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THE PHARMACOLOGICAL STUDIES OF PROCYANIDINE ISOLATED FROM CRATAEGUS AZAROLUS (IRAQI ENDOGENOUS)

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ABSTRACT

Procyanidine was isolated and identified from fruit of Crataegus azarolus (Iraqi endogenous). In vitro and in vivo pharmacological actions of procyanidine were studied. Toxicological and histopathological studies of procyanidne on the mice indicated its safety. The LD_{50} of procyanidine estimated to be about 100mg/kg.

No antibacterial and antiinflammatory effects were seen with procyanidine.

The antagonistic effect of procyanidine on the adenosine receptor was observed on the isolated jejunum of the rabbit. Procyanidine produced quite clear positive inotropic effect on the isolated, stimulated left atrium and papillary muscle of the rabbit, without any significant effects on the heart rate and blood pressure of experimental rats.

Also procyanidine produced marked diuretic and natriuretic effects without any significant actions on the potassium excretion in the rat (16mg/kg I:V) and volunteers healthy subjects (50mg orally).

The positive inotropic and diuretic actions of procynidine, most probably, are related to its antagonistic effects on the adenosine (Al) receptors.

These dual (positive inotropic and diuretic) effects of procyanidine make it a good drug for the treatment of congestive heart failure.

INTRODUCTION

The use of plants for healing purposes predates human history and forms the origin of much modern medicine. Many conventional drugs originate from plant sources: a century ago, most of the few effective drugs were plant based. Much of the medicinal use of plants seems to have been developed through observations of wild animals, and by trial and error (Vickers and Zollman, 1999).

Plants represent today, and are expected to represent in the future, a first class source for new medicaments in both industrialized and developing countries. Medical plants are the raw material from which the following derivatives are prepared (William, 1999).

1. Decoctions and infusions.
2. Total extracts and tinctures prepared in accordance with the pharmacopoeias.
3. Purified and standardized extracts.
4. Chemically pure active products.

Each of them represents a step in the passage from traditional to modern medicine; but each has its own value and function, and not necessarily the chemically pure products are the most active form of the plant.

Generally, therapeutic agents obtained from plant kingdom are considered to be less toxic as compared to those of synthetic origin. Virtually every pharmacological class of drug includes natural product prototype' that exhibits the classical effects of the pharmacological category in question, most of them are plants derived for example:

Cardiotonic – cardiac glycosides (e.g., lanatosides)

Analgesic:	alkaloids (e.gs. morphine, codeine, etc.).
Antihypertensive:	alkaloids (e.gs., reserpine).
Antimalarial:	alkaloids (e.g., quinine)
Catartic:	anthraquinones (eg. sennosides) (Bowman & Rand, 1980 and Laurance, 1990).

There are more than 50 categories of secondary organic constituents known from the world of higher plants. The prospects of investigations of plants are promising, since it has been estimated that only 6-8 % of the world's flora (approximately 600000 plants) and less than 10% of the organic constituents are known, and 90% remains for discovery and investigations (A1-Baytar, 1246 and Aljeboory, 1995).

Herbal Medicine is the use of herbs for their therapeutic or medicinal value. The herb is a plant or part of plant valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body (Mills, 1993). However, the pharmacologist may be interested in the studying of both phytochemistry and phytopharmacy which, deals with isolations and identifications of chemical constituents of medicinal plants (Weiss and Fintelmann, 2000).

The pharmacological evaluation has been developed through observation of wild animals since humanity originated. Pharmacological works on medicinal plants and their knowledge developed in the world and continued on wild animals and human being till now in which the pharmacological trials and errors, established by many researchers (Vickers and Zollman, 1999).

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. For instance, ephedra is a herb used in traditional Chinese medicine for more than two thousand years ago to treat asthma and other respiratory problems. Atropine was obtained from atropa belladonna (Higley, 2000). Another example of the use of a herbal preparation in modern medicine is the foxglove plant. This herb has been in use since 1775. Other examples include Salicylic acid, a precursor of aspirin, which was originally derived from white willow bark and the meadow sweet. Cinchona bark is the source of malaria-fighting quinine. Vincristine, is used to treat certain types of cancer and comes from periwinkle (Tyler *et al.,* 1988; Vickers and Zollman, 1999; Weiss and Fintelmann, 2000).

Beside their use and advantages as a source for drugs, many plants are toxic. There are reports about cases of serious ad verse events after administration of herbal products. For instance, several women developed rapidly progressive interstitial renal fibrosis after taking Chinese herbs which were prescribed by a slimming clinic. Several herbal products interact with conventional drugs such as Echinacea with anabolic steroids, garlic with warfare and Liquorice with Spironolactone (Mills, 1 993 and Newall *et al.,* 1996).

These plant-derived drugs and others, which are in use nowadays and still are drugs of choice for special diseases, are preferred on synthetic drugs for the following reasons:

1. Have less side effects and toxicity than synthetic drugs.
2. There is less resistance from micro-organisms against these drugs.
3. Can be obtained from cheap resources.
4. They are pure drugs of natural origin.

In our study we have chosen the fruits of crataegus azarolus (Iraqi endogenous) which belong to hawthorn group because very little information and studies are available about the medicinal effects of this plant which is present mainly in Kurdistan valleys.

MATERIALS AND METHODS

Phytochemical Studies

Isolation of procyanidine from Crataegus azarolus

Crataegus azarolus fruit was gathered from Himreen valey in the beginning of autumn. The fruit was cut into slices, then 400 gm of the slices extracted with 50% of ethanol and left for 24 hours, then filtered by ordinary filtration. The plant material was thrown away. The filtrate put in autoclave under 40^{o}C for 48 hours then extracted with distilled water. The filtrate extracted with n-hexane (lipophilic fraction) then the hydrophilic phase extracted with chloroform and dried under Freez-drying and the 40 gm powder was kept for the pharmacological tests (Fig. 1).

Ethanol was used to precipitate extremely polaric compounds, Terpene, and etc. Chloroform was used to take steroidal parts of plant. Hexane was used to isolate saponins.

Identification of Procyanidine isolated from crataegus azarolus:

The powder is extracted with ethanol 90%, the solvent concentrated and separated with sephadex LH chromotography (elution with methanol). This gives 5 fractions and under chemical screening procyanidine, identified according to British herbal pharmacopeia 1996. Identification test (Figure 2) which carried out for thin layer chromotography (British Herbal Pharmacopeia, 1996 and Svedström, 2002) involves, the following:

Apply 20 µ1 of each of the following solutions separately to the plate:

Solution (1): warm 10 ml of methanol containing 1 gm of procyanidine on a water bath for 10-15 minutes cool and filter

Solution (2): 0.025% rutin in methanol

Spray the plate with spray reagent A* and examine in ultraviolet light 366nm.

Major bands relative to rutin are as follows:

turquoise 2.25. yellow 1.65** turquoise 1.5* yellow 1.0.

*These bands may coalesce in high concentration to give the appearance of a green band at their overlap.

*Spray reagent A is consist from a 1% w/v solution of diphenylboric acid 2-arninoether ester in methanol, followed by separate application of a 5% w/v solutions of polyethyl glycol 4000 in ethanol (96%).

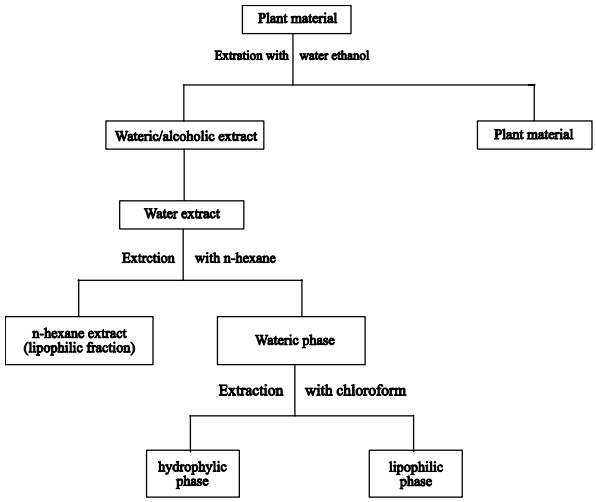

Fig. 1: Isolation of procyanidine from Crataegus azarolus.

