

*Full Length Research Paper*

# Investigation of the effects of *Prosopis farcta* plant extract on Rat's aorta

K. Asadollahi\*, N. Abassi, N. Afshar, M. Alipour and P. Asadollahi

Department of Epidemiology, Faculty of Medicine, Ilam Medical Sciences University, Ilam, Iran.

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Ischemic heart diseases are the most common causes of death in the developed and developing societies. Because vegetable based medications have lower side effects and are well adapted to body's physiology, a global trend to use these kinds of medications for cardiovascular diseases is increasing. To investigate the effect of *Prosopis farcta* plant extract (P.F.P.E) on rat's thoracic aorta. *P. farcta* plant extract was firstly prepared. Then 2 centimetres of rat's thoracic aorta was dissected and was divided into 4 pieces of 5 mm. After contraction of these pieces by phenylephrine (1  $\mu$ m), different dosages of plant extraction (0.5, 1, 2 mg/ml) were examined and the effect of P.F.P.E on rat's aorta with and without endothelium layer was measured. Different dosages of *P. farcta* extract (1, 2 mg/ml) at the presence and absence of *L-NAME* (a nitric oxide synthetas inhibitor) was examined. *P. farcta* plant extract showed a dose dependent relaxing effect on contracted aorta. The relaxing effect of P.F.P.E on aorta with endothelium was more significant than that on aorta without endothelium in the different dosages ( $p < 0.05$ ). The relaxing effect of P.F.P.E in the presence of *L-NAME* was reduced significantly ( $p < 0.05$ ). The relaxing effect of P.F.P.E was more than that by acetylcholine ( $p < 0.05$ ). Relaxing effect of acetylcholine was inhibited by atropine but it did not show any effect on the relaxing effect of P.F.P.E ( $p < 0.001$ ). *P. farcta* plant extract showed a dose dependent as well as an endothelium dependent relaxing effect on thoracic aorta in rats. Endothelium layer showed an important role in the relaxing effect of P.F.P.E on rat's aorta. Also nitric oxide had a significant role on the relaxing effect of P.F.P.E.

**Key words:** Aorta, endothelium layer, *prosopis farcta*, relaxing effect.

## INTRODUCTION

Cardiovascular diseases are the main causes of death among developed and developing countries. More than 50% of cardiovascular deaths are related to coronary artery diseases (CAD) (American heart association, 2009; Vafadarafshar et al., 2005; Jiang He et al., 2005; Ali et al., 2004). 36% of causes of deaths in Iran are due to cardiovascular diseases particularly due to myocardial infarction (Sadr et al., 2005).

Beta-blockers, Ca channel blockers and nitrites are used as the main medications for treatment of cardiovascular diseases. Because of lower side effects, trend to use of plants medications are increasing.

Plants have been used for medical purposes since many centuries. Different cultures have tried different kinds of plants and many papers and books have been published in this field. A lot of current medications have also a plant based structure (Table 1).

After producing of chemical medications in 16<sup>th</sup> century, use of plants medications decreased gradually. However, due to a least side effect, trend to use of plants medications have been increased recently (Deputy, 2002). Plants medications are the base of modern pharmacology (Shahin and Akhondzadeh, 1999). Also, during Almaty announcement, WHO accepted to put plants medications in the general health program until year 2000 (Deputy, 2002). The 20<sup>th</sup> century has been introduced as the century of return to the nature and the century of using plants medications by pharmacology scientists (Omidbagi, 1994).

Nowadays, more than 50% of different medications from western countries have been extracted from plants

\*Corresponding author. E-mail: masoud\_1241@yahoo.co.uk.  
Tel: 00988412227126. Fax: 00988412227120.

