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Effects of *Prosopis farcta* Root Extract in the Vascular Reactivity of Isolated Goat Coronary Artery

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Abstract

Prosopis species is a medicinal plant, well-known for its beneficial effects in treating various smooth muscles disorders, and its phytochemical analysis revealed the presence of different bioactive compounds in different parts of the plant, most of which show a great role in reducing cardiovascular risks. In the present study, the cardiovascular effect of Prosopis farcta Root Extract (PFRE) was investigated in vitro for possible mechanisms of the extract effect in the vascular reactivity of isolated goat coronary artery (CA) using Organ bath and PoweLab Data Acquisition system.

The results of the recording and analyzing of the effect of the PFRE in isolated CA, showed the negative inotropic activity of the extract in CA rings with intact-endothelium, while in CA ring with hyperglycemic-induced endothelium dysfunction the extract tended to vasodilate the CA ring non-significantly, and the extract induced dose-dependent vasodilation in CA rings pre-constricted with high concentration of (30 mM)KCl and showed no effects on contractions induced by ($1X10^{-3}$-$1X10^{-5}$) Phenylephrine PE, which is an indicator for its blockade activity on L-type voltage-dependent Ca^{+2} channel and non-interfering of the extract with the receptor-operated Ca^{+2} channel. The mechanical recording of the CA ring activities, revealed different potassium (K^{+}) channels including selective calcium-activated potassium channel, ATP-sensitive potassium channel, and different endothelium-derived relaxing factors (EDRF) including nitric oxide and Prostacyclin (PGI_2) seems to have no role in the relaxation effects of the extract, while the endothelium derived-hyperpolarizing factor (EDHF); epoxy eicosatrienoic acid (EET) showed significant participation in the vasodilation effects of the extract. On the other hand, the extract tended to relax the CA rings through its antagonizing of Ca^{+2}, reducing

and inhibiting Ca^{+2} influx and release from internal stores and interfering with the voltage-operated Ca^{+2} channels through its alkaloid and flavonoid active compounds which suggested to be the most predicted mechanisms for the maintenance of vasodilating tone and coronary circulation of the PFRE in coronary artery.

Keywords: *Prosopis farcta*, vascular reactivity, K^+ channels, Ca^{+2} channels, endothelium-derived relaxing factors, endothelium derived-hyperpolarizing factor

1. INTRODUCTION

From the Global Burden of Disease Study's survey, it is apparent that the CVDs mainly the heart disease and stroke will result in an increasing number of death all over the world and regardless of progression in biology and medicine and efforts in translational scientific for improving the diagnosis and therapeutic strategies over the past 20 years, CVDs remain to be a main global health problem [1], so increasing the number of patients suffering from the CVDs is a key indicator for the invention of the innovative strategies to be most effective with lesser side effects in the treating and preventing the CVDs [2].

Nowadays, the modern costly curative synthetic remedies associated with multiple side effects resulting in patient non-compliance, therefore there is a need to explore some alternative remedies especially from plants and herbal based formulations as are less cost-effective and possess minimal side effects [3]. For controlling and treating various diseases because of their remarkable pharmacological actions with no or rare side effects when used at a specific dose [4], and in general many secondary metabolites from plants [5] including alkaloids, flavonoid, tannins, phenolic compounds, steroids, resins, fatty acids, and gums regarded as an important bioactive compounds of the plant extracts that are capable of producing significant physiologic effects in the body [6].

Prosopis farcta regarded to be one of the important local plants with a great value for medicinal purposes [7], leaves and bean extracts of this plant gained the commonest use in traditional medicine [8]. From surveying literature it was revealed the use of *P. farcta* for treating many neurological diseases and disorders [9, 10], and it has anti-inflammatory effects, gastric ulcers treatment, fetus abortion, dysentery, arthritis, heart pains, and asthma [11].

Extracts of *P. farcta* tended to be with famous use for treating diabetes and its complication, as it is widely used by the general for healing lesions in Iran particularly in Sistan-Baluchistan zone especially healing processes of diabetic wounds through speeding up the inflammation, cell proliferation and hypoglycemia [12] and without any academic confirmation, its extract used to treat diabetes by the tribal people in Jordan [13] and this hypoglycemic activity may due to the containing of a large proportion of unsaturated fatty acids with linoleic, oleic acids as well as β-sitosterols [14] and flavonoids which inhibit the activity of cAMP phosphodiesterase, accompanied by an insulin secretion which in turn subsequently reduces glucose level [15].

Phytochemical investigations have been demonstrated that the flavonoids are the main active constituent of *P. farcta* [16, 17]. Quercetin (flavonoids), tryptamine, apigenin 5-hydroxytryptamine (alkaloids), L-arabinose, and Lectin regarded to be the most active compounds that exist in different parts of *Prosopis* plant [16, 18], and the vicenin-2, apigenin C-glycoside, iso-orientin, vitexin, luteolin 7-O-glucoside, isovitexin, quercetin 3-O-glucoside,

rutin, kaempferol 3-O-rutonoside, caffeic acid derivative, and luteolin are strong antioxidant phenolics of the plant [16], and this antioxidant activity of *P. farcta* played a significant role in improving the serum lipid parameters in hypercholesterolemic rabbits [19]. The flavonoids in *P. farcta* act as a strong antioxidant and decreased total cholesterol, triglyceride, malondialdehyde, and increased HDL-C in a tetrachloride induced hepatotoxicity rats [20] with no change in HDL in acetaminophen-induced hepatotoxicity in Wister rats [21]. For this, *P. farcta* has long been used as a traditional remedy for angina pectoris [22] and as a therapeutic agent in treating cardiovascular disorders [23] and reducing cardiac or chest pain and for managing cardiovascular disorder in Iran [19]. Different parts from different species of this plant contains an enormous concentration of quercetin which acts as an excellent scavenger for collecting and removing free radicals [24], protect cellular damage against any oxidative stress including reactive oxygen species (ROS) [25] and regenerate the injured pancreatic islets in diabetic models and probably increases insulin release in streptozotocin-induced diabetic rats [26]. In a recent study, revealed the potential ability of *Prosopis farcta* in treating colon cancer through synthesizing gold nanoparticle (Au-NPs) that capable to destroy colon cancer lines and thus regarded to be a suitable candidate for cancer treatment[27]. Since a toxicological point of view, is that it is important to remark the capability of some species of Prosopis to accumulate heavy metals like chromium, lead, arsenic, cadmium, manganese, molybdenum, and zinc [28].

The present study was conducted to investigate the efficacy of *Prosopis farcta* root extracts in coronary artery vascular reactivity and find out the possible mechanisms of the extract in interfering with different potassium and calcium channels and the EDRFs AND EDHF in coronary arteries.

2. METHODS AND MATERIALS

Prosopis farcta root collection and extraction

Syrian mesquite (*Prosopis farcta*) root collected from Koya District / Erbil City/ Kurdistan Region of Iraq (GPS coordinates: 36°04'41.55" N, 44°27'38.99" E), with an average elevation of about 563 m above sea level in December 2015. A voucher (7701) was deposited after authentication by botanists in the Biology Department, College of Education, Salahaddin University Herbarium (ESUH)/ Kurdistan Region of Iraq.

The root samples of *Prosopis farcta* were cleared from the clay and cleaned through washing with tap water and then shade dried at room temperature (23-25 °C) for 25 days. The dried root is insulated from bark of the root and the remained root cut into small pieces, pulverized mechanically to a coarse powder and grounded using electrical mill into fine powder and the powder sieved through a stainless steel mesh of 200 mm diameter size to ensure its uniformity then stored in a cloth bags at 5º C until extraction.