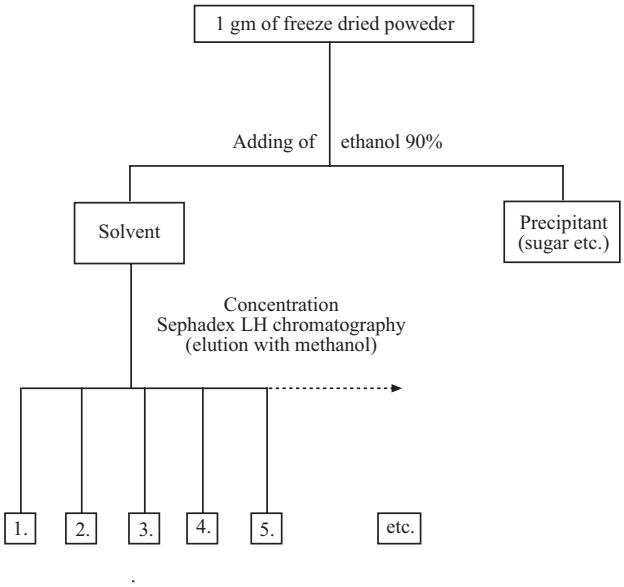

Fig. 2: Identification of procyanidine prextracted from Crataegus azarolas.

IN VITRO STUDIES

Tension studies

Isolated pulmonary arteries:

Pulmonary arteries were obtained from the freshly killed rabbits,, fibrillated with KCl and immersed in (KH) Krebs-Henseleit solution (composition in g/L being NaC1 6.9; KC1 0.35; $CaC1_2$ 0.28; KH_2PO_4 0.16; $MgSO_4$ 0.29; $NaHCO_3$ 2.1 and glucose 2). The vessels were either used immediately or after storage for a maximum period of 2 hr in Krebs-Henseleit. Spiral strips (2-3 cm in length) were set up vertically in an organ bath containing KH solution gassed with oxygen and maintained at 37^{o}C.

Isolated jejunum of the rabbit:

Jejunum was obtained from freshly killed rabbits. The gut was cut 5-10 cm below the stomach and a length of jejunum was obtained and placed in a petri dish containing Tyrode solution (composition in gm/L: NaCl 8.0; KCl 0.42; $CaC1_2$ 0.2; $NaHPO_4$ 0.05; $MgCl_2.6H_20$; 41.2; $NaHCO_3$ 1 and glucose 1) which supplied by oxygen. The tissues were set up vertically in an organ bath containing Tyrode solution gassed with oxygen and maintained at 37^{o}C.

Isolated bronchial muscle:

3-4 cm of bronchial muscle was obtained from freshly killed rabbits. Spiral strips (2-3 cm in length) were set up vertically as previously described.

Isolated atrial and papillary muscle of the rabbits:

Rabbits of both sex were killed. The thorax was rapidly opened and the left atrium and the papillary muscle freed from ventricular and connective tissues. Preparations of left atrium as well as of papillary muscle suspended in a 30m1 organ bath containing (KH) solution at 37^{o}C were used, and continuously gassed with O_2 and CO_2. The isolated preparation was impaled on a platinum electrode and set up vertically as described above. 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 student stimulator.

Resting tension of *0.5* g was maintained in all preparations and contraction were recorded. The isolated tissues were allowed to equilibrate for 1-2 hr during this period the physiological solution was replaced every 15-20 minutes. Tension variations were recorded with a two channel Oscillograph, (Washington 400 MD2, Bioscience, England) recorder and transducer (Washington Transducer type D Bioscience searle).

Bacteriological studies

Disk containing 10μ, 20μ, and 50μ, of procyanidine which extracted from Crataegus azarolus in aseptic condition were prepared. Culture and sensitivity test done, to observe the antimicrobial activity of procyanidine against, three types of microorganism, *E. coli*, Staphylococcus aurous and Pseudomonas eurogenosa.

IN VIVO STUDIES

Animal preparations

Rats:

9 Wistar female Albino rats were used in the present study. Their weight ranged from 175-260 gms (mean ± standard error of mean=1.44±0.05). The rats were kept in the animal house of the college of medicine in a comfortable room temperature of 25^{o}C. They were maintained on palatable food containing all essential components of rodent's diet.

Mice:

Twenty four adult mice weighing 20-32 gm have been used in this study. They were divided into Four groups, group 1 as a control and the other groups received 10mg/kg. 50mg/kg and 100mg/kg of procyanidine by oral administration, respectively. The animals were given procyanidine at 11 am each day for about 15 days. These mice were used for acute toxicity and histopathological studies.

Rabbits:

Experiments were performed on local domestic rabbits *(Oryctolagus cuniculus)* n=48. Before experimental use, the animals were kept in the animal house and maintained on normal available food (barley and vegetables) and in suitable room temperature (18-25^{o}C). The weight of animals ranged from (1.0-1.8 Kg) (mean ± standard error of mean=l .44±0.05).

Anaesthesia:

Rats were anaesthetized by intraperitoneal injection of a combination of ketamine (ketamine hydrochloride, Rotex media GMBH, TRITTAU, Germany) in a dose of 75mg/Kg body weight with xylazine (2%, Ceva sante a ninale-laBallastiere, 33501 Libourne Cedex-france) in a dose of 10mg/Kg body weight (Laber-Laird *et al.,* 1996). This combination provided perfect surgical anesthesia, and supplementary small doses of the combination were given as necessary to maintain the level of anesthesia.

Intravenous infusion:

Intravenous administration of physiological saline and procyanidine into rats was performed via the tail vein. One of the tail veins was made prominent by obstructing the blood flow in it temporarily downstream and massaging the vein gently in the direction of the tail tip. This procedure with some practice and experience enabled us to insert a 27 gauge dental needle into the tail vein. The needle was connected by polythene tubing (PP25, Portex Ltd, Kent, England) to a syringe that was fixed on an infusion pump.

Tracheostomy:

A longitudinal incision of about 3-5cm was made through the skin, at the ventral side of the neck. The muscles below were pulled apart by forceps to expose the trachea. A small transverse cut was made through it, and then a suitable polythene tubing was inserted into the trachea and secured in place by ligature. The tracheal cannulation allows free ventilation and is necessary for long-term anesthesia in rats to avoid tracheal obstruction due to secretion of mucus. Any secretion appeared in the trachea was removed by suction using a syringe connected to a polythene tube.

Collection of urine samples:

In female rats, the urinary bladder was

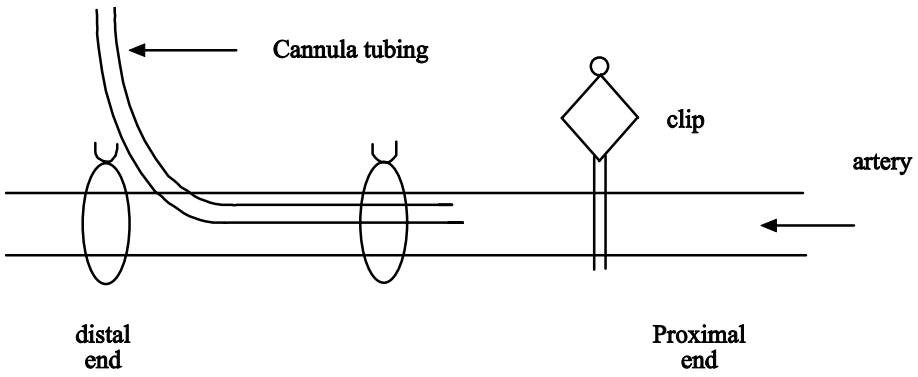

Fig. 3: A diagram showing cannulation of the common carotid artery in rats (Dizaye, 1998).

