Journal of Medicinal Plants Research Vol. 6(7), pp. 1361-1366, 23 February, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1558
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Evaluation of prunes for hypotensive, angiotensin converting enzyme (ACE) inhibitory and diuretic activities in rats

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Accepted 22 December, 2011

Prunes are dried fruits of several species of Prunus, including that of *Prunus domestica* Linn (Rosaceae) and are used for the treatment of hypertension in traditional systems of medicine in India and Pakistan. We investigated the blood pressure lowering, angiotensin converting enzyme (ACE) inhibitory and diuretic activities of the aqueous-methanolic crude extract of dried fruits of *P. domestica* (Pd.Cr.) in rats. Intravenous administration of the Pd Cr decreased mean arterial blood pressure, systolic blood pressure (SBP) and diastolic blood pressure (DBP) of the ketamine-diazepam anesthetized normotensive rats dose-dependently, at the dose range of 1 to 30 mg/kg. *In vitro*, the extract was fond to have serum ACE inhibitory activity, with IC_{50} value of 1.102 mg/ml. The extract did not increase urine output in rats. The study concludes that the crude extract of prunes has hypotensive and ACE inhibitory activities, which provides scientific justification for its traditional uses as cardioprotective and antihypertensive remedy.

Key words: Prunes, *Prunus domestica,* hypotensive, angiotensin converting enzyme (ACE) inhibition, diuretic.

INTRODUCTION

High blood pressure is the leading preventable cause of cardiovascular diseases such as stroke, myocardial infarction, left ventricular hypertrophy, nephropathy, retinopathy and dementia. In middle and old age groups, every 20 mmHg of systolic or 10 mmHg diastolic increase in blood pressure above 115 mmHg/75 mmHg results doubling of mortality from ischemic heart disease and stroke (Lewington et al., 2002). Plants have been used for prevention and treatment of various diseases since ancient times. About 80% of the developing World's population is relying on traditional medicines especially plant drugs because of their easy accessibility and affordability. In developed countries, use of different plants in the form of botanical drugs, nutraceuticals, dietary supplements, functional foods and medicinal foods is also increasing (Raskin et al., 2002). Prunes are considered as healthy food because of lower fat contents and contain considerable amount of important nutrients like carbohydrates, vitamins and minerals.

Traditionally prunes are used in blood circulation problems, measles, digestive problems, diabetes and obesity. Scientific studies proved that plums and prunes have antioxidant, anticancer, antihyperlipidemic, anxiolytic activities and prevent osteoporosis (Jabeen and Aslam, 2011). Prunes are used for the treatment of hypertension in Unani system of treatment since long (Usmanghani et al., 1997). To support its ethnic use in the treatment of hypertension, only one scientific study is available which reported that chronic administration of prunes in stroke prone spontaneously hypertensive rats (SHRSP) prevented elevation of blood pressure and caffeic acid, which is an important polyphenol present in prunes, decreased reactive oxygen species in angiotensin-II treated vascular smooth muscle cells obtained from SHRSP (Negishi et al., 2007). Purpose of

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the present study was to investigate the effects of aqueous-methanolic extract of prunes on blood pressure of normotensive rats and to explore the possibility of involvement of angiotensin converting enzyme (ACE) inhibitory and diuretic activities for its blood pressure lowering effects.

MATERIALS AND METHODS

Animals

Sprague-Dawley rats of either sex weighing between 250 to 300 g were used in the experiments. Animals were housed at the animal house of the Department of Pharmacy, the Islamia University of Bahawalpur, Pakistan, kept in housing cages with saw dust (renewed after every 48 h), maintained at temperature of 25 ± 3°C and exposed to 12 h light/12 h dark cycles. The animals had free access to water and a standard diet. The “Guide for the care and use of laboratory animals” was followed in this study (Institute of Laboratory Animal Research, Commission on Life Sciences, NRC, 1996).

Plant material

Prunes were purchased from a reputed local herbal shop, that is, Shadab Dawakhana, Shahi Bazar, Bahawalpur, Pakistan. The plant material was identified and authenticated by Mr. Abdul Hameed, Botanist, Cholistan Institute of Desert Studies, The Islamia University of Bahawalpur as dried fruits of *Prunus domestica Linn* subsp. *Insitita* (family Rosaceae). A sample of the plant material was deposited in the herbarium of the Pharmacology Section, Faculty of Pharmacy and Alternative Medicine, the Islamia University of Bahawalpur and voucher No. PD-FT-03-10-003 was assigned to it.

