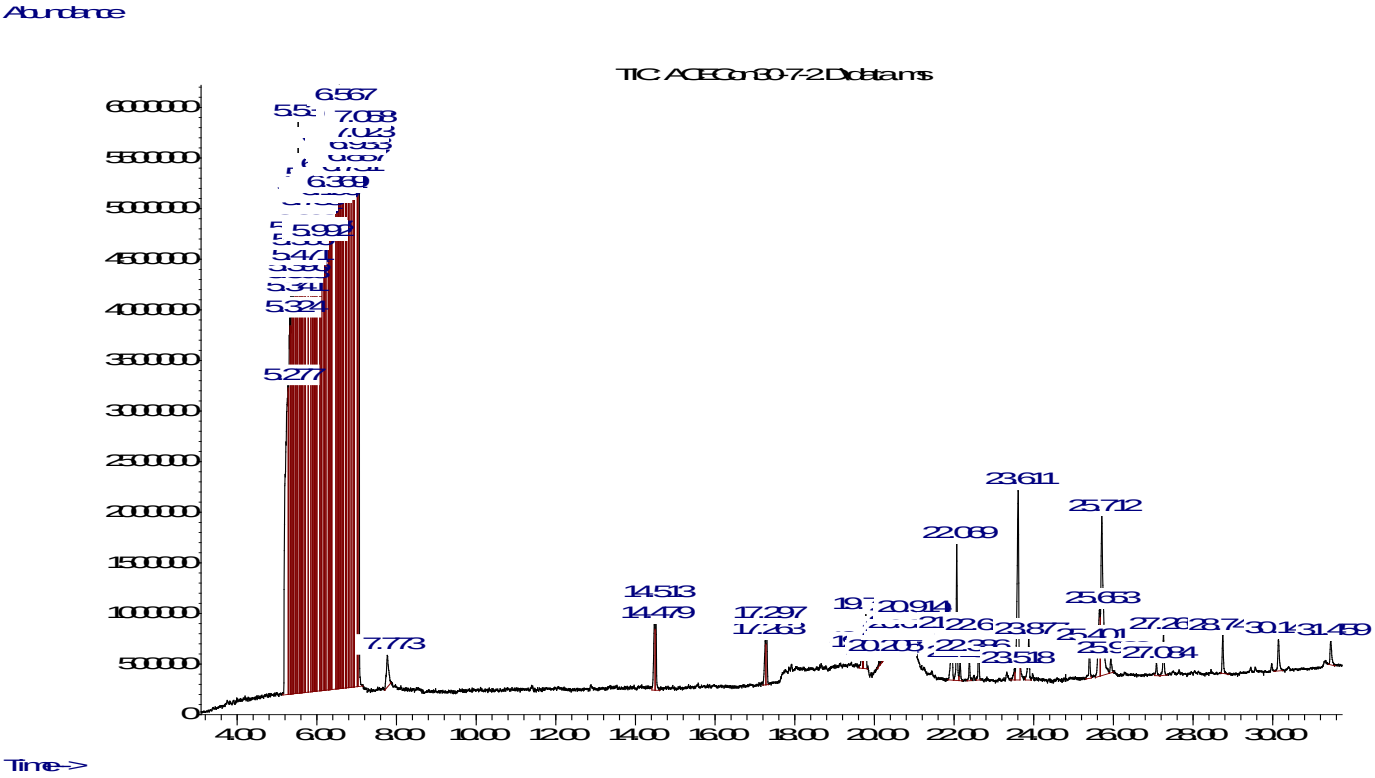

Figure 12 GC/MS chromatogram of *A. capillus* represented by retention times

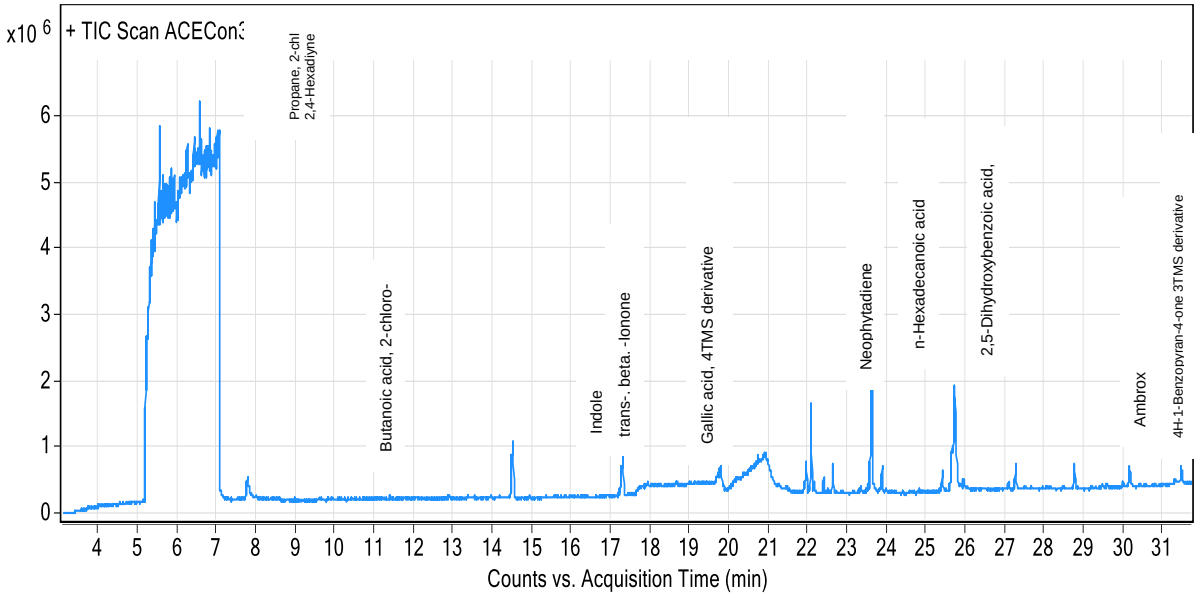

Figure 13 GC/MS chromatogram of A. capillus represented by compound names

Discussion

Vasorelaxant effect of *Adiantum capillus-veneries extract* in renal artery

The current study was the first in evaluating the pharmacological effects of the *Adiantum capillus-veneries extract* (ACVE), and very little information and studies are available about its physiological action on the renal artery. In addition, the study aimed to identify the active ingredients of ACVE by using GC/MS.

The GC/MS analysis in the current study of the alcoholic extract of *Adiantum capillus-veneries extract* (ACVE) shows that the analysed sample contained many bioactive compounds such as alkaloids, terpenoid, fatty acid, gallic acid, phenol, 2-chloro- Propane, 2,4-Hexadiyne, n-Hexadecanoic acid and isoflavone. Previously Khodaie *et al*. (2015) and El Aali (2018), showed that the phytochemical screening of ACVE were carvone, carvacrol, hexadecanoid acid, thymol, aceton, hexahydrofarnesyl, terpenoids, flavonoids, phenylpropanoids, tannins and n-nonanal.