Preparation of the plant root extract

The powdered root material (1Kgm) of *Prosopis farcta* extensively degreased at room temperature using petroleum ether then re-extracted through inundation in a measured volume of ethyl acetate solvent (5g of plant root sample in 50ml of ethyl acetate) for 1 day in a container kept away from light and putting the mixture on the magnetic stirrer for one hour. The extract was filtered before repeating the process three times to obtain more extracts, each time with fresh ethyl acetate solvent. The filtrates were collected, combined then concentrated and the solvent was removed through using a rotary evaporator at 40°C and 150 rpm then stored at 4 °C till using it in our physiological studies.

Phytochemical screening
The *Prosopis farcta* root extract was preliminarily screened for the qualitative presence of various classes including alkaloids and flavonoid phytochemical compounds using chemical tests for identification of these compounds following methods describes by[29].

Test for Flavonoids
5 ml of the extract is mixed with 3ml of 1% Aluminum chloride, appearance of yellow color is an indicator of the presence of flavonoid.

Test for Alkaloids
10 ml of aqueous HCl stirred well with 2 ml of the extract on a steam bath and filtered while it was hot. Few drops of (potassium bismuth iodide) which represent the Dragendorff's reagent added to bout 2m of the filtrate

Animal hearts and isolation of coronary artery rings
Male and female domestic goat (*Capra hircus*) hearts were collected from Koya slaughterhouse within 20-30 min of slaughter in an ice-cold, aerated modified Kreb′s Henseleit solution of the following compositions in mM: 118 NaCl, 4.7 KCl, 2.5 $CaCl_2.2H_2O$, 1.2 $MgSO_4.7H_2O$, 11.9 $NaHCO_3$, 1.2 KH_2PO_4 and 11.1 D (+) Glucose and were carried immediately to the Science and Health Research Center (SHRC) laboratories / Faculty of Science and Health / Koya University, from December 2016 to November 2017.

The left anterior descending coronary artery (CA) was isolated within 10 min from about (34) goat hearts, following the procedure of [30] with some modifications to take care during isolation of the tissue to avoid any damage of endothelium, then the artery transferred to a petri-dish filled with ice-cold modified Kreb′s Henseleit solution, aerated with carbogen (95% oxygen (O_2) and 5% carbon dioxide (CO_2)) and was cleaned of fat and connective tissues, the coronary artery tissue was then cut into 4–5 rings of about 3 mm length and 1.5–2 mm external diameter [31].

Vascular reactivity setup
The viability of the isolated CA segments was confirmed through contracting the tissue with KCl and relaxing it by acetylcholine (Ach). The prepared CA rings individually suspended between two stainless steel angular hooks and mounted in an organ bath (Automatic Organ bath-Panlab Harvard Apparatus-USA, AD instrument PowerLab 8/35-Australia) containing 10 ml of oxygenated Krebs solution (95% O2 and 5% CO2) maintained at 37 C˚ (pH 7.4). Passive tension of 1.5 gram was applied to CA rings and the change of the tension measured isometrically by force transducer. The rings were allowed to equilibrate for about 90 min during which the Krebs solution changed every 15 min and the tension was continuously readjusted to the optimum force before starting the experiments. Following equilibration, for the coronary artery smooth muscle and endothelial integrity respectively, the rings were stimulated to sub-maximal contraction with (30 mM KCl) [32]. Once reached the plateau, coronary artery rings relaxed by ($1x10^{-5}$ M) Acetylcholine (Ach). Endothelium regarded to be intact when Ach caused ≥ 75 % relaxation from the maximal contraction of coronary artery rings caused by 30 mM KCl (data not shown in the study), while the lack of relaxation indicates non-functional endothelium. Then the CA rings were washed and re-stabilized at the optimum tension for at least 45 min before applying other effective vasoactive agents.

Experimental Design
The effect of Prosopis farcta root extract (PFRE) firstly was determined on the resting baseline of CA rings for its vasoconstriction activity. Later the plant root extract tested for its interfering ability with contractions induced by 30 mM KCl and different doses of phenylephrine ($1X10^{-3}$-$1X10^{-5}$) respectively; the tissue has shown no response to PE , that an indicator of the non-involvement of receptor-operated Ca^{+2} channels. The extract relaxant effect on the pre-

contraction induced by KCl, represents the involvement of L-type voltage-dependent Ca^{+2}channel, the tissue incubated for 30 min with different concentrations of PFRE, ranging from (0.0001, 0.001, 0.01, 0.1, 1.0, and 10 mg/ml) then subjected to KCl for maximum contraction and relaxed with different doses of SNP ($1x10^{-7}$ -$1x10^{-4}$M).

To investigate the role of endothelium in vascular response to SNP and PFRE, cumulative doses of SNP ($1x10^{-7}$ -$1x10^{-4}$M) and different concentrations of PFRE were applied to the CA rings with an intact endothelium and CA with hyperglycemic induced endothelium dysfunction (hyperglycemic Krebs solution is the solution contains raised concentration of glucose and prepared according to [33] by equimolar substitution of glucose (44 mM) for NaCl.

Test of the involvement of different CA potassium channels in the relaxatory effects of PFRE were investigated through incubating the CA rings with an intact endothelium for 20 minutes with a selective calcium-activated potassium channel blocker; TEA (1mM), ATP sensitive potassium channel blocker, Glibenclamide (30 µM) and non-selective potassium channel blocker, BaCl2 (1mM) before the cumulative doses of SNP ($1x10^{-7}$ -$1x10^{-4}$ M) were applied, in another set of the experiment to determine effects of PFRE on potassium channel involved in vascular response to SNP, CA were pre-incubated with PFRE for 30 min before applying the cumulative doses of SNP in the presence of the above blockers separately.

The role of endothelium-derived relaxing factors (EDRF); nitric oxide (NO) and Prostacyclin (PGI_2) and endothelium-derived hyperpolarizing factors (EDHF); epoxy eicosatrienoic acid (EET) in the relaxatory effects of PFRE also were tested through pre-incubating an intact endothelial CA rings for 20 min with NO synthesis inhibitor (L-NAME), non-selective COX inhibitor (Indomethacin) and non-selective P450 cytochrome inhibitor (Clotrimazole) before cumulative doses of SNP ($1x10^{-7}$-$1x10^{-4}$M) were applied, while to test the effect of the PFRE on the above enzyme involvement in vascular response to SNP, CA rings with intact endothelium were pre-incubated with PFRE for 30 min before applying cumulative doses of SNP in the presence of the above inhibitors separately.

Also, the role of calcium (Ca^{+2}) ion in the relaxatory effects of PFRE also investigated in different sets of experiments. Firstly, the involvement of L-type Ca^{+2} channel in the relaxatory response of CA was investigated both in the presence and absence of PFRE, the intact endothelium CA rings were incubated with nifedipine (10µM) before applying of cumulative doses of SNP. Then the role of low extracellular Ca^{+2} in relaxatory effects of PFRE, coronary artery ring exposed to reduced or low extracellular Ca^{+2} Kreb's solution was investigated through incubating coronary artery rings in low Ca^{+2} Kreb's solution (0.63 mM, *i.e* ¼ of Ca^{+2}) for 30 min before applying the cumulative doses of SNP [32], the results considered as a control group. Then in another set of experiment tissues incubated for 20 min with PFRE after 30 min incubation in reduced Ca^{+2} Kreb's solution. Lastly the PFRE interference with the influx of Ca^{+2} through voltage-dependent calcium channels (VDCC), CA rings were subjected to Ca^{+2} free Kreb's solution (normal Krebs solution component except that $CaCl_2$ was excluded and substituted for EDTA (0.1 mM)was investigated to eliminate extracellular calcium and ensure the retaining of intracellular Ca^{+2}) following procedure of [34]. Coronary artery rings after equilibration incubated for 60 min in Ca^{+2} free Kreb's solution, then two cumulative concentration-response curves for $CaCl_2$ ($1x10^{-5}$ -$1x10^{-2}$ M) were recorded. The first curve was without incubating of CA with PFRE and regarded as a control group while the second one was incubation of the CA rings with PFRE for 20 min.