**Abbreviations:** P.F.P.E.; *Prosopis farcta* plant extract.

**Table 1.** Some kinds of plants or trees which are used for medical purposes.

Name of plant/tree	Application	Location	Component of use	Reference
<i>Acacia aenegal</i>	Cutaneous and mucosal inflammations	Iran	Gum	(Ettarh and Emeka 2004)
<i>Acacia arabica</i>	Cough, bronchitis, dyspnoea	North Africa, Senegal, Sudan, Somalia, Iran	Gum	(Ettarh and Emeka 2004)
<i>Acacia franesiana</i>	Styptic, dysentery, neural pains	India, Guiana, Iran	Decoction	(Ettarh and Emeka 2004)
<i>Acacia catecha</i>	Styptic, diarrhoea, hematochesia	Thailand, Bengal, north Africa	Wood Decoction	(Ettarh and Emeka 2004)
<i>Adenanthera pavonina</i>	Rheumatoid arthritis	India, Philippine, china	Leaf	(Ettarh and Emeka 2004)
<i>Mimosa pedica</i>	Inflammation, healing, icter, haemorrhoid, kidney stones	Africa, warm climates	Root, leaf	(Ettarh and Emeka 2004)
<i>Albizzia antlementica</i>	Constipation	North Africa, Syria	crust	(Ettarh and Emeka 2004)
<i>Albizzia lebbeka</i>	Itching, dermatitis, haemorrhoid, asthma, gingivitis	Asia, Africa	peel, stem, root	(Ettarh and Emeka 2004)
<i>Prosopis spicigera</i>	Dysentery, asthma, bronchitis, lepra	South Asia, Iran, India, Afghanistan	crust	(Ettarh and Emeka 2004)
<i>Prosopis farcta</i>	Cardiac and chest pain, blood sugar lowering	Mountain climates	Decoction	(Afifi 1993)

(Iwu et al., 1999). About 25% of prescribed drugs in the USA are plants based medications (Farnsworth and Morris, 1976).

There is a long history of traditionally using the extraction of *P. farcta* plant (P.F.P) for treatment of angina pectoris in Ilam Province. Decoction of P.F.P has also been used traditionally to reduce cardiac or chest pain in this province. There is no report to show an academic research (*in vitro*, *in vivo*), to evaluate the anti angina effect of this plant so far. This study aimed to evaluate the mechanism and the effect of P.F.P extraction on rat's aorta (Figure 1).

The genus *Prosopis* comprises almost 50 species, 25 of which are on the list of federal noxious weeds. *Prosopis* are most often spiny trees or shrubs predominantly well adapted to hot, arid climates. It has been distributed in Southwest Asia, Africa; predominantly America, from western North America to Patagonia. Only four species are native to Southwest Asia and Africa. The remaining species in the genus are native to Mexico and South America, with the center of polymorphism in Argentina (Russell and Macbr, 2009).

*P. farcta* is a small, prickly shrub, 30 - 80 cm tall or "shrub-tree" 2 - 3 m or taller and is native to Northern Africa (Algeria (rare), Egypt and Tunisia) and much of southwestern Asia, from Kazakhstan south to the Indian subcontinent and west to the Middle East and Asia minor. Also found in the United States (Russell and Macbr, 2009).

## METHODOLOGY

A sample of *P. farcta* plant was collected and was kept in a standard condition. This sample was sent for Mashhad Faculty of Pharmacy and Faculty of Drug Sciences of Köln and it was identi-

fied and confirmed by both of these faculties. After drying, the root parts of plants powderized. 100 grams of plants powder was added to 1000 ml boiling water. Lowering the flame, the powder was mixed properly in water during a period of 15 min. After 15 min, via Buchner Funnel, whole content of mixture was firstly filtered by usual filter paper. The filtered solution was again filtered by no.1 of Whatman filtering paper. The filtered solution was transferred into the relevant apparatus for removing the surplus water and about 80% of water was removed. Then the ultimate solution was put into the bath of warm water (30°C). Male young rats (Wistar), mean age  $7 \pm 2$  and mean weight  $350 \pm 50$  g, were collected from Isfahan Faculty of Pharmacy and were put in the poly carbonate cages as groups of the same temperature and light conditions. They freely accessed food and water. Rats were anesthetised by injecting 50 mg/kg of ketamine and then 2 centimetres of their aorta were collected and were immediately put into cold and originated Krebs Henseleit solution. In the next step, each 2 centimetres of rat's aorta was divided into 4 pieces of 5 mm. Using needle no.18 with an irregular surface, the endothelial layers of some groups of dissected aorta were destroyed. The prepared aorta parts were put in the tissue bath (10 ml) between 2 horizontal pipes. A part of rat's aorta was constantly connected to the bottom of tissue bath and another part was connected via a string to an isometric transducer. Aorta contractions were recorded on a paper by universal Harvard Osillograph with a speed of 0/1 mm/s.