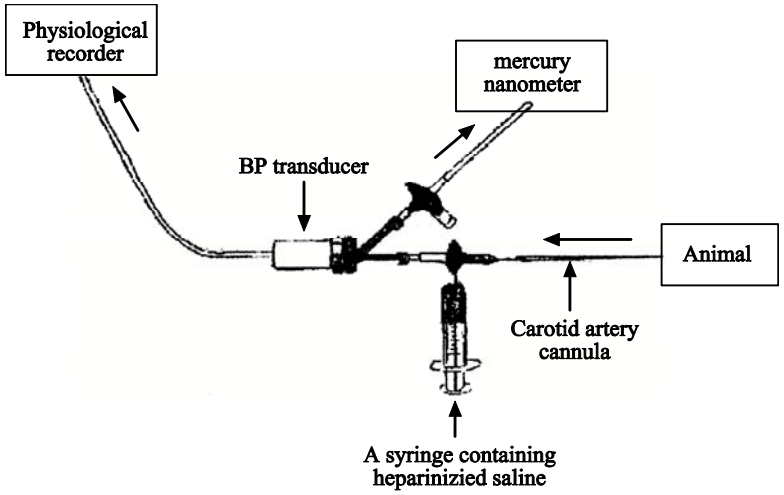

Fig. 4: A diagram showing the main aspects of arterial blood pressure recording (Dizaye, 1998)

catheterized via the urethra with PP 25 polythene tubing. A mark was made on the urethral cannula, about 2cm from its tip, when this mark is at the edge of the urethral opening, the tip of the cannula lies just inside the bladder. This helps to prevent damage to, and the consequent bleeding of the bladder during urine collection. The urethral cannula was inserted directly through the urethral opening by careful manipulation. The urethral cannula was held in place by a ligature tied around the external orifice of the urinary tract. Urine was collected in sample cups by applying gentle pressure to the lower abdomen above the bladder. The volume of urine was determined by weighing it, and assuming the specific gravity to be 1.00 (the specific gravity of rat's urine has been reported to be 1.018 (Saleh, 2001).

Cannulation of the common carotid artery:

The left and right carotid arteries run parallel with the vagus nerves dorsolateral to the trachea toward the head. One of the carotid arteries was exposed, freed and lifted gently by forceps. A ligature was made around the artery at its distal end at the level of mandibular angle, closing it completely. A loose ligature

was placed around the artery at its proximal end and the artery was clipped temporarily at this end. A length of about 15-25 mm of the artery formed the distance from the clip to the distal ligature. This part of the artery was raised slightly by a small curved forceps and a small transverse cut was made by using fine scissors. Then a piece of PP 25 polythene tubing attached to a syringe containing heparinized saline (Heparin Sodium, pharmaceutical products Ballerup, Dinamarca) was inserted into the artery against the direction of the blood flow. The tubing was pushed down to the clip and secured in place by the ligature. The clip was removed allowing blood to flush into the cannula in pulsations (see Fig. 3). This method of arterial cannulation was previously employed by Dizaye (1998).

Determination of arterial blood pressure and heart rate:

The arterial ABP was recorded by connecting the common carotid artery cannula to a blood pressure transducer (Washington, Pt 400, S/No. 304, supplied by Electomatic Ltd., England). The arterial cannula connection was through a 3-way stop-cock attached to a syringe containing heparenized isotonic saline. The blood pressure transducer was in turn connected to a two channel Oscillograph (Washington 400 MD2, Bioscince, England), and to a mercury manometer for calibration. The process of calibration allows the determination of the range of pressure in which the blood pressure of the animal is recorded (see Fig.8).

It is rather difficult to accurately determine the heart rate in the rat by palpation. The method described by Dizaye (1998) was therefore used to record the heart rate (HR) from the blood pressure trace. Each pulse in the artery is transmitted to the physiologic recorder as an upward deflection representing the systolic pressure followed by a downward deflection representing the diastolic pressure. A cardiac cycle, on the recording paper consists of an upward deflection and a downward deflection. The number of cardiac cycles (beats) per unit of time can be determined from the speed at which the paper of the recorder is running. For this purpose, the speed of the recorder was increased for few seconds from 0.25mm/sec to 25 mm/sec

Assay of electrolytes:

Urine was analyzed for sodium and potassium by flame photometry (Jenway, PFP7). The urinary excretions of Na^+ and K^+ were calculated as follows:

Excretion rate (μEq/min) = urine concentration (mEq/L) x urine flow (ml/min).

Human subjects:

Two groups of subjects participated in the present study as follows:

Group 1:

Seven patients aged (60-70) years of both sexes with heart failure who attended the Rizgary teaching hospital in Erbil as inpatients in the medical ward. Four patients of them have taken classical treatment of heart failure [like frosemide ACEI (Angiotensine converting enzyme inhibitor) and digoxine] with placebo as a new drug, and the three other patients have taken single daily dose of 50 mg (orally) of procyanidine in addition, to the half dose of the classical drugs which are used ordinary for treatment of heart failure. Sign and symptoms of heart failure of the patients were observed during 5 days of their admission in the medical ward.

Group 2:

Six healthy male volunteers aged (22-28), have taken a single dose of 50 mg (orally) of procyanidine at morning for one day, Urine were collected for analysis and compared with urine which collected after taking placebo.

RESULTS

In vitro studies:

Effects of drugs on the isolated pulmonary and coronary artery:

KC1 produced dose dependent 10-30 mEq

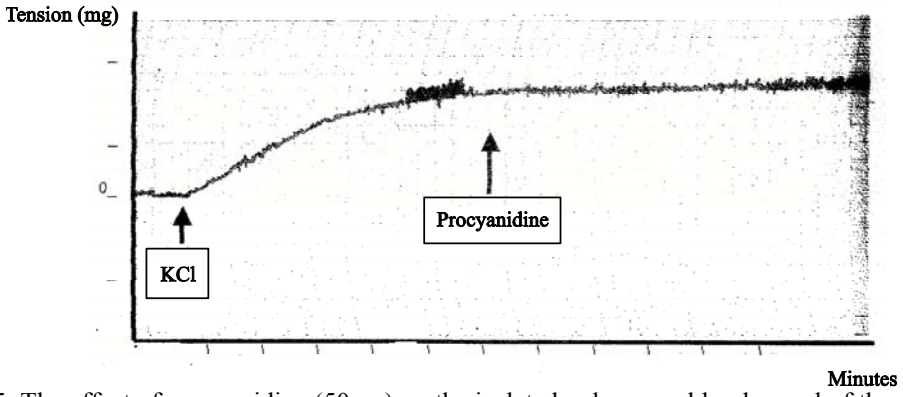

Fig. 5: The effect of procyanidine (50 µg) on the isolated pulmonary blood vessel of the rabbit pretreated with KCl (30 mEq). n=6.