The statistical analysis was performed to compare between the control and studied drugs through using two-way analysis of variance (ANOVA) supported by Sidak' post hoc test which used for comparing the same doses of different groups. Data are expressed as mean±SE of means (SEM) of the number of coronary artery rings used in all studied groups. Results were

analyzed using an independent Student's t-test for comparing potency difference (PIC_{50}) between the studied groups.

3. RESULTS

Phytochemical screening

The qualitative phytochemical study carried out on the root extract of *Prosopis farcta* and the results revealed the presence of some bioactive phytochemicals including flavonoid alkaloid compounds.

Effects of PFRE on coronary artery

Isolated and pre-contracted coronary artery rings with 30 mM KCl subjected to incubation for 30 minutes with different concentrations of PFRE (0.0001, 0.001, 0.01, 0.1, and 1.0, 10 mg/ml), as shown in table (1), (0.1 mg/ml) of the extract showed the highest percentage of relaxation with E_{max}=89.04±9.638% and the potency PIC_{50}=4.364±0.160, respectively (Fig. 1)

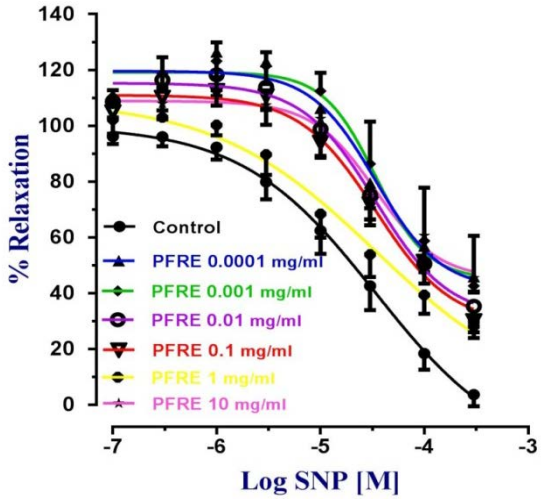


Figure 1: Concentration-relaxation curve for PFRE in isolated intact coronary artery rings

Table 1: Shows maximum relaxation response (E_{max}) and the potency from the coronary artery to KCl in the presence of different concentrations og PFRE

Concentrations (mg/ml)	E_{max} (%)	PIC_{50}	IC_{50}	(95% Cl) of IC_{50}
0.0001	73.72±4.757	4.565±0.134	$2.723X10^{-5}$	4.836 to 4.293
0.001	78.22±11.726	4.392±0.23	$4.056\ X10^{-5}$	4.855 to 3.929
0.01	84.01±7.983	4.476±0.159	$3.346\ X10^{-5}$	4.793 to 4.158
0.1	89.04±9.638	4.364±0.160	$4.327\ X10^{-5}$	4.686 to 4.042
1.0	81.72±5.421	4.827±0.186	$1.488\ X10^{-5}$	5.202 to 4.452
10.0	62.66±5.885	4.497±0.168	$3.184\ X10^{-5}$	4.84 to 4.154

The ability of the extract to relax the contraction induced by KCl represents the involvement of L-type voltage-dependent Ca^{+2}channel at the same time the tissue not shown any response to PE (data not shown in the study), indicating non-involvement of receptor-operated Ca^{+2} channels in the response of the tissue.

To detect the involvement of coronary artery endothelium in the relaxation response to SNP, cumulative doses of SNP ($1X10^{-7}$-$3X10^{-4}$M) were applied to both the intact and hyperglycemic-induced coronary artery endothelium dysfunction pre-contracted with 30 mM KCl. Results showed that incubation of coronary artery rings with hyperglycemic Krebs solution for 3hrs remarkably but non-significantly decreased the efficacy of SNP to relax the coronary artery rings (E_{max}: 112.54±10.662, intact *vs* E_{max}: 82.95±13.781dysfunction) (Fig. 2A), with no potency difference between them (PIC_{50}: 4.552±0.252, intact *vs* 4.491±0.512, dysfunction) respectively (Fig. 2B).

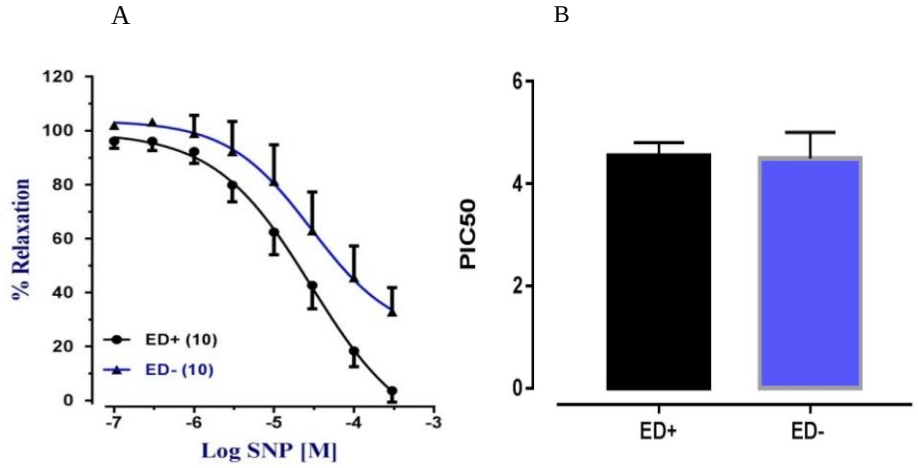


Figure 2: Coronary artery responsiveness to SNP **(A)** Hyperglycemic-induced endothelium dysfunction effects on coronary artery response to SNP **(B)** Graph shows the potency difference between intact and hyperglycemic-induced endothelium dysfunction coronary artery. The number of coronary artery rings used in the study is in parentheses. ED+: Endothelium intact coronary artery, ED-: coronary artery with hyperglycemic induced endothelium dysfunction

To assess the role of PFRE in relaxation responsiveness of coronary artery rings to SNP, CA rings with intact and hyperglycemic-induced endothelium dysfunction separately pre-incubated with PFRE (0.1 mg/ml), and induced for maximal contraction with 30mM KCl. PFRE shifted the relaxation curve to the right and significantly ($P< 0.01$) attenuated the maximum relaxation response in intact coronary artery rings in compare with the control group (E_{max}: 89.04±9.638%, PFRE *vs* 112.54±10.66, control) and PIC_{50} (4.364±0.160, PFRE *vs* 4.373 ± 0.117 control) respectively (Fig.3A), while in CA rings with endothelium dysfunction, PFRE non-significantly increased the maximum relaxation response of the tissue to SNP in comparison to CA rings with endothelium dysfunction (E_{max}: 82.95±10.662%, ED- control *vs* 94.37±2.386%, PFRE) with increase in potency (PIC_{50}: 4.491±0.512, ED- control *vs*; 5.061 ±0.14, PFRE) respectively (Fig.3B). Fig. 3C accentuate the difference in relaxation effects of PFRE in coronary artery rings to SNP both in intact and hyperglycemic induced endothelium dysfunction, (E_{max}:94.37±6.646%), PIC_{50} (5.061±0.14) in comparison to coronary artery rings with intact endothelium (E_{max}: 89.04±9.638%) and PIC_{50} (4.364±0.160), with significant ($P<0.01$) potency difference between them (Fig. 3D).

