Cardiovascular effects of vitexin isolated from *Prosopis farcta*

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ABSTRACT

Vitexin was isolated and identified from fruit of *Prosopis farcta* (Iraqi indigenous). Cardiovascular actions of vitexin were studied in vitro and in vivo. Vitexin produced a positive inotropic effect on isolated atrium of the rabbit which was not related to Beta 1 adrenergic receptor activation. Vitexin had no vasodilator activity nor it could reverse the vasoconstrictor responses of the isolated pulmonary artery of the rabbit to potassium chloride and phenylephrine.vitexin has produced a significant increase in urine flow and urinary sodium and potassium excretions in healthy and mild hypertensive volunteers and in rabbit. Moreover, vitexin significantly reduced mean arterial blood pressure of the mild hypertensive volunteers and rabbits. This hypotensive effect of vitexin is not related to a direct vasodilatation or to blocking alpha and Beta adrenergic receptors. The most likely mechanism of action of vitexin as a hypotensive compound is through its diuretic effects.

الخلاصـــة

استخلصت وعينت هوية مادة الفتكسين (vitexin) من ثمرة نبات Prosopis farcta (عراقية المنشأ). ولقد درست التأثيرات القلبية الوعائية لهذه المادة في الزجاج وفي الجسم الحي. وكان تأثير مادة الفتكسين إيجابيا على انقباض عضلة القلب. وهذا التأثير ليس له علاقة بتحفيز مستقبل بتا١ (β1) الادرينالييني. ولم يكن هناك تأثير موسع للشريان التنفسي لمادة الفتكسين ولم تعكس مادة الفتكسين الاستجابة الانقباضية للشريان التنفسي لكل من مادة كلوريد البوتاسيوم ومادة الفنيسل فرين. سببت مادة الفتكسين زيادة في سرعة جريان البول وطرح الصوديوم والبوتاسيوم في المتطوعين الأصحاء والمرضى المصابين بفرط الدم الشرياني وكذلك في الأرانب. قد يكون سبب هذه التأثيرات هو تثبيط نقل الصوديوم والبوتاسيوم في القنوات القاصية في الكلية. بالإضافة إلى ذلك، قللت مادة الفتكسين معنوياً ضغط الدم في المرضى المصابين بفرط الدم الشرياني المتوسط وكذلك في الأرانب. ولم يكن التأثير المخفض لفرط الدم الشرياني بسبب التأثير المباشر على الأوعية الدموية أو نتيجة غلق مستقبلات الفا (α) أو بيتا، والأكثر احتمالية كان التأثير المخفض لفرط الدم الشرياني من خلال التأثير المدرر.

Substances derived from the plants remain the bases for a large proportion of the commercial medications used today for the treatment of heart disease, high blood pressure, pain, asthma, and other problems.[1]

Plants represent today and probably in the future, a first class source for new medicaments in both industrialized and developing countries. Generally, therapeutic agents obtained from plant kingdom are considered to be less toxic as compared to those of synthetic origin.[2]

Prosopis farcta is one of the important local plants which are of value for medicinal purposes. *Prosopis farcta* is a small, prickly shrub, 30-80 cm tall or "shrub-tree" 2-3 m or taller. In its native range, it is widespread, and a weed of wheat and cotton fields, invading by root suckers. Although it is not eaten by livestock (because of its spines), other herbivores eat the fruits, thus most likely aiding seed dispersal.[3]

Al-jeboory in 1984 found that Vitexin extracted from *Prosopis farcta* produced antihypertensive effects in dogs and monkeys.[4]

This study was undertaken to evaluate the cardiovascular effects of vitexin which is isolated from *Prosopis farcta*.

Figure 1. *Locally indigenous plant of Prosopis farcta*

Materials and methods

Plant material: The plant fruits were collected from north region of Iraq Kalar district, dried and identified by the staff of Iraqi national herbarium center in Abu-ghreab.