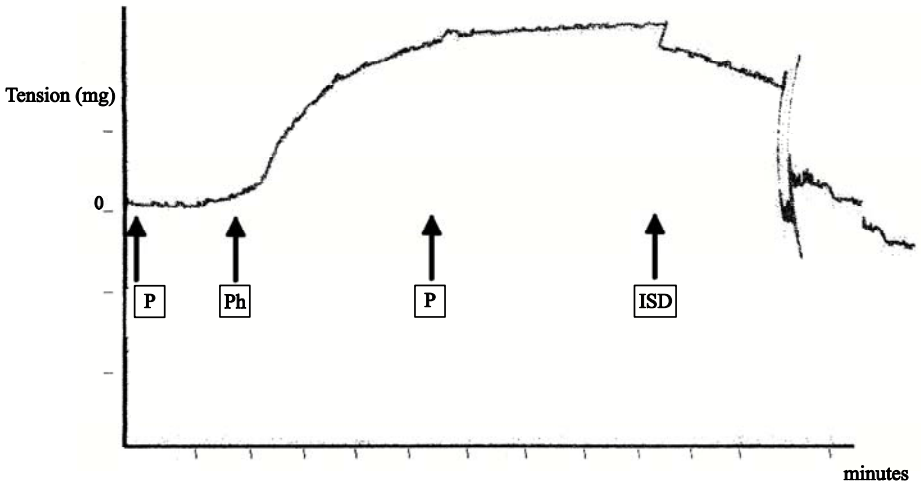

Fig. 6: The effect of procyanidine (50 µg/ml), phenylphrine (20 µg/ml) and isolated dinitrite (50 µg/ml) on the isolated pulmonary blood vessel of the rabbit. n=6.

P = Procyanidine Ph = Phenylphrine ISD = Isosorbide dinitrite

contraction of both coronary and pulmonary spiral segments of arteries which was not affected by procyanidine (50ug) in a dose up to 120 as shown in Fig. 5

We tried in other vasoconstrictor phynelphrine (20 ug) which produce dose dependent contraction of the isolated pulmonary artery of the rabbit which not affected by procyanidine While this contraction was antagonized and reversed to dilatory effect by isosorbide dinitrite (50ug) (Fig. 4).

These results show that procyanidine did not affect the adrenergic receptors.

Effects of procyanidine on the pendular movements of the isolated jejunum of the rabbit:

Figs. 11 and 12 clearly, show that, procyanidine produced an increase in the

Table-1
Mean and standard error of mean of different doses of the procyanidine on the pendular movements of the isolated jejunum of the rabbit. n=6

Parameters	Control	Procyanidine 20µg/ml	Procyanidine 40µg/ml	Procyanidine 80µg/ml
Mean ± S.E.M.	110 ± 9	395 ± 8	491 ± 28	626 ± 21

LSD = 85.18

Table-2
Analysis of variance for the effects of different doses of procyanidine on the pendular movements of the jejunum of the rabbit. n=6

Source of variation	df	MS	F
Treatment	2	81238.9**	30.95
Error	15	39366.6	
Total	17		

** = Highly significant

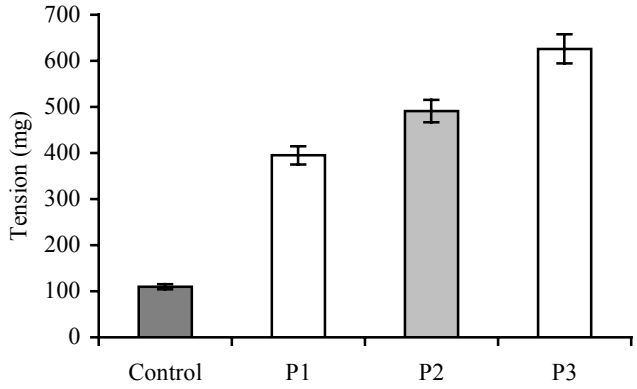

Fig. 7: The effects of different doses of procyanidine on the pendular movements of the jejunum of the rabbit. n=6.
P1 = Concentration of 20 µg/ml; P2 = Concentration of 40 µg/ml; P3 = Concentration of 80 µg/ml

spontaneous contractility of the rabbit's jejunum and the effect was dose dependent (20-80) µg/ml.

Adenosine in a dose of (1µg/ml) caused an inhibition of the activity of the pendular movements of the isolated jejunum, but procyanidine could reverse its' effect. This effect is quite clear in the Fig. 7. The effect of adenosine was also reversed by using of 10µg/ml of aminophylline (Fig. 8).

Effects of procyanidine on the isolated atrium and papillary muscle of the rabbit:

From the studies of isolated right and left atria of the rabbit and left stimulated atria alone, procyanidine produced dose dependent positive inotropic effects (20µg, 40µg, 80µg), as shown in Tables 3 and 4 and Fig. 10. This effect was clear in papillary muscle of the

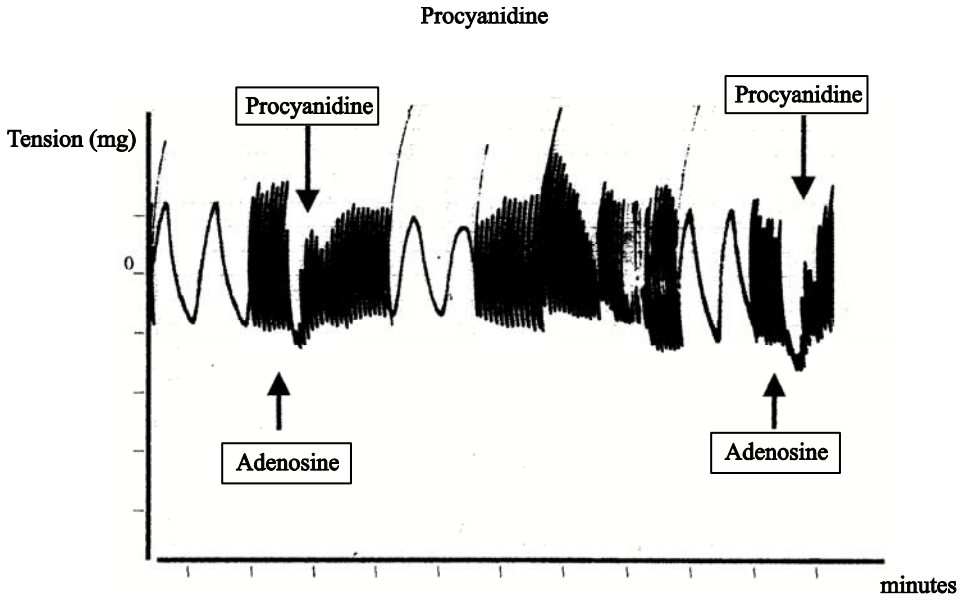

Fig. 8: The counteractive effects of procyanidine (20 µg/ml) on the inhibitory effect of Adenosine (1 µg/ml) on the pendular movements of the jejunum of the Rabbit. n=6.

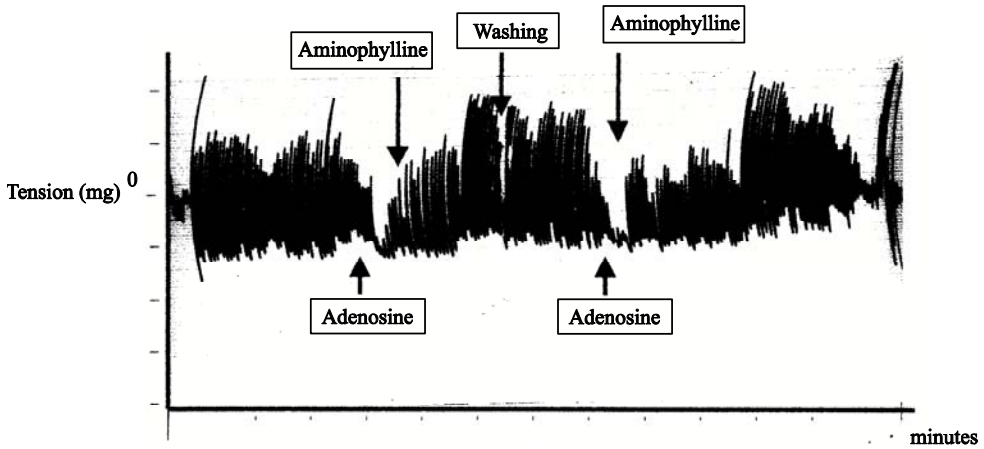

Fig. 9: The counteractive effects of Aminophylline (10 µg/ml) on the inhibitory effect of Adenosine (1 µg/ml) on the pendular movements of the isolated jejunum of the Rabbit. n=6.

rabbit as shown in Figs. 20 and 21. This response of procyanidine was compared with adrenaline (10 µg) and digoxin (l0 µg). The positive inotropic effects of these drugs are clear in Figs. 22, 23 and 24 and Table-5.