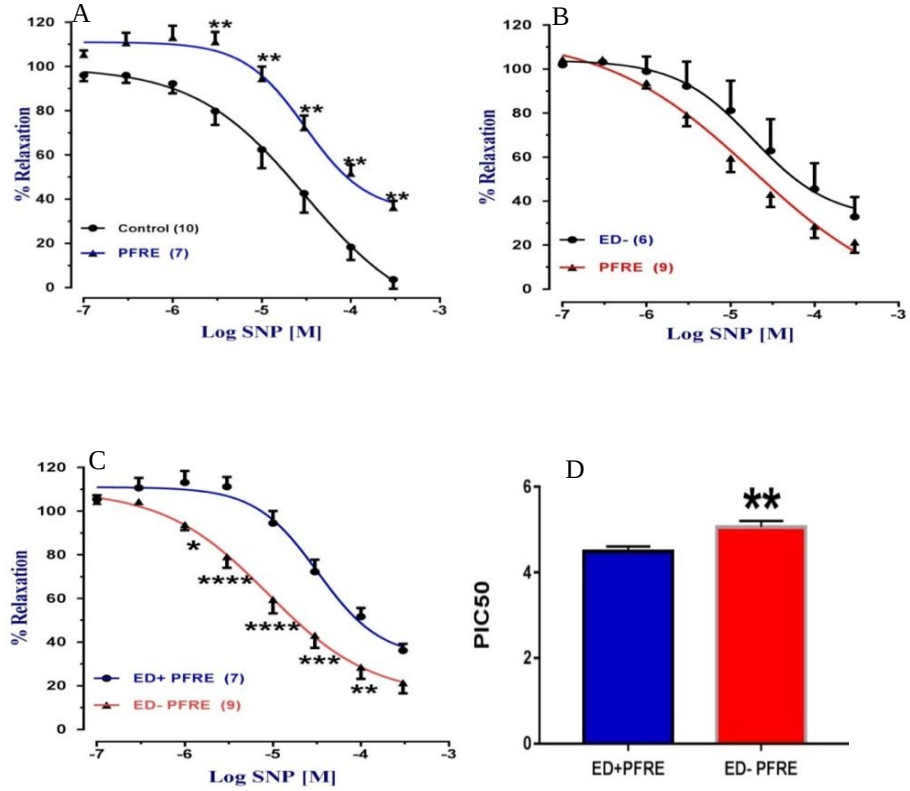


Figure 3: PFRE (0.1 mg/ml) effects on CA responsiveness to SNP. (**A**) The effect of PFRE on CA with intact endothelium. (**B**) The effect of PFRE on CA with hyperglycemic induced endothelium dysfunction. (**C**) Difference in the effect of PFRE on both intact and induced dysfunction CA. (**D**) The graph shows the potency difference between intact and denuded endothelium studied CA. * symbolize statistical differences at P< 0.05, ** symbolize statistical differences at P< 0.01, *** symbolize statistical differences at P< 0.001, **** symbolize statistical differences at P< 0.0001,Number of used coronary artery ring in the study is in parentheses.

Role of potassium channels in coronary artery vascular responsiveness to PFRE

Experiments with potassium channel blockers conducted on the CA with an intact endothelium through pre-incubation of CA ring with selective calcium-activated potassium channel blocker TEA (1 mM), ATP-sensitive potassium channel blocker Glibenclamide (10 µM), and non–selective potassium channel blocker $BaCl_2$ (1 mM). In TEA treated groups, results in table (2) show the significant (P< 0.001) reduction in relaxation response of the CA artery to SNP (E_{max}:78.3±7.399%) and PIC_{50} (4.399±0.15) respectively in comparison to control groups (E_{max}: 112.52±10.661%) and PIC_{50} (4.552±0.252) respectively, while in PFRE treated groups pre-incubated with TEA, the relaxation response of the tissue non-significantly increased to about (E_{max}: 89.04± 9.638%) with no change in PIC_{50} (4.332±0.164) respectively (Fig.4A) in compare to TEA treated control group. Blocking ATP-sensitive potassium channels with glibenclamide (30 µM), significantly (P< 0.01) shifted the DRC to the right, while it tended to reduce the maximum relaxation response of the tissue to SNP (E_{max}: 69.33±4.671%) when compared to control groups (E_{max}: 112.52±10.661%), with no significant increase in potency of glibenclamide treated groups in comparison to control one (PIC_{50}: 4.682±0.16 *vs* 4.552±0.252) respectively as shown in table (2), meanwhile, PFRE treatment of coronary artery rings pre-incubated with glibenclamide resulted in no significant increase in relaxation response (E_{max} : 69.33±4.671%) for Glib and (E_{max}: 99.03±7.649%) of Glib and PFRE respectively with no significant decrease in potency of Glib and PFRE treated groups in compare to Glib treated control one (PIC_{50}: 4.551±0.143 *vs* 4.682±0.160) respectively (Fig. 4B). pre-incubation of

constricted coronary artery rings by KCl with $BaCl_2$ (1 mM), non-significantly and slightly reduced the percentage of relaxation induced by SNP to (E_{max}: 108.46±9.808%) and from (E_{max}: 112.52±10.661%) of untreated control group and with no significant change in the potency (PIC_{50}: 4.532±0.167, $BaCl_2$; *vs* PIC_{50}: 4.552±0.252, control). Incubation of the coronary artery ring with $BaCl_2$ and PFRE for 20 min, shifted the DRC to the right and accentuated non-significant reduction in maximum relaxation of the tissue to SNP with (E_{max}: 75.19±6.22%) as shown in table (2) in comparison to $BaCl_2$ treated groups with no significant change in PIC_{50} (Fig. 4C)