Preparation of Vitexin: Freshly collected dried plant fruits (400g) were ground into course powder and extracted at the room temperature with 20% petroleum ether (1000 ml of each). The solvent was evaporated in vacuum at 20 °C to give the dry extract. Vitexin in the dried extract was isolated and separated with Sephadex LH chromatography (elution with methanol). The procedure was used in accordance with previously reported results[5] to separate Vitexin from *Prosopis farcta* (Figure2).

Drugs: Furosemide, isosorbid dinitrate, phenylephrine, timolol, KCl and vitexin

Drugs were freshly prepared in order to prevent hydrolysis of the components of the drugs during long storage.[6]

In Vitro studies

Tension studies (isolated rabbit tissues):

Isolated pulmonary arteries: Pulmonary arteries were obtained from the freshly sacrificed rabbits, fibrillated with KCl and immersed in Krebs-Henseleit (KH) solution. Spiral strips (2-3 cm in length) were set up vertically in an organ bath containing KH solution gassed with air and maintained at 37°C.

Isolated atrial muscle: Rabbits of both sexes were sacrificed. The thorax was rapidly opened and the left atrium freed from ventricular and connective tissues. The preparation of left atrium was suspended in a 30 ml organ bath containing KH solution at 37°C, and continuously gassed with O_2 and CO_2. The isolated preparation was impaled on a platinum electrode and set up vertically in the organ bath. Contractions of left atrial preparations were obtained by square wave pulses (frequency 2-5 Hz, duration 5 ms) of twice threshold voltage (usually 5-10 V) delivered by a student stimulator.

In Vivo studies

In rabbits:

Experiments were performed on local domestic male rabbits (*Oryctolagus cuniculus*) weighing 1.5–2 Kg. The rabbits were anaesthetized by intraperitoneal (I.P.) injection of pentobarbital. Arterial BP of each rabbit was measured from the femoral artery connected to a mercury manometer on kymograph. The ear vein was cannulated for administration of drug solutions.

The heart rate of the rabbit was recorded from the blood pressure trace, after increasing the speed of recording paper to make the wave and pulsations apparent. Urine samples were collected via the urethra by catheterization of the urinary bladder by small size pediatric feeding tubes.

Human subjects:

Twelve patients,their ages ranged 60-70 years of both sexes with stage I hypertension. Group 2: Six healthy male Two groups of subjects participated in the present study as follows: Group 1: volunteers their ages ranged 22-28 years of both sexes.

Both groups were given a single dose of 30 mg of vitexin orally (PO) at morning for

seven days. Mean blood pressure of the hypertensive volunteers were measured. Urine samples (per 12 hours) of both groups were collected for analysis and compared with urine which was collected before taking vitexin.

The data were expressed as mean ±SE. Differences between means were evaluated statistically using student's t – test for paired samples and also by using complete randomized design (CRD) with different replicates, then the least significant difference (LSD) test was used for comparisons between means.

Results

Tension studies

Effects of *vitexin* on isolated tissues of the rabbit

KCl produced contraction of spiral segments of pulmonary artery which was not affected by vitexin (30μg/ml) as shown in figure (3). Also the same dose of vitexin failed to reverse the vasoconstrictor effect of phenylephrine (20μg/ml). Whereas, the contraction response to phenylphrine was antagonized and reversed to dilatory effect by Isosorbide dinitrate (50μg/ml) (figure 4). Figures (5 & 6) show positive inotropic effects of vitexin (30μg/ml) and (30μg/ml) adrenaline on the isolated atrium of the rabbit. The cardiotonic effect of vitexin was not inhibited by using Timolol (0.5mg/ml).

Effects of vitexin on the kidney function, heart rate and arterial BP in the rabbit

Intraperitoneal (I.P.) injection of 1mg/kg of vitexin in the rabbits produced significant increases ($P<0.01$) in the urine flow and urinary excretion rates of sodium and potassium in rabbits (table 1). The same dose of vitexin significantly reduced the arterial BP which was statistically similar to the hypotensive effects of 40μg /kg of furosemide (table 1). Whereas the diuretic effects of furosemide statistically ($P<0.01$) was more potent than the diuretic effects of vitexin. Meanwhile, the injection of this dose of vitexin caused no significant changes in heart rate of the rabbit (table 1).