The results of the present study reveal the first detailed investigation on concentration-dependent vasorelaxation of *Adiantum capillus-veneries extract* (ACVE) in goat renal artery rings. *Adiantum capillus-veneris* extract has been recommended in ancient literature of the Unani medicine as an important ingredient of many formulations for the treatment of urolithiasis (Ahmed *et al.*, 2013). According to Iranian traditional medicine (ITM), Maidenhair fern has been prescribed as a single medicine or in poly, herbal formulations for the treatment of many diseases and among them respiratory and urogenital diseases were the most important ones (Dehdari and Hajimehdipoor, 2018).

The *Adiantum capillus-veneries extract* (ACVE) have been found to be spasmolytic, and may be mediated through blockade of Ca^{2+} channels. *In vitro* anti-lithiasis activity of hydroalcoholic extract of ACVE was significantly inhibited crystallization and aggregation (Ahmed, 2012). In a previous study on male rats, the hydroalcoholic extract of ACVE showed anti-urolithiasis activities and showed a significant reduction in the number of crystals in the urine. It also reduced the level of the serum Ca^{2+}, phosphorous and blood urea significantly (Al-Snafi, 2015). *Adiantum capillus-veneries* extract has produced a significant increase in the urine flow and urinary Na^{+} and K^{+} excretion in rabbit (Mahwi *et al*., 2011). As reported by some researchers, ACVE contains flavonoids; the anti-urolithiasis activity may also be reported because of this constituent (Ahmed *et al*., 2013).