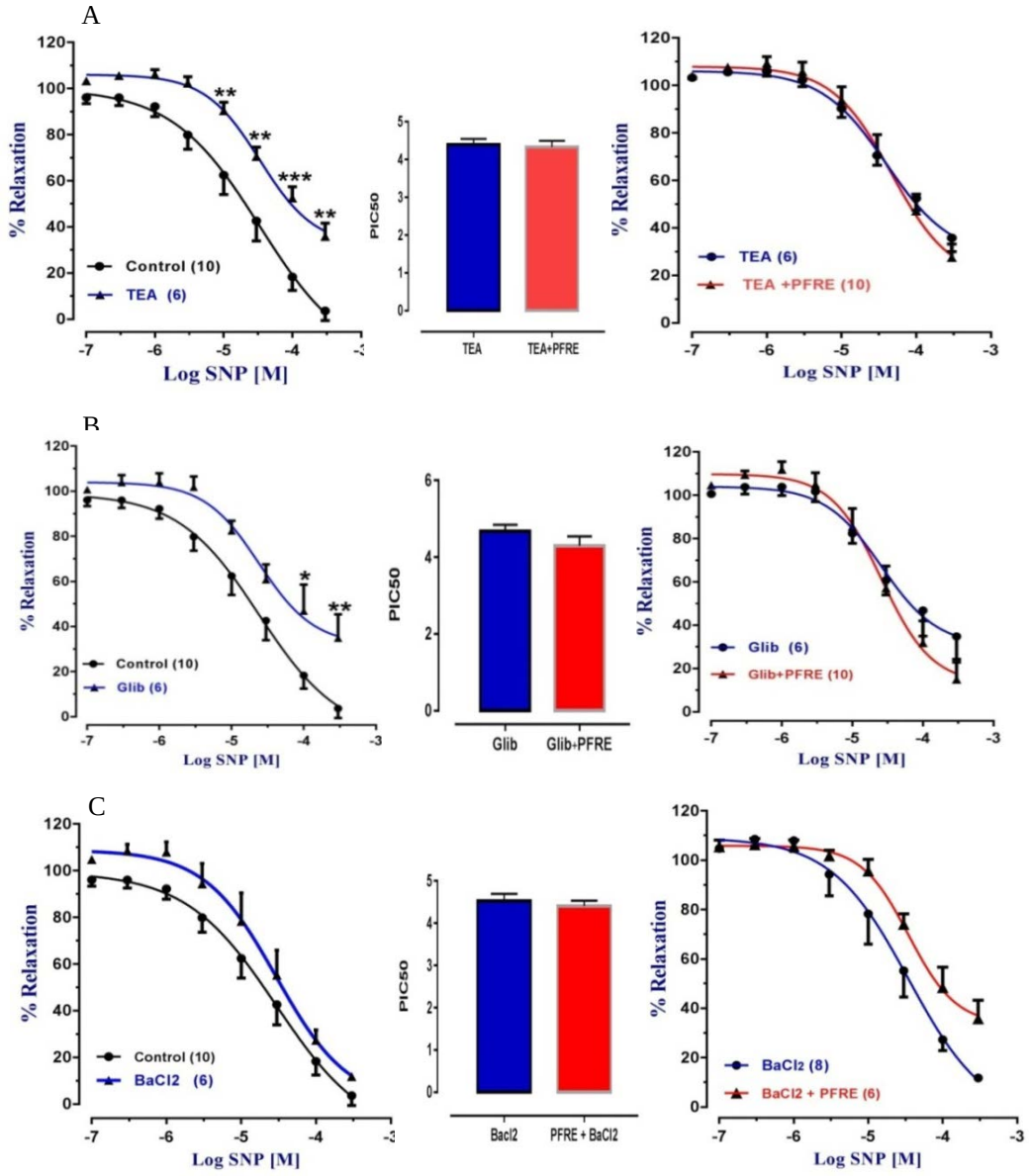


Figure 4: Cumulative-concentration relaxant effects of SNP on CA rings pre-treated with different K^+ channel blockers. (**A**) The effect of PFRE (0.1mg/ml) on CA pre-incubated with TEA (1mM), the inserted graph shows potency difference between CA (0.1mg/ml) on CA pre-incubated with Glib (30µM), the inserted graph shows potency difference between CA rings treated with Glib and Glib with PFRE together. (**C**) The effect of PFRE (0.1mg/ml) on CA pre-incubated with $BaCl_2$ (1mM), the

inserted graph shows potency difference between CA rings treated with $BaCl_2$ and $BaCl_2$ with PFRE together * symbolize statistical differences at P< 0.05, ** symbolize statistical differences at P< 0.01, *** symbolize statistical differences at P< 0.001, Number of used coronary artery ring in the study is in parentheses.

Role of EDRF and EDHF in coronary vascular relaxatory responsiveness to PFRE

From the data in table (2), it is obvious that maximum relaxation response of the coronary artery rings with (E_{max}: 112.52±10.661% and PIC_{50}: 4.552±0.252) slightly and non-significantly reduced to (E_{max}: 107.544±5.988 % and PIC_{50}: 4.815±0.165) when pre-incubated with the non-selective inhibitor of the COX enzyme; Indomethacin (30µM). In another experiment, treatment of Indomethacin pre-incubated CA rings with PFRE, shifted the relaxation response curve of SNP to the left and magnified the relaxation of the coronary arteries non-significantly with (E_{max}: 120.477±2.569 %) and caused no significant change in (PIC_{50}: 4.974±0.097) (Fig. 5A).

Irreversible inhibition of the coronary artery eNOS with L-NAME (300µM) shifted the response curve to the left and non-significantly reduced the maximum relaxation of the tissue with (E_{max}: 96.73±0.112% and PIC_{50}: 5.244±0.110) respectively in compare to untreated control CA rings , while treating these rings with PFRE (0.1 mg/ml) in the presence of the inhibitor, tended to reduce the relaxation response of the tissue to about (E_{max}: 86.33±0.262%) with no significant change in PIC_{50} (Fig. 5B).

Incubating CA rings with non-selective inhibitor of cytochrome P450; Clotrimazole (30µM) induced no change in maximum relaxation response of the studied CA rings (E_{max}: 112.356 ±15.644 % and PIC_{50}: 4.592±0.305) in comparison to untreated control groups (E_{max}: 112.52±10.661% and PIC_{50}: 4.552±0.252), while treating CA rings pre-incubated with the above inhibitor with PFRE, the extract tended to shift the relaxation response curve to the left and significantly (P<0.01) potentiated the relaxation of the tissue (E_{max}: 124.24±6.583 %) and increased the potency (PIC_{50}: 4.873±0.163) (Fig. 5C).