Effects of vitexin on the kidney function of healthy volunteers

The effects of 30 mg of Vitexin (PO) on the urine flow and urinary electrolyte excretion rates in healthy volunteers are shown in table 2. Vitexin induced marked and significant ($P<0.01$) increases in urine flow and sodium and potassium excretion rates of healthy volunteers.

Effects of vitexin on the kidney function, heart rate and BP of hypertensive volunteers

Thirty mg of vitexin (PO) produced a significant increase in the urine flow and sodium and potassium excretion rate in mild hypertensive volunteers (table 3). Moreover the same dose of vitexin significantly reduced mean BP of hypertensive volunteers (table3).

In vivo studies

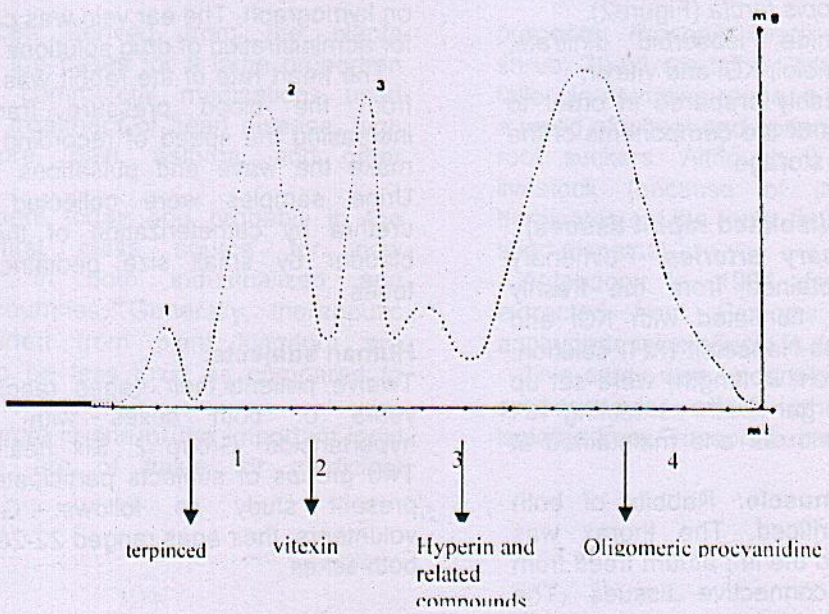

Figure 2. *Flow diagram for the extraction and fraction of Prosopis farcta.*

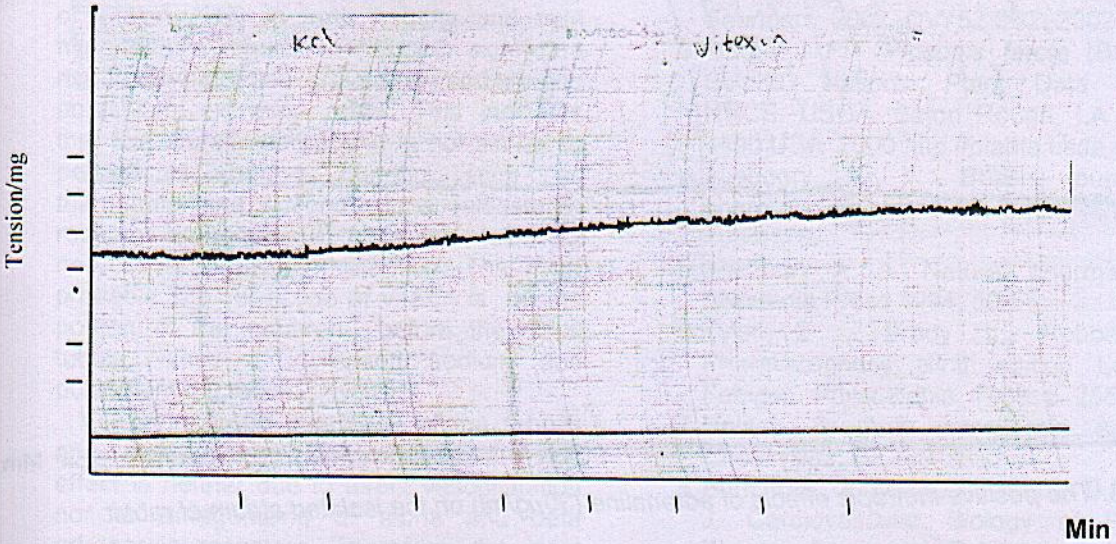

Figure 3. *The effects of vitexine (30 μg/ml) on the isolated pulmonary artery of the rabbit pretreated with KCl (50 mEq/ml).*

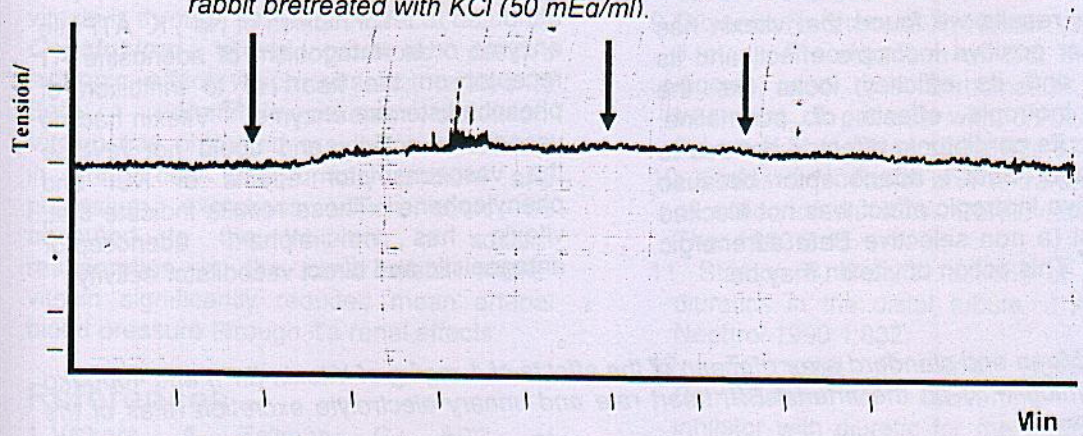

Figure 4. *The effects of vitexine (50μg/ml), phenylephrine (20μg/ml) and Isosorbidedinitrate (50μg/ml) on the isolated pulmonary artery of the rabbit.*