The result of the current study demonstrated that the cumulative addition of ACVE exhibited greater relaxant effects on the contractions induced by PE than KCl. The vasorelaxant effect of ACVE decreased with KCl-induced vasoconstriction. The plant-induced vasodilatation was attenuated by the constriction caused by PE, this may be due to its Ca^{2+} channel blockade like mechanisms (Janbaz *et al*., 2014). Phenylephrine is selective α_1-AR agonist and it induced an initial transient phasic contraction followed by a tonic contraction; the initial contraction is mediated by intracellular Ca^{2+} release (Shekha, 2010). The relaxant effect of ACVE in isolated renal artery rings precontracted with PE, and the result of this effect may be due to the presence of active ingredients in ACVE, such as triterpenoid. It was previously demonstrated that triterpenoid may have a relaxant effect (Al-Habib *et al.*, 2015). Furthermore, alcoholic extract of *colocynth pulp* inhibited normal contraction in a concentration dependent manner, and free calcium solution completely closed muscle response to alcoholic extract, it was also observed that this extract gradually inhibited acetylcholine-induced contraction (Kakey and Sundus, 2006).

The phytochemical screening of ACVE showed detections of monoterpenoids, sesquiterpenoids, terpenoids, fatty acids, alkaloid, tannin, isoflavone, phenol, phenolic acid, chloro-Propane and 2,4-Hexadiyne. The presence of these active compounds in ACVE could account for its vasorelaxation activity. Previously have been demonstrated that the branched-chain of fatty acid causes relaxation of colonic smooth muscle via PKA pathway, activation of PKA and/or PKG plays a key role in smooth muscle relaxation (Blakeney, 2018).

Role of Potassium channels in the *Adiantum capillus-veneries* extract action

Pre-treatment of renal artery rings with K^{+} channel blockers significantly enhanced the vasorelaxant effect of ACVE as compared to its effect in renal artery pre-treated with TEA, $BaCl_2$ and 4-AP, while there were no significant changes in the vasorelaxant action in renal artery pre-treated with Glib.

The outcome of the present study on ACVE exhibited statistically significant dose-response curve shifted to the left with TEA and potent inhibitory effect with $BaCl_2$ and 4-AP. These results indicate that the vasodilatory response of ACVE is mediated through a negative role of K_{Ca}, K_{Ir} and K_V channels, this may suggest that they present an active ingredient in ACVE. Flavonoid, is an ingredient of ACVE, which can modulate the ion channels activity of VSMCs (K^{+} and Ca^{2+} channels) and regulates the vascular tone (Scholz *et al*., 2010). Furthermore, other studies have shown that flavonoids are able to increase the conductance of the K^{+} channel by activating various K^{+} channel subtypes in VSMCs which induce vasodilation (Al-Habib *et al*., 2015). The findings of the current study are also supported by the findings of Chen *et al*. (1998), who has been reported that K_{ATP} channels showed no role in vasorelaxation action of *Crataegus* extract in rat arterial SMCs.

Studies have been reported that some of the medicinal plants or plant-based remedies exhibited an inhibitory effect on spontaneously contracting smooth muscles through K^{+} channel activation, K^{+} channel openers (increase in K^{+} efflux) and Ca^{2+} antagonists (inhibition of Ca^{2+} entry). They are known to cause intestinal smooth muscle relaxation by decreasing $[Ca^{2+}]i$, through respective mechanisms of membrane hyperpolarization (Janbaz *et al.*, 2015). Another active compound, alkaloids, may exert antispasmodic or relaxation activities in different smooth muscles through subsequent closure of K_{ATP} channels, membrane depolarization, Ca^{+2} influx, and change in Na^{+} transport across sarcolemma (Sadraei *et al*., 2003).

Role of endothelium nitric oxide, prostaglandin I2 and

epoxyeicosatrienoic acid in Adiantum capillus-veneries extract action

To study the role of endothelium/NO, PGI_2 and EET, the three major enzymes responsible for the release of relaxant factors in the vascular beds, renal artery rings were pre-incubated either with L-NAME, indomethacin or clotrimazole. Then the effect of ACVE was evaluated. The results of the current study indicate that indomethacin and clotrimazole have vasorelaxant effect when induced by ACVE, while L-NAME do not play any role in the vasodilation induced by ACVE in precontracted renal artery rings with PE. The application of indomethacin and clotrimazole revealed that both PGI_2 and EET have potent activity on the vascular relaxation process. In our knowledge, this study is the first attempt done in this context as no review papers were found about studies that have deal with goat renal artery rings pre-incubated with indomethacin, L-NAME and clotrimazole and their mechanisms of action. This suggests that the relaxant effects of ACVE may involve PGI_2, and EET from the endothelium.