The inability of Timolol (a non selective Beta blocker) to block the procyanidine's positive inotropic activity on the isolated atrium is shown in Figs. 25 and 26 and Table-6.

Effects of procyanidine on the kidney function of the rat:

Intravenous injection of 16mg/kg of procyanidine on the rat produces a significant increase in the urine flow as shown in Table-7 and Fig. 4). On the other hand in the same

Table-3
Mean and standard error of mean of the positive inotropic effects of different doses of procyanidine on the isolated atrium of the rabbit. n=10

Parameters	Control	Procyanidine 20 µg/ml	Procyanidine 40 µg/ml	Procyanidine 80 µg/ml
Mean ±	46 ± 7	293.5 ± 22.5	416 ± 20	503 ± 26.4

LSD = 84.298

Table-4
Analysis of variance for the positive inotropic effects of different doses of procyanidine on the isolated atrium of the rabbits. N=10

Source of variation	df	MS	F
Treatment	2	111270.8**	20.85
Error	27	5337	
Total	29		

** = Highly significant

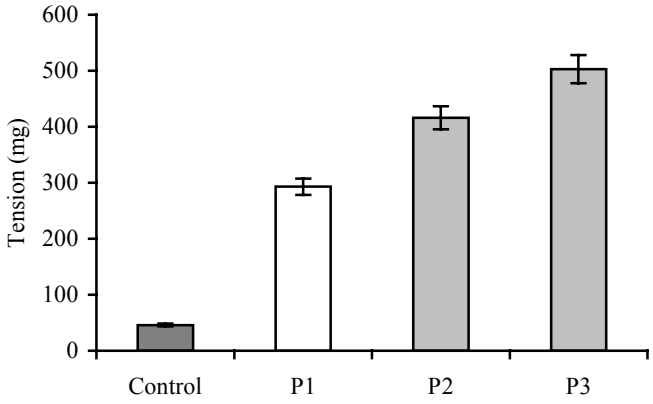

Fig. 10: The positive inotropic effect of different doses of procyanidine on left atrium of the rabbit. n=6.
P1 = Concentration of 20µg/ml, P2 = Concentration of 40µg/ml, P3 = Concentration of 80µg/ml

dose, procyanidine induced a marked and highly significant increase in the sodium excretion rate of the rats (Fig. 5 and Table-7).

We have noted in these series of experiments there was no significant hypokalaemic effects produced by intravenous injection of procyanidine in the dose of (16 mg/kg IV), as shown in the Table-7.

Effects of procyanidine on the kidney function of the healthy volunteers:

The effects of 50 mg (orally) of procyanidine on the urine electrolyte and urine flow in healthy volunteers are shown in Table-8. Procyanidine induced marked and significant increases in urine flow and sodium excretion rate (Figs. 17 and 18). There urinary K^+ excretion rate also increased insignificantly

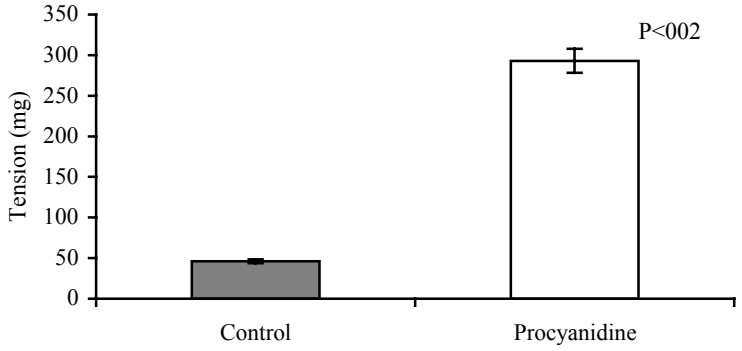

Fig. 11: The effect of procyanidine (20-80 μg/ml) on the contraction of the isolated papillary muscle in the rabbit. n=6.

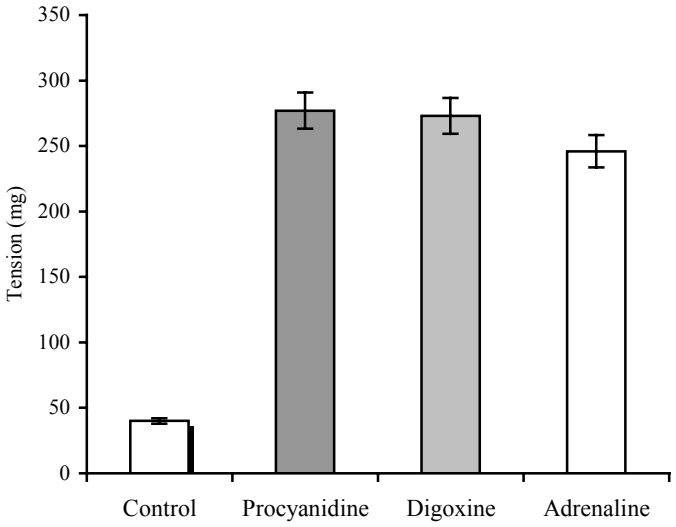

Fig. 12: The positive inotropic effects of procyanidine (20μg/ml), digoxin (10μg/ml) and adrenaline (10μg/ml) on the isolated atrium of the rabbit. n=6.

but, to a lesser extent than Na^+ excretion.

Effects of procyanidine on patients with heart failure:

Figs. 19, 20, 21, 22 and 23 show the responses of daily Mean arterial blood pressure, diastolic blood pressure, systolic blood pressure, pulse pressure and heart rates of patients with heart failure in both groups (patients treated with classical treatment of heart failure and patients treated with 50 mg of procyanidine with half of the doses of ordinary drugs which were used for heart failure.

DISCUSSION

In vitro studies:

Effects of drugs on the isolated pulmonary and coronary arteries:

Procyanidine isolated from Crataegus azarolus has no vasodilator activity and couldn't reverse the vasoconstrictor effects of

Table-5
Mean and standard error of mean of the positive inotropic effects of procyanidine, digoxine and adrenaline on the isolated atrium of the rabbit. n=6

Parameters	Control	Procyanidine	Digoxine	Adrenaline
Mean ±	40 ± 8	277 ± 35	273 ± 21	246 ± 16.6

Table-6
Mean and standard error of mean of the positive inotropic effects of procyanidine on the isolated atrium of rabbit pretreated with timolol. n=6

Parameters	Procyanidine	Timolol
Mean ±	266 ± 35.7	279 ± 21

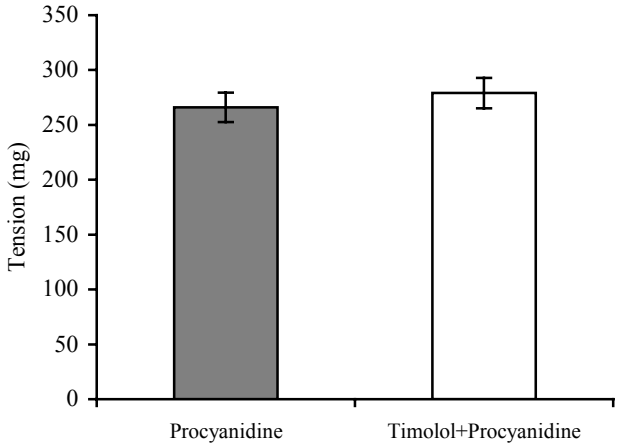

Fig. 13: The effects of procyanidine (20 µg/ml) on the isolated atrium of the rabbit pretreated with timolol (20 µg/ml). n=6

KCl and phenylphrine (Figs. 5 & 6). These results indicated that, procyanidine has no alpha-1 adenoceptor antagonistic and direct vasodilator activity. This was in contrast to Weikl, 1993 and Loew, 1994, who suggested that the extractions of some of the Crataegus species, like Crataegus oxyacantha, Crataegus monogyna caused vasodilation and improved coronary artery blood flow.