Krebs Henseleit solution had a temperature of 37°C, a pH of 7.4 and its contents comprised NaCl (1/8), KCl (4/7), CaCl (2/52), MgSO<sub>4</sub> (1/24), KH<sub>2</sub>PO<sub>4</sub> (1/18), NaHCO<sub>3</sub> (Farnsworth and Morris, 1976) and glucose (5/5), (Godini, 2002). A constant oxygenation was performed at the bottom of the bath. After 60 min of adaptation period the bath solution was changed. In the all stages of this study, for marking a healthy endothelial layers, phenylephrine with a density of 1 µm was added to the bath and after some contractions, acetylcholine (1 µm) was added (Hong et al., 2000; Puebla et al., 2002). If the relaxation of aorta, due to acetylcholine effect, was more than 60%, the aorta piece was considered as aorta with a healthy endothelial layer (Barriere et al., 2001). In the next step, phenylephrine (1 µm) and then one of the different dosages of P.F.P.E were added onto the both groups of aorta with and without endothelial layers. After using of one dosage of P.F.P.E, the percentage of aorta's relaxation was measured. For testing another



Figure 1. *P. farcta* flowering and fruit.

dosage of P.F.P.E, the bath solution was changed. Waiting for 10 min and returning the aorta tonicity to the basic state, phenylephrine and then the new dosages of P.F.P.E was added. For evaluating the role of nitric oxide (NO) on the effect of plants extraction, the percentage of aorta relaxation was firstly measured for the dosages of 1 and 2 mg/ml of P.F.P.E and 5 min after adding the 100  $\mu$ m of nitric oxide synthetase inhibitor (L.NAME), the percentage of aorta relaxation was measured again (Kim et al., 2000). To see if the P.F.P.E had a cholinergic effect, some groups of aorta with the endothelial layers were firstly contracted with 1  $\mu$ m of phenylephrine. Then the percentages of aorta relaxation due to 1 mg/ml of P.F.P.E, at the presence of 1  $\mu$ m of atropine (Legssyer et al., 2002) were measured in each group.

Using SPSS version 12.0, 2-tailed t-test was applied and the mean relaxation effects of different dosages of P.F.P.E were compared between groups with and without endothelium and also at the presence and absence of atropine, phenylephrine and L.NAME.

This study adheres to rules for animal research as reviewed and approved by institutional appointed committee and it was also approved prospectively by Ilam Medical Sciences University's Ethics Committee.

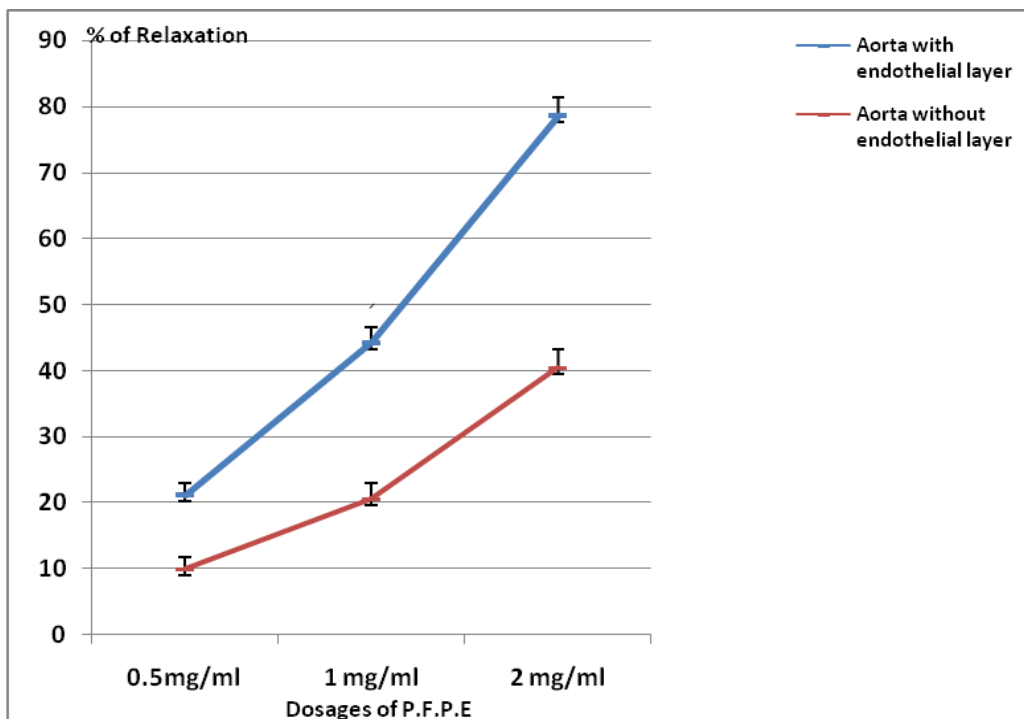
## RESULTS

Different dosages of P.F.P.E (0.5, 1, 2 mg/ml) have been tested on rat's aorta and as the dosage increased, the