The PGI_2 causes relaxation of VSMCs through the stimulation of G-protein-coupled receptors, which in turn, activates AC and thus elevate the level of cAMP that induces vasodilation (Luna-Vázquez *et al*., 2013). Furthermore, Hildebrand *et al*. (2013), have been indicated that the cGMP activates PKG, which in turn mediates vascular relaxation through phosphorylation of different targets. Cerqueira *et al*. (2012) reported that activated PKG causes lessening of $[Ca^{2+}]i$ and disassociation of actin and myosin filaments, which ultimately leads to relaxation of the SMCs.

Researchers have demonstrated that the relaxant effect of *Spilanthes acmella* extract containing phenolic and triterpenoids is produced partially via endothelium induced PGI_2 production in rat thoracic aorta (Wongsawatkul *et al.*, 2008). Other studies have revealed that *Crataegus* extract contains several classes of phenolic substances, the vasorelaxation effect may be attributed to the presence of phenolic compounds (Liu, 2012).

Since no attempt has been made to study the effect of *Adiantum capillus-veneries extract* (ACVE) on renal artery pretreatment with previously described blocker, it is not possible to compare the results.

Role of Calcium channel blockers in *Adiantum capillus-veneries* extract action

The results of the current study with respect to Ca^{2+} channels, demonstrated that the cumulative addition of ACVE enhanced the relaxation in renal artery rings pre-contracted with PE. The vasodilatory response may be due to the presence of active ingredients in ACVE such as flavonoids. It is previously demonstrated that flavonoid may act as Ca^{2+} channel antagonists (Kheder, 2013).

Pretreatment of renal artery rings with both nifedipine, L-type CCB and ACVE, significantly altered the vasoconstriction response in the presence of $CaCl_2$. These results suggest that the vasoconstriction response of ACVE is mediated through blockade of voltage-dependent Ca^{2+}channels, due to the presence of bioactive constituents in ACVE, like flavonoids that may act as Ca^{2+} channel antagonist. Recently, Dehdari and Hajimehdipoor (2018), have reported in a study that the active ingredients of ACVE such as tannins, terpenoids, flavonoids, alkaloids, and steroids possess vasorelaxant response as Ca^{2+} channel antagonist activity. Moreover, the relaxation activity may be through non-hydrolysable tannins and hydrolyzable monomers of gallic and ellagic acids that relax smooth muscles but did not alter muscle tones (Rasheed, 2008).

Furthermore, El Aali (2018), showed that ACVE contain flavonoids and triterpenoids as major bioactive compounds. In SMCs, flavonoids exert a potent effect on Ca^{2+} current, which mediates vasorelaxation (Scholz *et al.*, 2010). On the other hand, Yuan (2015), showed that triterpenoids can act as a Ca^{2+} channel antagonist. Since no more data available on the effect of alcoholic extract on the isolated renal artery, it's difficult to compare the result.

From the present study outcomes, it was found that *Adiantum capillus-veneris extract* (ACVE) has a relaxant effect in the renal artery and this result is the first record of this drug in the renal artery. Additionally, ACVE block potassium and calcium channels, which are mediated possibly through blocking of K_V, K_{ATP}, PGI_2, EET and voltage-dependent calcium channels.

Furthermore, *Adiantum capillus-veneris extract* contain many bioactive ingredients such as (monoterpenoids, sesquiterpenoids, terpenoids, fatty acids, alkaloid, tannin, isoflavone and phenol) according to the GC/MS phytochemical analysis.

Conclusion

From the present study outcomes, it was found that ACVE has a relaxant effect in the renal artery and this result is the first record of this herbal drug in the renal artery. Also, ACVE blocks potassium and calcium channels, which are mediated possibly through the blocking of K V , K ir , K Ca , PGI 2 , EET, and voltage-dependent calcium channels. Additionally, the ACVE contains many bioactive ingredients according to the GC-MS phytochemical analysis

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