Effects of procyanidine on the pendular movements of the isolated jejunum of the rabbit:

From the Figs. 7 and 8, it is clear that there is a relation between the effect of procyanidine and aminophylline on the adenosine receptor, which may present in the smooth muscle of the jejunum of the rabbit. This is in agreement with Holtje who used computer-assisted model analysis to observe the structural similarity of flavanoid extracted from Hawthorn to papaverine and theophylline (Adenosine receptor antagonism), two chemical agents known to inhibit phosphodiesterase. (Nasa, 1993 and Holtje, 1993). This indicates that procyanidine, a flavanoid derivative, has some adenosine receptor antagonistic activity.

Table-7
The effects of intravenous injection of 16mg/kg of procyanidine on the renal function, heart rate and Blood pressure of rats (n=6).

Parameters	Control	Procyanidine	% Change	Statistical evaluation t-test for paired samples
Urine Flow µl/min./Kg	285 ± 51.8	548.8 ± 826	+ 92.3	P<0.05
Na^+ Exc. Rate µl/min./Kg	20.6 ± 9.9	126.7 ± 40.8	+ 515	P<0.05
K^+ Exc. Rate µEq/min./Kg	15.1 ± 3.7	19.4 ± 4.3	+ 28	N.S.
Heart rate	259 ± 15.7	253 ± 14.1	- .2	N.S.
Arterial BP mmHg	75	73	- 2.7	N.S.

P<0.05 = Significant, N.S. = Non-significant.

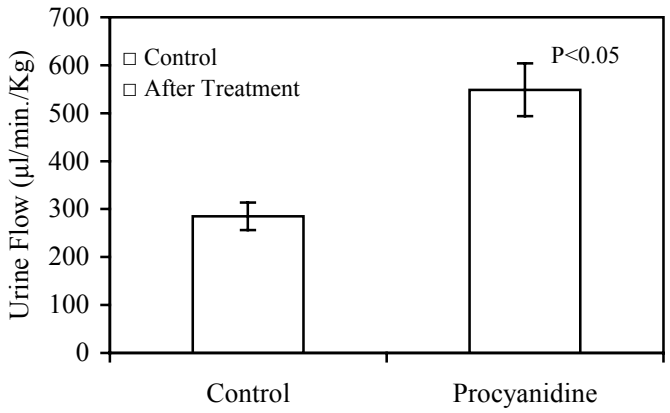

Fig. 14: The effects of intravenous injection of 16mg/kg of procyanidine on the urine flow of rats (n=6).

Effects of procyanidine on the isolated bronchial muscle:

The effect of procyanidine on the bronchial muscle was not clear. However, the effects of procyanidine on the bronchial muscle, pretreated with adenosine (Figs. 9, 10) indicated that, procyanidine and unlike aminophylline has no any actions on Adenosine A_{2B} receptors in which its activation leads to degranulation of autacoids, like histamine and prostaglandin from the mast cells (Choi *et al.,* 1988; Burnstock *et al.,* 1998; Bertil *et al.,* 2001). And also procyanidine has no actions on the muscarine (M_3), and Histamine (H_1) receptors (Fanta *et al.,* 1986; Goyal 1988; Chapman, 1990). This gives evidence that there was no role of procyanidine in the treatment of bronchial asthma (Hauck 1976; Flatt *et al.,* 1990 and Owen *et al.,* 1993).

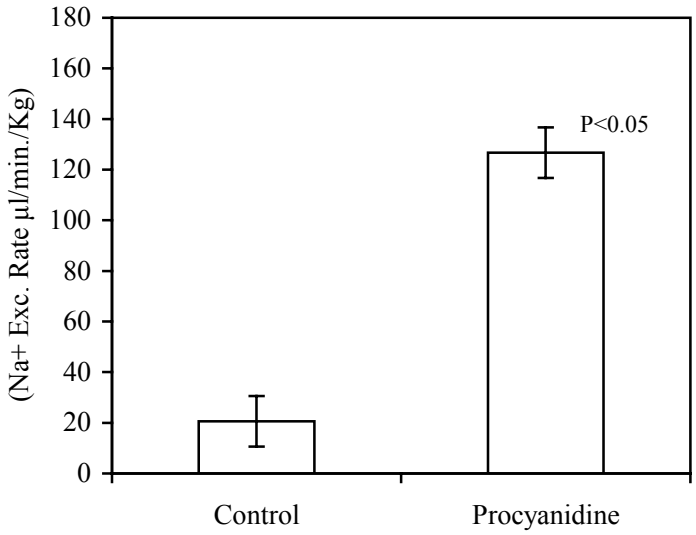

Fig.15: The effects of intravenous injection of 16mg/kg of procyanidine on the sodium excretion rate of rats (n=6).

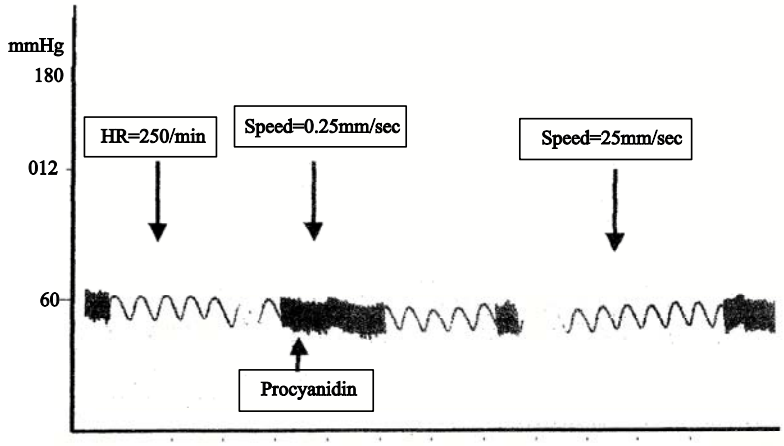

Fig. 16: The effects of 16mg/kg IV injection of procyanidine on the blood pressure and heart rate of the rat (n=6).

Effects of procyanidine on the isolated atrium and papillary muscle of the rabbit:

From our results we found that procyanidine has quite clear positive inotropic effect and its potency and its efficacy looks like the positive inotropic effects of cardiac glycoside and adrenaline. Its positive inotropic effects is not due to activation of Beta-1 adenoceptor because its effect was not blocked by timolol (a non selective Beta adrenergic blocker) (Schussler *et al.,* 1995). And also this positive inotropic effects of procyanidine is not due to the increase in the intracellular levels of cAMP or phosphodiesterase inhibition (Muller *et al.,* 1999; Schwinger *et al.,* 2000). Therefore, the most probable mechanism of action of procyanidine as a positive inotropic drug may contribute to the antagonism of adenosine Al receptor present mainly in the heart, kidney and brain, in which

Table-8
The effects of 50mg of procyanidine on the urine output and urine electrolyte of healthy volunteers (n=6)

Parameters	Control	Procyanidine	% Change	Statistical evaluation t-test for paired samples
Urine flow µl/Kg/hr	0.7 ± 0.06	1.48 ± 0.23	111.4	P<0.01
Na^+ Exc. Rate µl/Kg/hr	148.07 ± 14.7	290 ± 48.5	95.9	P<0.02
K^+ Exc. Rate µl/Kg/hr	39.15 ± 17.4	69.25 ± 19.7	67.9	N.S.