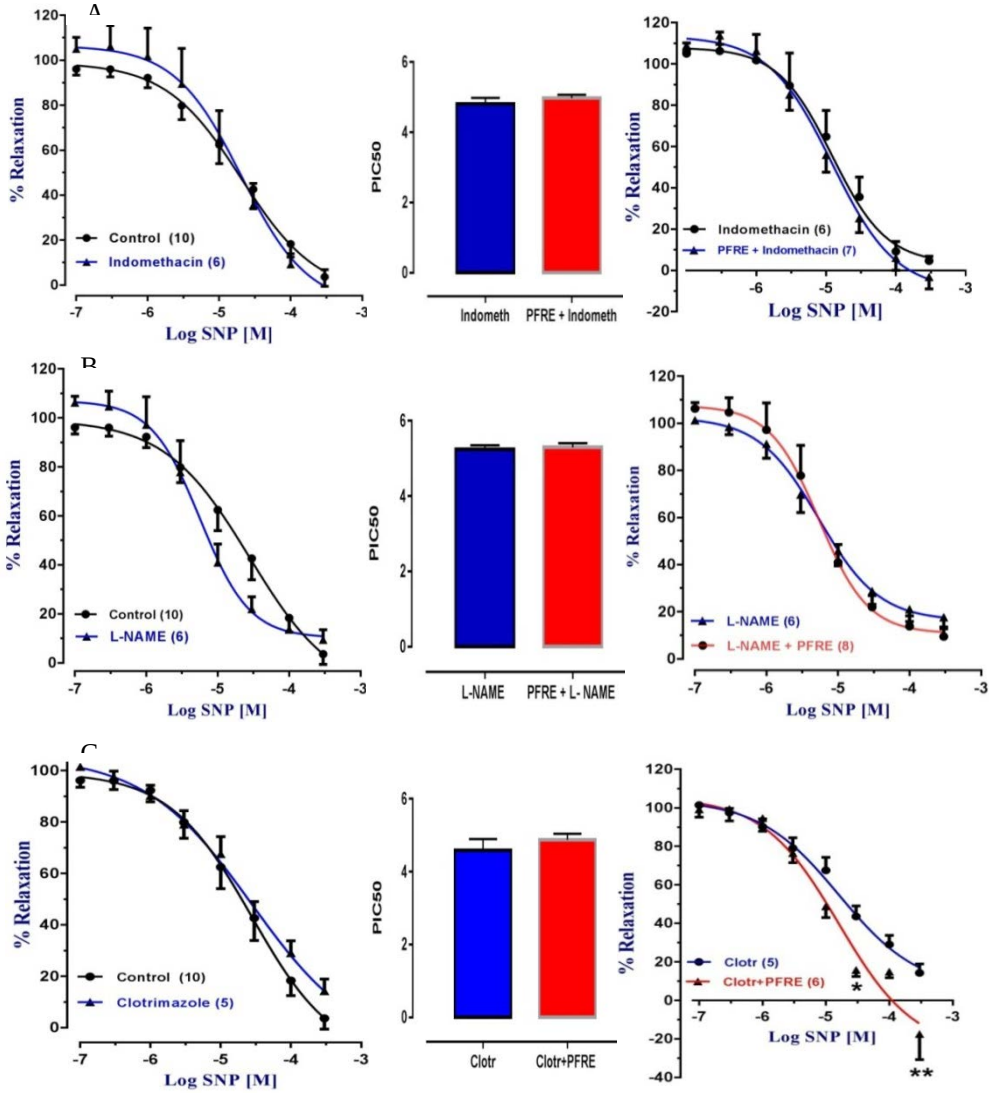


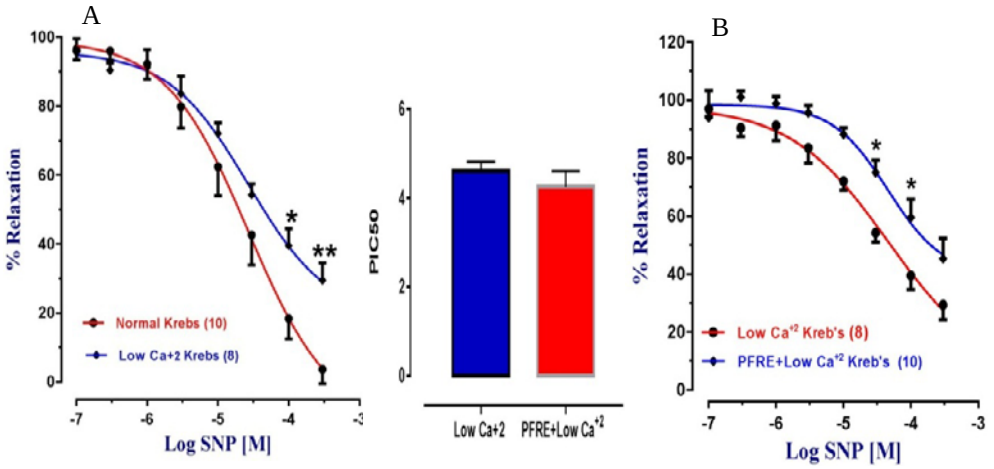
Figure 5: Cumulative-concentration relaxant effects of SNP on CA rings pre-treated with different endothelium enzyme inhibitors (**A**) The effect of PFRE (0.1mg/ml) on CA pre-incubated with Indomethacin (1mM), the inserted graph shows potency difference between CA rings treated with Indomethacin and Indomethacin with PFRE together(**B**) The effect of PFRE (0.1mg/ml) on CA pre-incubated with L-NAME (30µM), the inserted graph shows potency difference between CA rings treated with L-NAME and L-NAME with PFRE together (**C**) The effect of PFRE (0.1mg/ml) on CA pre-incubated with Clotrimazole (1mM), the inserted graph shows potency difference between CA rings treated with Clotrimazole and Clotrimazole with PFRE together * symbolize statistical differences at P< 0.05, ** symbolize statistical differences at P< 0.01, Number of used coronary artery ring in the study is in parentheses.

Table 2: Shows the potency and maximum relaxation response (E_{max}) from the PFRE treated and untreated coronary artery to KCl

Drugs	Untreated Coronary Artery		PFRE treated Coronary Artery	
	E_{max} (%)	**PIC_{50}**	**E_{max} (%)**	PIC_{50}
Control	112.52±10.661	4.552±0.252	89.04±9.638**	4.364±0.160
TEA	78.3±7.399***	4.399±0.15	89.96±10.36	4.332±0.164
Glibenclamide	69.33±4.671**	4.682±0.160	99.03±7.649	4.551±0.143
$BaCl_2$	109.72 ±13.255	4.516±0.215	75.19±6.221	4.408±0.127
L-NAME	96.73±0.112	5.244±0.110	86.33±0.262	5.3±0.109
Clotrimazole	112.356±15.643	4.592±0.305	124.24±6.583	4.873±0.163**
Indomethacin	107.544 ±5.988	4.815±0.165	120.477±2.569	4.974±0.097
Nefidipine	120.86±7.4	5.028 ±0.284	79.771±8.651	6.155 ±0.159**

From the data shown in table (3), it is apparent that there is a clear trend of significant (P<0.01) decreasing in maximum relaxation response of CA rings to SNP bathed in Low Ca^{+2} Krebs solution (LCKS) (E_{max}: 75.15±7.8.5% and PIC_{50}: 4.607±0.207) in comparison to the CA rings in normal Krebs solution (E_{max}: 112.52±10.661% and PIC_{50}: 4.552±0.252) (Fig. 6A),, while treating CA rings with 0.1 mg/ml PFRE in LCKS, significantly (P<0,05) reduced the maximum vascular relaxation to about (E_{max}: 60.11±16.197% and PIC_{50}: 4.332±0.164) in (Fig. 6B),

Figure 6: Comparative effects of Cumulative-concentration relaxant effects of SNP in CA rings incubated in (A) Normal and low Ca^{+2} Krebs Solution (B) PFRE treated in low Ca^{+2} Krebs Solution. The inserted graph shows potency difference between CA rings treated with nifedipine and nifedipine with



PFRE together * symbolize statistical differences at P< 0.05, ** symbolize statistical differences at P< 0.01, the number of used coronary artery rings in the study is in parentheses

Table 3: Shows the potency and maximum relaxation response (E_{max}) from the PFRE treated and untreated coronary artery to KCl bathed in normal and low Ca^{+2} krebs solution

Drugs	Normal krebs solution		Low Ca^{+2} krebs solution	
	E_{max} (%)	PIC_{50}	E_{max} (%)	PIC_{50}
Control	112.52±10.661	4.552±0.252	75.15±7.805	4.607±0.207**
PFRE	78.3±7.399	4.399±0.15	60.11±16.197	4.332±0.164*

The possible role of L-type Ca^{+2} channels in the relaxation response of CA muscles to SNP investigated through incubation of the CA rings with, Nifedipine (30µM). The results in table (2) showed a leftward shift of the curve and non-significant increase in the magnitude of the relaxation of CA rings pre-incubated with nifedipine in comparison to the untreated control rings (E_{max}: 120.86 ±7.4%, Nifedipine *vs* 112.52±10.661% control) with increase in potency (PIC_{50}: 5.028±0.284, *vs*; 4.552±0.252, control) respectively (Fig.7A) and PFRE treatment in the presence of the above blocker shifted the curve to the left and significantly P< 0.001 reduced the relaxation response of tissue to SNP with (E_{max}: 79.771 ±8.651%) while the potency significantly P< 0.01 increased in compare to Nifedipine treated control groups (PIC_{50}: 6.155±0.59 PFRE, *vs*; 5.028±0.284, Nifedipine) (Fig.7B)

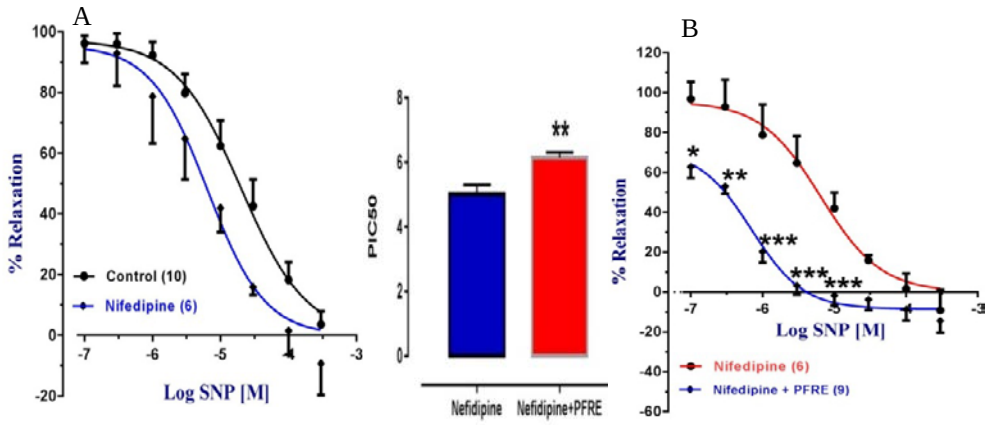


Figure 7: Cumulative-concentration relaxant effects of SNP in CA rings pre-treated with L-type Ca^{+2}channel blocker nifedipine. (**A**) The effect of nifedipine (30µM) on CA precontracted with 30mM KCl. (**B**) The effect of PFRE (0.1mg/ml) on CA pre-incubated with Nifedipine (30µM), the inserted graph shows the potency difference between CA rings treated with Nifedipine and Nifedipine with PFRE together. * symbolize statistical differences at P< 0.05, ** symbolize statistical differences at P< 0.01, *** symbolize statistical differences at P< 0.001, the number of used coronary artery ring in the study is in parentheses.