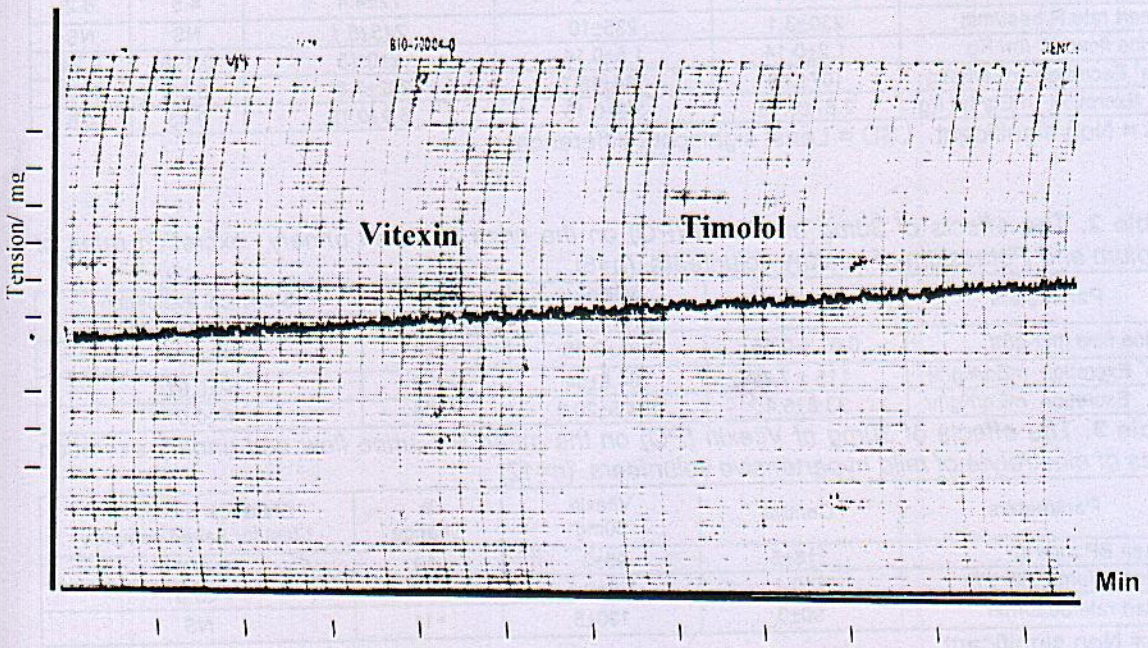

Figure 5. *The effects of Vitexine (30μg/ml) and Timolol (50μg/ml) on the isolated atrium of the rabbit.*

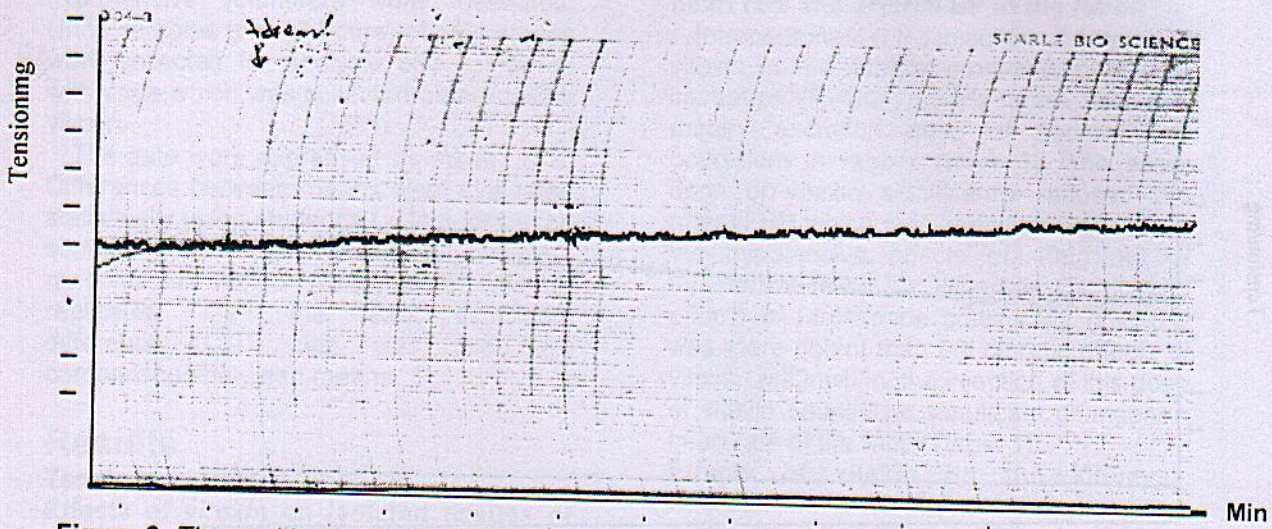

Figure 6. *The positive inotropic effects of adrenaline (30μg/ml) on the isolated atrium of rabbit.*