percentage of relaxation of aorta increased. Destroying the endothelial layer of aorta caused the relaxation effect of P.F.P.E on aorta to be decreased. There was a significant difference between the mean relaxation effect of P.F.P.E on rat's aorta with and without endothelial layer in all different dosages ( $P < 0.0001$ ) (Figure 2).

At the presence of L.NAME, the percentage of relaxation effect of P.F.P.E was decreased. The difference between the mean relaxation effect of P.F.P.E on rat's aorta at the presence and absence of L-NAME was statistically significant ( $P < 0.01$ ) (Table 2).

To see if the P.F.P.E components have any cholinergic effect like acetylcholine on aorta, some groups of aorta with the endothelial layers were firstly contracted with 1  $\mu$ m of phenylephrine. Then the percentages of aorta relaxation due to 1 mg/ml of P.F.P.E, at the presence of 1  $\mu$ m of atropine (Legssyer et al., 2002) were measured in each group. By the same way the percentages of aorta relaxation due to 1  $\mu$ m of acethylcholine at the presence of 1  $\mu$ m of atropine was measured and the results were compared (Table 3). Atropine showed an inhibiting effect on the relaxation effect of acetylcholine but did not show the same effect on the relaxation effect of P.F.P.E. (Table 3).



**Figure 2.** Comparison the mean relaxation effect of different dosages of P.F.P.E on aorta with and without endothelial layer ( $p < 0.0001$ ). Bars = Standard deviations from the mean.

**Table 2.** Comparison between the mean relaxation effect of rat's aorta in different dosages of P.F.P.E at the present and absence of *L-NAME*.

Dosage of P.F.P.E	Mean relaxation effect %	SD %	P value
1 mg/ml, L- NAME absence	50	3.7	0.01
1 mg/ml, L- NAME present	12.9	2.3	
2 mg/ml, L- NAME absence	70	1.6	0.01
2 mg/ml, L- NAME present	30.7	1.9	

**Table 3.** Comparison the inhibiting effects of atropine on relaxant effect of P.F.P.E and acetylcholine.

Dosage of different variables	Mean relaxation effect %	SD %	P value
Relaxant effect of acetylcholine (1 $\mu$ m)	34	0.024	0.001
Relaxant effect of P.F.P.E (1 mg/ml)	-21	0.024	
Relaxant effect of acetylcholine (1 $\mu$ m)	34	0.024	0.001
Inhibiting effect of atropine (1 $\mu$ m) on relaxant effect of acetylcholine	85	0.038	
Relaxant effect of P.F.P.E (1 mg/ml)	-21	0.024	0.12
Inhibiting effect of atropine (1 $\mu$ m) on relaxant effect of P.F.P.E	-0.9	0.014	

## DISCUSSION

According to the results of the current study, the liquid extract of the P.F.P, inhibited the contraction effect of phenylephrine on the healthy rat's aorta. The inhibited

effect of P.F.P.E, after phenylephrine contraction, was reduced when the endothelium layer of aorta was destroyed. The relaxation effect of the P.F.P.E showed a dose dependent phenomenon on aorta with endothelium. The relaxation effect of P.F.P.E on aorta with and without

endothelium showed a significant difference ( $p < 0.05$ ). Therefore it can be concluded that the relaxation effect of P.F.P.E on rat's aorta may be related to the endothelial layer. Both aorta with and without endothelium layer were contracted by phenylephrine, therefore the inhibition effect of P.F.P.E was not due to the difference of the contraction intensity of aorta.

Using key words *P. farcta* and aorta, *P. farcta* and heart, *P. farcta* and hypertension, *P. farcta* and muscle relaxation, search engines of Google, ISI web of knowledge, Ovid and Athens were interrogated and there were no published papers including these terms in this field.