Figure (8) shows that in Ca^{+2}-free Krebs solution, $CaCl_2$ induced concentration-dependent contraction of CA rings with (E_{max}: 119.67±13.52% and PEC_{50}: -2.932±0.307), while incubating these CA rings with PFRE, shifted the concentration-response curve to the right and accentuated non significant decline in the magnitude of the contraction with (E_{max}: 91.28±3.99% and PEC_{50}: -3.113±0.162), data shown in table (4).

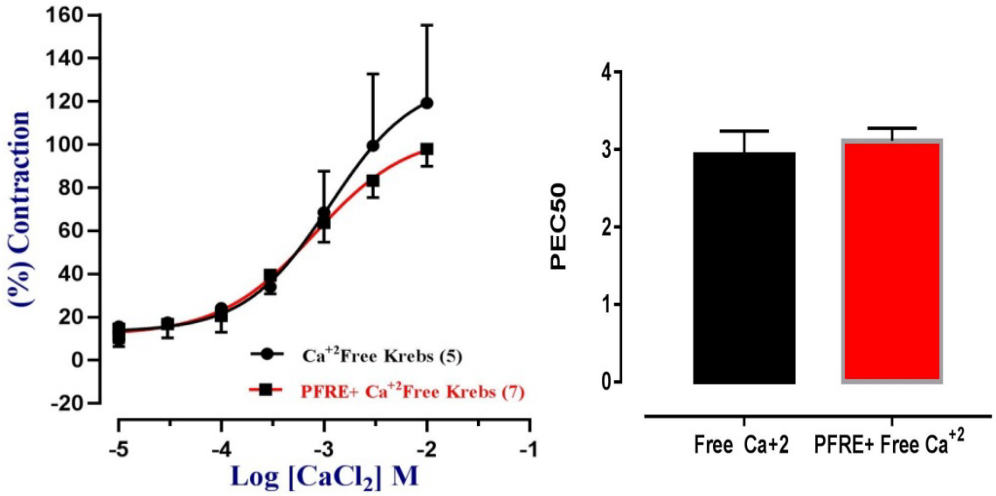


Figure 8: Cumulative-concentration response curve of (**A**) PFRE effect on $CaCl_2$-induced contraction in Ca^{+2}- free Krebs Solution. (**B**) the graph shows the potency difference between CA rings in Ca^{+2}- free Krebs Solution and CA rings in Ca^{+2}- free Krebs Solution incubated with PFRE (0.1 mg/ml), the number of used coronary artery ring in the study is in parentheses.

Table 4: Shows the potency and maximum relaxation response (E_{max}) from the PFRE treated and untreated coronary artery to KCl bathed in free Ca^{+2} Kreb's solution

Drugs	Free Ca^{+2} krebs solution		PFRE + Free Ca^{+2} krebs solution	
	E_{max} (%)	PEC_{50}	E_{max} (%)	PEC_{50}
Control	119.67±13.52	-2.932±0.307	91.28 ±3.99	-3.113±0.162

4. DISCUSSION

Findings of the present study showed PFRE appear to be devoid any vasoconstriction agent because it did not show any contraction activity on the resting baseline of bathed coronary artery in normal physiological Krebs solution, and the results reveal the negative inotropic effects of the plant root extract and the vasorelaxant activity of PFRE at concentrations ranging from (0.0001, 0.001, 0.01, 0.1 mg/ml), while concentrations (1.0 and 10 mg/ml) showed less relaxation effects in isolated coronary artery rings of the goat however, it is of possibility to speculate that maintaining the coronary circulation and vasodilatory activity of PFRE may due to its phenolic compounds [35], and also reported that most of the plant's physiological activities brought about through its alkaloid content [36],

The unanticipated finding of this study was that the extract induced significant concentration-dependent relaxation in intact endothelium arteries in comparison to that of control untreated arteries, so it can be argued that the vasodilatory effect of the plant is endothelium-dependent. This strange result is supported by some earlier studies on mice aorta [22] and rat aorta [37], meanwhile the results somewhat conflicts previous study which revealed that the liquid *Prosopis farcta* Pod extract inhibited the contraction effect of phenylephrine on the healthy rat's aorta but is endothelium-independent [22]and mice aorta [38], the root extract of the plant also able to increase the expansion rate of rat aorta [12]. The affirmation of the relaxing effect of PFRE in coronary artery may implicate important implementation of the plant in patients with myocardial and coronary artery disease, that's why *P. farcta* has long been used as a traditional remedy for angina pectoris [22], reducing cardiac or chest pain and for managing cardiovascular disorder in Iran [19], as a therapeutic agent in treating cardiovascular disorders [23] and as re-

emerging health aiding plant for cardiovascular diseases [39] . However, the result somewhat conflicts the earlier finding of [22]

Vascular contractions implicate the control of K^+ channels, the opening of K^+ channels in VSMCs results from the outward current of K^+ and membrane hyperpolarization which in turn results in a reduction in Ca^{+2} entry as a result from the closure of VDCC, and vasodilation [40]. In contrast, closure of K^+ channels leads to membrane depolarization and vascular contraction. Pre-incubation of the coronary artery with K_{ca} channels blocker; TEA significantly reduced the E_{max} and PIC_{50} of the artery, this may due to the fact that blocking of most K_{ca} channels with TEA resulted in coronary artery cell membrane depolarization which in turn increase the driving force of outward K^+ current and cell contraction [31] this in agreement with the findings of [41] in which blocking of K_{ca} potentiated the contraction altitude induced by hU-II in rat aorta, so it is of great importance to note that K_{ca} blocking results in augmentation of vascular basal tone and contractile response to phenylephrine in rat aorta [42] however, seemingly, K_{ca} not to involved in relaxing effect of PFRE, because treatment of CA with PFRE in the presence of TEA, resulted in slight but non-significant increase in relaxation response of tissue which is in accordance to that of [40] who demonstrated that K_{ca} channels may not involve in vasodilation activity of PFRE.

The K_{ATP} channels regarded as the type of K^+ channel that its activity reduced with the increase of ATP at the intracellular side of the plasma membrane, so decreasing the ATP level through reducing or inhibiting glycolysis, sufficient for the coronary artery K_{ATP} channels to open and dilate the tissue. Thereby, these channels considered to be an important link between blood flow and metabolism through vascular tone regulation, not at least in the coronary artery [43]. K_{ATP} channels have been demonstrated in vascular smooth muscles [43] and act as an important modulator of vascular relaxation and in turn activated by AMPK, which also involved in activation of BK_{ca} [37]. In the present finding, blocking K_{ATP} with glibenclamide resulted in significant attenuation in the maximum relaxation response of the artery in comparison to the untreated control arteries, this is consistent with some previous studies that revealed the activating K_{ATP} channels in rat coronary artery act as eminent dilating mechanism [44], also from an earlier study on cultured macrophage, it was revealed that glibenclamide tended to inhibit induction of iNOS [45]; meanwhile in the current study, incubation of the coronary artery with glibenclamide before the PFRE did not abolish the relaxation effect of the extract, this in accordance with that of [44] on rat coronary artery.