N.S. = Non-significant, P<0.02 =Significant, P<0.01 Highly significant

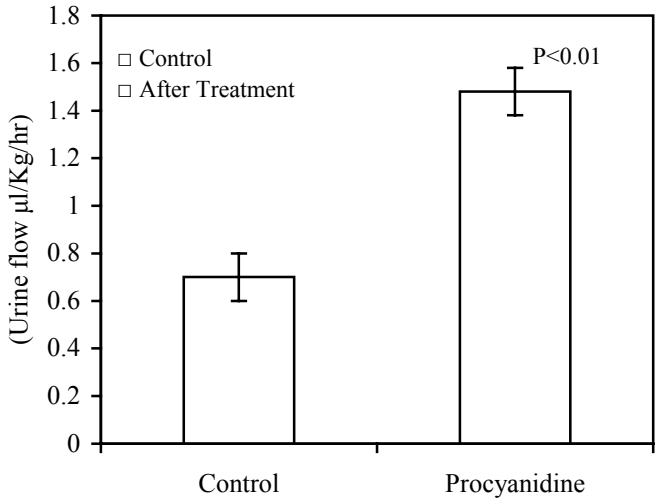

Fig. 17: The effects of 50mg of procyanidine on the urine flow of healthy volunteers (n=6).

activation of this receptor by adenosine leads to negative inotropic activity (Burnstock *et al.,* 1998).

In vivo studies:

Patho-toxicological studies of procyanidine on the mice:

It was clear that procyanidine has no high toxic activity. Its LD_{50} was about 100mg/kg which indicated that this substance is safe drug with large therapeutic index (Haddad and Winchester, 1990). In addition to that, and from histopathological studies, there was no clear evidence that this drug has any histopathological abnormality in the liver and kidney of the experimental mice (Al-Talabani *et al.,* 1990).

Effects of procyanidine on the kidney function of the rat:

Data from Table-7 and Figs. 4 and 5 clearly show that intravenouse injection of procyanidine induced statistically significant (P<0.05) rise in the urine flow and marked and

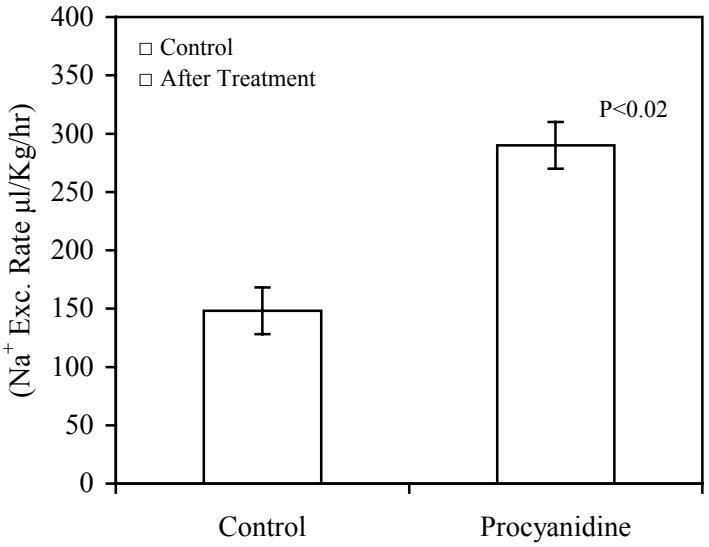

Fig. 18: The effects of 50mg of procyanidine on the sodium excretion rate of healthy volunteers (n=6).

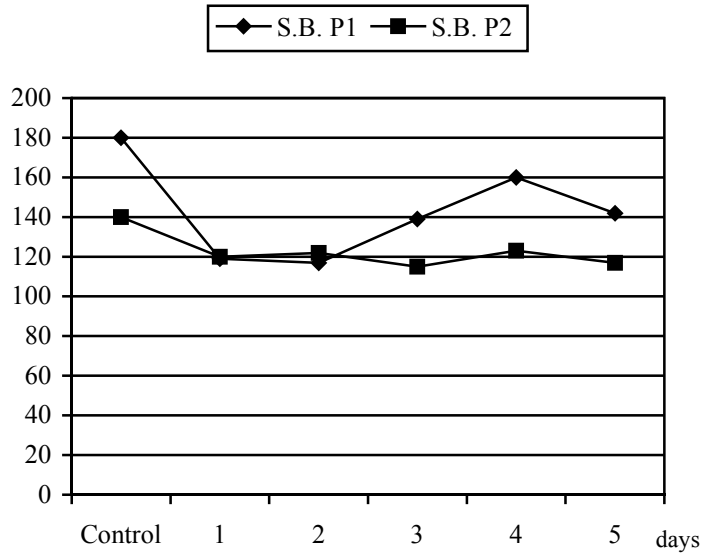

Fig. 19: Daily mean systolic blood pressure of:
♦ - Patients with heart failure treated without procyanidine (n=4).
■ - Patients with heart failure treated with procyanidine (n=3).

significant increase in sodium excretion rate ($P<0.05$), which devoid from any significant increase or decrease in the excretions of potassium, which makes this drug superior to the other diuretic drugs as thiazides, loop diuretics and Potassium sparing diuretics

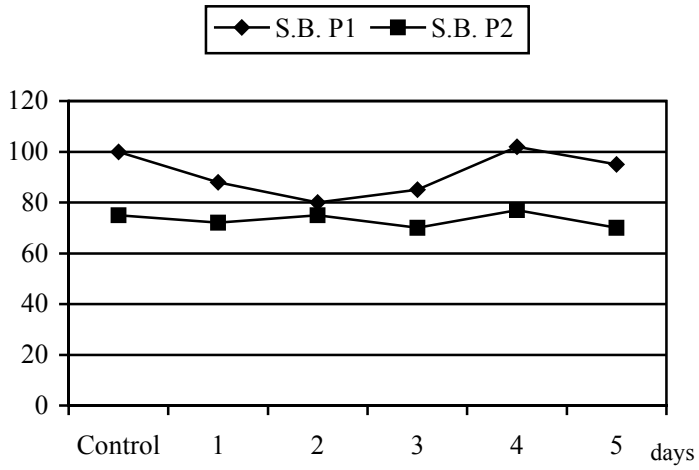

Fig. 20: Daily mean systolic blood pressure of:
♦ - Patients with heart failure treated without procyanidine (n=4).
■ - Patients with heart failure treated with procyanidine (n=3).

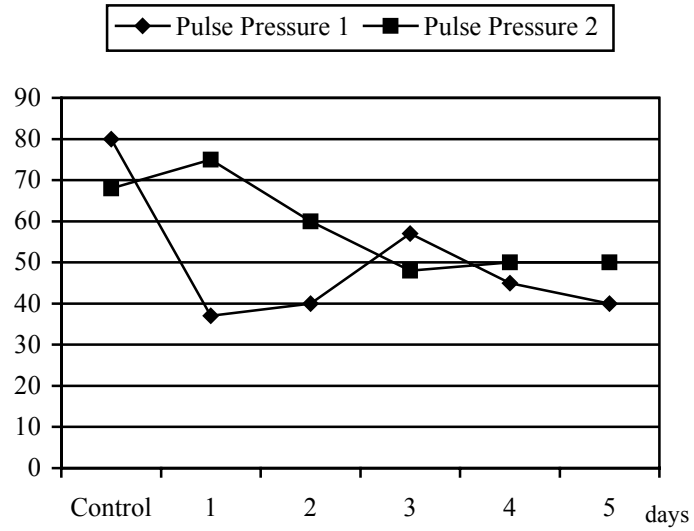

Fig. 21: Daily mean systolic blood pressure of:
♦ - Patients with heart failure treated without procyanidine (n=4).
■ - Patients with heart failure treated with procyanidine (n=3).

(Stein *et al.,* 1972). And this is important and encouraging looking for a new diuretic drug which does not has hypokalaemic or hyperkalaemic activity (Hollenberg and Mickiewice, 1989) so, there is no need to add potassium supplements or using potassium sparing diuretics (Further studies need to be done to prove this phenomena of diuretic effects).