Previous studies have showed a contraction effect of phenylephrine on aorta without endothelium layer (Tunctan et al., 2000). The presence of the endothelium layer for the inhibition effect of P.F.P.E on the contracted aorta in rats is important and this effect is corresponded to the effects of procyanidine of the grape on human's aorta reported by previous studies (Soares et al., 2002; Aldini et al., 2003; Kamanyi et al.). Also, the relaxant effect of P.F.P.E on rat's aorta was in accordance to the same effect by liquid extraction of *Gassia Occidental* on rat's aorta reported by Ibadon biomedical communication (Ajagbonna et al., 2001). The importance of endothelial layer for the P.F.P.E is in accordance to the effect of escaping oils extracted from *Elaeis guineensis* plant (Abeywardena et al., 2002). Another study reported the importance of endothelium layer of the relaxation effect of rat's aorta and this was in accordance to the results of the current study. The same results were reported for the effect of Nitric acid and methylxanthins on endothelial layer of aorta (Tare et al., 1990; Hatano et al., 1995).

A study from India reported that the *Arbutus unedo* plant had a relaxant effect on aorta and by this phenomenon showed an antihypertensive effect (Kamanyi et al.). Using the results of the above mentioned study can guess that *Prosopis farcta* may have an antihypertensive effect and this should be investigated by future studies. It seems that during the process of relaxation of rat's aorta by P.F.P.E, alpha receptors are occupied by special elements in the P.F.P.E. *L-NAME* prevents the NO synthesis from the endothelial cells of aorta by inhibiting the nitric oxide synthetase enzyme (Moncada et al., 1991). The effects of 1 and 2 mg/ml of the P.F.P.E on contracted aorta at the presence and absence of *L-NAME* were examined for 2 min. The results showed a lower relaxation effect of the P.F.P.E at the presence of *L-NAME* with a significant difference ( $p < 0.05$ ).

Previous studies have showed a relaxation effect of *Quercetin* on aorta via NO syntetase activation, which was reduced at the presence of *L-NAME* (Diaz et al., 1989; Kubota et al., 2001). Therefore there may be a similar effect of special elements in the P.F.P.E and this *Flavonoid*.

The current study showed that *L-NAME* caused a decrease of the relaxation effect of P.F.P.E via a decrease

of releasing of the NO from the endothelial layer. According to the above results, it can be concluded that the relaxation effect of P.F.P.E may be due to an increase of the production of NO and CGMP. The effect of P.F.P.E at the presence of *L-NAME* and its relevant results are in accordance to the effect of Xanthorrhizol on rat's aorta reported by another study (Campos et al., 2000). The same effect at the presence and absence of *L-NAME* has been reported for *Morinda Lucid* plant extract (Ettarh and Emeka, 2004).

To investigate the possible interaction effect of atropine on the relaxation effect of P.F.P.E, aorta was firstly contracted by phenylephrine (1  $\mu$ m) and adding 1 mg/ml P.F.P.E caused a relaxation on aorta and atropine could not prevent or reduce the effect of P.F.P.E ( $p < 0.001$ ). However, by the same way atropine could reduce the relaxation effect of acetylcholine on rat's aorta ( $p < 0.001$ ).

There was an assumption that some of the P.F.P.E components have cholinergic effect like acetylcholine on aorta. In this study atropine showed an inhibiting effect on the relaxation effect of acetylcholine but did not show the same effect on the relaxation effect of P.F.P.E. therefore, it can be concluded that P.F.P.E does not have any cholinergic element in its component. The same conclusion was reported by another study when the effect of *Vitis Vinifera* plant's extract was examined on frog's heart (Kazem et al., 2004). The researchers reported that atropine reduced the relaxation effect of acetylcholine on frog's heart palpitation but did not on the relaxation effect of *V. Vinifera* plant extract.

## Suggestions

- P.F.P.E may have an anti angina effect in human and this effect should be investigated by future studies.
- Because P.F.P.E showed a relaxation effect on aorta, it may have a curable effect on hypertension and this should be investigated by future studies.
- As P.F.P.E showed a relaxation effect on smooth muscles, this plant could be used in treatment of some diseases such as migraine, Reynaud phenomenon, asthma etc for which contraction of smooth muscles are the main aetiology and this should be investigated by future studies.

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