Barium chloride as a non-specific selective blocker of variety potassium channels, their blocking activity somewhat depends on its external concentration[41]. In the present study, the predominance of these channels in endothelial cells and their critical roles in modulating vascular relaxation made them be used as a pharmacologic target for studying the signaling pathways of different vasodilating agents [41]. The current findings represent the failing of $BaCl_2$ to antagonize the coronary vasodilator response to SNP, which consistent with that of [32], while applying the extract to the coronary artery in the presence of the blocker, potentiates the blocking action of $BaCl_2$ and non-significantly reduced maximum relaxation of the tissue to SNP with no significant change in their potency. As the PFRE reduced the artery response SNP, in the presence of blocker, it was suggested that this extract may interact with K^+ influx or modulate Ca^{+2} influxes, because blocking K^+ channels conductance induce cell membrane depolarization and results in increasing the influx of transmembrane Ca^{+2} and eliciting the contraction response of the artery.
It is well known that vascular endothelium plays an important role in the regulation of vascular tone through the synthesis and release of EDRF include NO and PGI_2 and EDHF. Prostacyclin is an EDRF synthesize from both vascular smooth muscle cells and endothelium. It is a lipid compound that is enzymatically derived from fatty acids and discovered first earlier than NO. In the current study, it was decided to find out the effects of prostacyclin in the relaxation response of coronary artery to SNP and the results showed no significant attenuation in the

relaxation response of coronary artery rings pre-incubated with a non-selective inhibitor of COX enzyme; indomethacin. This is in accordance to results of [46] revealing non-contribution of the prostacyclin in coronary artery vasorelaxation, however, treating coronary arteries with PFRE in the presence of indomethacin resulted in non-significant potentiation of the tissue relaxation. This supports the previous study of [38] suggesting that the relaxant effect of the extract is not endothelium-derived prostacyclin dependent. Meanwhile, our finding somewhat conflicts the result of [40] in that the inhibition of COX enzyme and dysfunction of the endothelium not reduced the relaxation response significantly.

It is of great importance to note that there are many vasodilators that are responsible for vascular relaxation; some include an increase in NO production as an EDRF, while others require an intact endothelium such as acetylcholine. It is well-known that L-NAME is a novel irreversible inhibitor for the synthesis of NO from endothelial cells which act through inhibition of the endothelial nitric oxide synthase enzyme. In the present investigation, there was no significant change in E_{max} and PIC_{50} for coronary artery rings pretreated with L-NAME. However, treating these rings with PFRE in the presence of the inhibitor induced non-significant attenuation in the tissue relaxation, this implies non-involvement of the endothelium-derived NO synthesis in the relaxation effects of the extract. This supports the previous study of [40] and [38] who revealed non-involvement of the endothelium NO in the vasodilatory effect of some plant extracts. To the contrary, [22] revealed the involvement of endothelial NO in the relaxation effects of *Prosopis farcta* extract on rat's aorta.

Regulation of vascular tone through endothelium-derived hyperpolarizing factors involves the cytochrome P450 synthesis of epoxyeicosatrienoic acids (EETs). From previous studies, it was reported that EET generation has contributions in endothelium and cardiovascular function [47]. Selective inhibition of coronary artery rings with specific cytochrome P450 inhibitor; clotrimazole in the present study not showed any significant change in vascular activity, it seems to be due to the fact that overproduction of NO by SNP may involve the suppression of CYP450 activity [48], meanwhile the strange evidence of the study was that PFRE showed significant potentiation of relaxation in coronary artery rings incubated with clotrimazole. Because clotrimazole selectively inhibit CYP450 activity, so it is of possible plausible to interfere with coronary VSMC and endothelial activity. Some previous studies reported the ability of clotrimazole to interfere with both Ca^{+2} and Ca^{+2}-dependent K^+ channels [49] and IK_{Ca} channels activity [41].

Because most of arterial K_{Ca} channels are activated as a result of an increase in intracellular calcium concentration $[Ca^{+2}]_i$ and cell membrane depolarization which results in cell contraction, thus the possibility of interfering the PFRE with this type of channel and Ca^{+2} is investigated through either incubating the tissue in low Ca^{+2} physiological saline solution to reduce $[Ca^{+2}]_i$ or blocking some Ca^{+2} channels with nifedipine. Our findings depict the significant attenuation in maximal vasorelaxation induced by SNP, resulted from lowering the opening probability of K_{Ca} channels which is consistent with an earlier study of [32] suggesting the opening of large conductance K_{Ca} channels and adequate $[Ca^{+2}]_i$ resultant hyperpolarization of vascular cell membrane involved in SNP induced vasorelaxation in goat coronary artery. In the present study, lowering $[Ca^{+2}]_i$ through incubation of CA rings in low PSS markedly antagonized the relaxant effect of SNP in the tissue, which is consistent with the study of [32]. Similarly, PFRE appears to interfere with Ca^{+2} for its vasodilation in CA rings. So for the CA rings incubated in low Ca^{+2} Krebs, PFRE markedly induced significant reduction in tissue relaxation response to SNP; suggesting the decrease in extracellular Ca^{+2} diminishes the effect of the relaxation effects of PFRE.

It is well known that calcium level control vascular smooth muscle cell contraction [2], and this contraction is regulated by both the intracellular Ca^{+2} and contractile element's sensitivity and the agonist-induced contraction results from an increase in $[Ca^{+2}]_i$ through the release of

$Ca^{+2}$from SR stores which is of critical importance during agonist-stimulated contraction coupling [50] and the Ca^{+2} source seems to vary depending on the agonist-induced contraction species and vessels. The unique characteristic of smooth muscle, even when extracellular Ca^{+2} depleted to zero, it is able for the pharmaco-mechanical coupling depending upon inducing the release of intracellular stores [51]. Epicardial coronary arteries regarded to be the major therapeutic target in general for different calcium antagonists [52].

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In the present finding, depletion of intracellular Ca^{+2} level through blocking of L-type Ca^{+2} channel with nifedipine, non-significantly potentiated the relaxation response of CA to SNP, this may due to the fact, the NO donor SNP induce relaxation through inhibition of SR Ca^{+2} release [50]. Meanwhile, this vasorelaxation of SNP in the presence of PFRE significantly decreased, suggesting that the extract may induce relaxation through reducing and inhibiting Ca^{+2} influx and release of it from internal stores, consistent with the results of [40] The ability of the extract to relax the contractions induced by KCl is an indicator for its blockade activity on L-type voltage-dependent Ca^{+2} channel [34].

However, there may be different mechanisms by which plant or herbal extracts could modulate contraction or relaxation. For vasodilation facilitating and vascular system remodeling, herbal plants try to block the contractile and structural protein or may through myosin light chain protein phosphorylation [40]. PFRE non-significantly shifted $CaCl_2$-curve to right in of CA bathed in Ca^{+2}-free Krebs solution, seemingly the extract tend to induce relaxation of $CaCl_2$ induced contraction in CA rings through antagonizing mechanism of Ca^{+2}, and interfering of the plant extract with the voltage-operated Ca^{+2} channels similar to [30, 53] and often connected with the alkaloid active compound ability to control calcium channels, as the synthetic substances/drugs do [36].

5. CONCLUSION

In conclusion, to the best of our knowledge, this is the first study to report about the in vitro effects of *Prosopis farcta* root extract in coronary artery vascular reactivity. Results of the study show devoid of vasoconstriction activity and negative inotropic effects of PFRE in coronary vascular tissues while tend to exhibit the vasodilating effects through EDHFs and its antagonizing of Ca^{+2}, through reducing and inhibiting Ca^{+2} influx and release from internal stores and blocking of the voltage-operated Ca^{+2} channels which are suggested to be the most predicted mechanisms for the endothelium-dependence maintenance of vasodilating tone and coronary circulation of the PFRE in coronary artery.

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