These actions of procyanidine may attribute to its antagonistic effects on

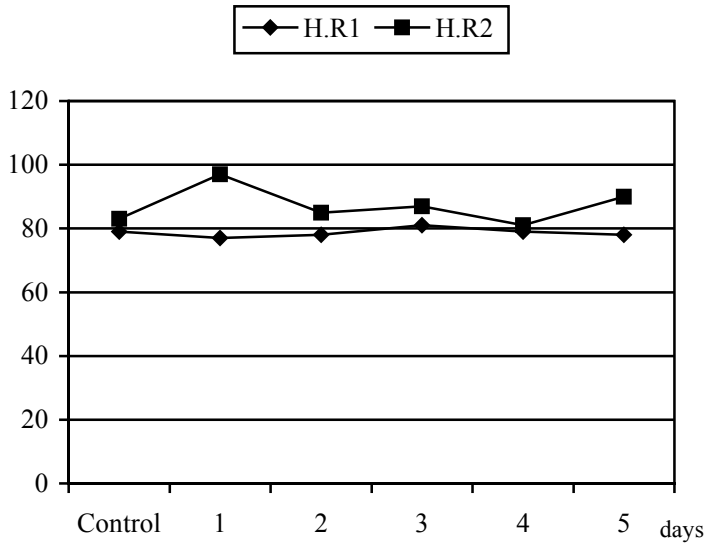

Fig. 22: Daily mean systolic blood pressure of:
♦ - Patients with heart failure treated without procyanidine (n=4).
■ - Patients with heart failure treated with procyanidine (n=3).

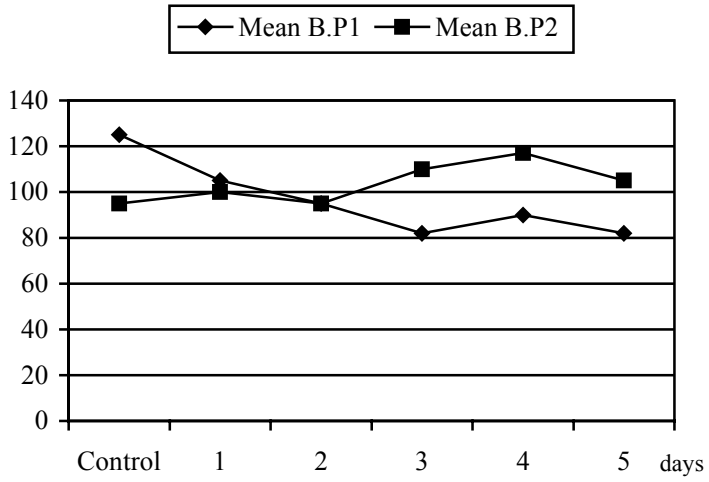

Fig. 23: Daily mean systolic blood pressure of:
♦ - Patients with heart failure treated without procyanidine (n=4).
■ - Patients with heart failure treated with procyanidine (n=3).

Adenosine Al receptor since adenosine plays a comparable role in renal regulation of fluid homeostasis in normotensive rats (Kost *et al.*, 1998).

The actions of procyanidine as a diuretic

drug, looks like the diuretic and natriuretic effects of selective A_1 antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) and CVT-124 drugs which produce diuresis and natriuresis without significantly increasing potassium excretion (Balakrishnan *et al.,* 1993; Gellai *et al.,* 1998 and Curtis *et al*., 2000).

On the other hand, we observed from our studies that, Procyanidine as a diuretic drug has a prolonged duration of action, which may be attributed to the metabolism of procyanidine to an active metabolite (Melmon *et al.,* 1992).

Effects of procyanidine on the blood pressure and heart rate of the rats:

In spite of its diuretic effects, procyanidine did not cause significant changes in the ABP of the experimental rats (Table-7 and Fig. 36). This effect was similar to the actions of FK-453, a non xanthine, selective adenosine A1-receptor antagonist which produces a significant increases in urine flow rate without any changes in the mean arterial blood pressure in healthy volunteers (Balakrishnan *et al.,* 1993). However arterial blood pressure effect of procyanidine was not agreement with (Rewerski, 1971) and (Cheng, 1993), who suggested that procyanidine isolated from crataegus Oxyacantha lowered mean blood pressure of cats from 160 to 110 mm/Hg and from (SHR) in a dose-dependent manner. This may be attributed to the fact that, this drug, and like some other drugs, do not reduce mean blood pressure in normotensive subjects.

Unlike to the other cardiotonic agents, Procyanidine did not cause any marked changes in the heart of the experimental rats. This was in agreement with (Josef *et al.,* 1995) who used guinea pig hearts to compare the influence of Crataegus extract with that of other inotropic drugs such as epinephrine, mirlinon and digoxin. They reported that all of the drugs except, Crataegus extract, shortened the effective refractory period in a concentration dependant manner.

Effects of procyanidine on the kidney function of the healthy volunteers:

From the experiments on the human being we found that, procyanidine induced significant diuresis and natriuresis in healthy volunteers which was similar to that produced with our experimental rats (Figs. 14 & 15 and Table 7).

Effects of procyanidine on the patient with heart failure:

The administration of Procyanidine to patients with heart failure needs their approval and in most cases it was difficult to persuade them to take the medication. Only 7 patients accepted to take new treatment for five days. Therefore the number of patients, statistically, was not acceptable and the duration of the treatment was very short to observe the optimum effects of procyanidine.

Fig. 19 shows that there are a reduction in the systolic blood pressure in both models of treatment but it is more marked in the classical treatment of heart failure as compared to the second model. This may be attributed to the inotropic effects of procyanidine which indirectly, lead to the rise in systolic blood pressure (Reynolds *et al.,* 1997).

However, diastolic blood pressure did not change in both models this indicate that the two models had no influence on the peripheral resistance and their vasodilation effects were not remarkable (Laurance, 1990). Pulse pressure and mean blood pressure were reduced in classical model more than the other model because of the greater effects of classical treatment on the systolic blood pressure (Figs. 21 & 22).

It was quiet clear that procyanidine had no effects on the heart rate of patients with heart failure (Fig. 23) this was agreement with our experiments on the heart rates of the rats (Josef *et al.,* 1995).

CONCLUSION AND RECOMMENDATION

Conclusion

In view of the results of the present study and their interpretations, and indications, the following conclusions are drawn:

1. Large quantity of procyanidine can be obtained from cheap a resource (Crataegus Azarolus), which is found in great quantity in Iraqi Kurdistan and can be exported to all over the world for good of humanity.
2. Procyanidine has quiet clear positive inotropic effects in which its efficacy looks like digoxin.
3. Unlike digoxin, Procyanidine has no any arrhythmic effects.
4. Procyanidine has a good diuretic and natriuretic effects without any significant effects on the excretion of Potassium.
5. The positive inotropic and diuretic actions of procynidine most probably, is related to its antagonistic effects on the adenosine (Al) receptors.
6. Procyanidine has no agonist and antagonistic activity on the muscarine (M1), histamine (H1) and adrenergic receptors.
7. Unlike aminophylline, Procyanidine has no antagonistic actions on the adenosine (A2) receptor. So it has no role in treatment of bronchial asthma.
8. Procyanidine has large therapeutic index, without any significant toxicity.
9. Procyanidine has both positive inotropic and diuretic effects, so it can be considered as a novel drug for the treatment of heart failure.

Recommendations

Further studies are recommended as follows:

1. Estimation and identification of the pharmacokinetic parameters of procyanidine as absorption, distribution, biotransformation and excretion.
2. Determination of half-life and the bioavailability of procyanidine.
3. Assay the administration of procyanidine in a large population of patients with heart failure.
4. Study of know-How of procyanidine as a drug.

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