Discussion

From our results we found that vitexin has quite clear positive inotropic effect, and its potency and its efficacy looks like the positive inotropic effects of adrenaline. However, its cardiotonic effect is not due to activation of Beta-1 adenoceptor because this positive inotropic effect was not blocked by timolol (a non selective Beta adrenergic blocker).[7] This action of vitexin may be attributed to the inhibition of Na^+, K^+ ATPase enzyme or to antagonism of adenosine A1 receptor on the heart or to inhibition of phosphodisterase enzyme.[8,9] Vitexin had no vasodilator activity and could not reverse the vasoconstrictor effects of KCl and phenylephrine . These results indicate that, vitexin has no alpha-1 adenoceptor antagonistic and direct vasodilator activity.

Table 1. *Mean and standard error of mean of the effects of 1 mg/kg of Vitexin (I.P.) and 40μg /kg of furosemide (I.P) on the arterial BP, heart rate and urinary electrolyte excretion rates of the rabbits. n=12.*

Parameters	Control	Vitexin 1mg/kg	Furosemide 40μg/kg	LSD	
				0.05	0.01
Arterial BP mmHg	83±4.8	74±5.2	72±4.4	4.5	6.2
Heart rate R beat/min	230±3.1	235±10	*245±5.1*	NS	NS
Urine flow ml/ /hr/ Kg	1.2±0.14	1.5±0.14	2±0.15	0.195	0.24
Na^+ Excretion mEq/hr/kg	107±3.8	134±16.7	202±7.8*	6.54	8,46
K^+ Excretion mEq/ hr/ Kg	2.87±0.13	3.8±0.15	*5.9±0.16*	0.43	0.6

NS = Non significant LSD = Least significant difference.

Table 2. *The effects of 30mg of Vitexin (PO) on the urine flow and urinary excretion rates of Sodium and Potassium of healthy volunteers. (n=6).*

Parameters	Control	Vitexin 30mg	% Change	Statistical evaluation t-test for paired samples
Urine flow ml/Kg/hr	0.67 ± 0.05	2.31±0.35.	+244	*P<0.01*
Na^+ Excretion mEq/Kg/hr	115 ± 7.7	364.8±54	*+217*	P<0.01
K^+ Excretion mEq/Kg/hr	33.8±5.4	134.5±20.3	+305	*P<0.01*

Table 3. *The effects of 30mg of Vitexin (PO) on the mean BP, urine flow and urinary excretion rates of electrolyte of mild hypertensive volunteers. (n=12)*

Parameters	Control	Vitexin 30mg	% Change	Statistical evaluation t-test for paired samples
Mean BP mmHg	118±5	95±3.	-19	*P<0.01*
Urine volume ml/kg/hr	2±0.1	3.4±0.1	*+70*	P<0.01
Heart rate beat/min	90±3	100±5	+11	*NS*

NS = Non significant

Data from tables 1, 2 & 3 clearly show that 1 mg/kg of vitexin (I.P.) in rabbits and 30 mg of vitexin (PO) in both healthy and mild hypertensive volunteers induced significant rise in the urine flow and urinary sodium and potassium excretion rates. This indicates that this diuretic compound is not similar to potassium sparing diuretic such as triametrine and spironolactone (aldosteron receptor antagonism)[10] because vitexin did not cause potassium retention. The most probable site of action of vitexin is on the portion of the nephron before the distal tubule, since it increased sodium and potassium excretion.[11]

Vitexin reduced mean BP of the rabbits and mild hypertensive volunteers. This effect is neither due to direct vasodilatation nor to antagonizing of alpha and beta adrenergic receptors. Therefore, the most likely mechanism of hypotensive effect of vitexin is through its diuretic effects.[12]

Conclusion: vitexin produced positive inotropic effect which was not related to Beta 1 adrenergic receptor activation. Vitexin has produced a significant increase in urine flow and urinary sodium and potassium excretions which could be attributed to the inhibition of sodium reabsorption in the renal distal tubule. vitexin significantly reduced mean arterial blood pressure through it's renal effects.

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