Chemicals

Acetylcholine, cyanuric chloride, dimethyl sulphoxide (DMSO) and hippuryl-L-histidyl-L-leucine (HHL) were purchased from Sigma-Aldrich, USA. Folin-Ciocaltue reagent was purchased from Merck, Germany. Ketamine injections (Ketalar®, Akahai Pharmaceuticals, Karachi), diazepam injections (Valium®, Roche Pharmaceuticals, Karachi), adrenaline injections (PDH Pharmaceuticals, Lahore), heparin injections (Heparol®-5000, China), captopril (Bristol-Myers Squibb, Pakistan) and frusemide injections (Lasix®, Sanofi-Aventis, Karachi) were used in the study. All other chemicals/solvents were of analytical grade.

Extraction procedure

The pericarp of the dried fruits was scraped with help of a sharp knife. Approximately 572 g of the eatable portion of the prunes was soaked in aqueous-methanol (30:70) at room temperature with occasional stirring for three days. It was filtered through muslin cloth and then through Whatman grade 1 filter paper. The procedure of soaking and filtration was repeated with the residue using fresh solvent for two more times.

All the three filtrates were combined and evaporated on rotary evaporator (Heidolph Laborota-efficient-4000, Germany) under reduced pressure at 40°C to a thick, jelly like mass of brown color; that is, the crude extract of dried fruits of *P. domestica* (Pd. Cr.). The yield of the extract was 46.5% (W/W). The extract was solubilized in normal saline for blood pressure and diuretic experiments and in 50% DMSO for ACE inhibitory assay.

Photochemical analysis

Quantitative determination of total phenolic contents was performed by Follin-Ciocaltue method using gallic acid as standard as described by Chang et al. (2001). Preliminary qualitative screening of major secondary metabolites for presence of alkaloids, saponins, flavanoids, tannins, anthraquinones, cyanogenic glycosides and coumarins was conducted by standard methods already described by various authors (Tanira et al., 1996; Francisco and Pinotti, 2000; Awoyinka et. al., 2007).

Blood pressure measurements in anesthetized rats

The blood pressure (BP) of the anesthetized rats was recorded by method described by Gilani et al. (2008) with some modifications. Animals were anesthetized with intraperitoneal (i.p.) injections of ketamine (50 to 80 mg/kg) and diazepam (5 mg/kg). Animals were fixed in supine position on a dissecting table. Temperature was maintained with the help of an overhead lamp. Trachea, right jugular vein and left carotid artery were exposed by a small mid tracheal incision. The trachea was cannulated with 18 gauge polyethylene tubing to facilitate spontaneous respiration. The right juggler vein was cannulated with polyethylene tubing PE-50 (outer diameter 0.97 mm, internal diameter 0.58 mm) for intravenous injection of drugs and the extract solutions. The left carotid artery was cannulated with polyethylene tubing PE-50 filled with heparinized saline (60 iu/ml) and connected to a pressure transducer (MLT0699 disposable BP transducer, AD Instruments, Australia) filled with the same solution. The pressure transducer was coupled with PowerLab 4/30 and LabChart Pro software (AD Instruments, Australia) for BP and heart rate (HR) recordings.

A system calibration was performed with the help of mercury manometer connected to pressure transducer before the start of first experiment every day. The exposed surface of the cannulation was covered with a piece of cotton swab moistened in warm saline. Heparinized saline (0.1 ml) was injected to cannulated rat to prevent blood clotting. Acetylcholine (1 µg/kg) and adrenaline (1 µg/kg) were used to check the hypotensive and hypertensive responsiveness of each animal before administration of the test substance. After 15 to 20 min of equilibration, 0.1 ml of the extract or drug solution was injected intravenously followed by 0.1 ml of saline flush. BP was allowed to return to the resting level before every next dosing. Pulse pressure was obtained by subtracting DBP from SBP. Mean arterial blood pressure (MABP) was determined by adding values of DBP and one-third of pulse width. Change in blood pressure was expressed as mean ± SEM of control values obtained immediately before administration of each dose of the test substance.

Angiotensin converting enzyme (ACE) inhibitory assay

The activity of serum ACE was determined using hippuryl-L-histidyl-L-leucine (HHL) as the substrate. The enzyme hydrolyzes this molecule to give hippurate in the presence of boric acid/NaOH buffer containing NaCl. The final concentrations in the incubation mixture were 80 mmol/L boric acid (adjusted to pH 8.3), 800 mmol/L NaCl and 4 mmol/L of HHL. The librated hippurate was reacted with the colour reagent; that is, cyanuric chloride/ dioxin (9 g/L) in the presence of phosphate buffer (200 mmol/L, pH 8.3) to yield a chromogen, which was quantified from its absorbance at 382 nm (Hurst and Lowell-Smith, 1981). Briefly, 0.1 ml of borate buffer was mixed with 0.05 ml of the extract solution and 0.05 ml of rat serum as source of ACE. After incubation at 37°C for 10 min, 0.05 ml of HHL solution (20 mM) warmed at 37°C was added to the reaction mixture and the reaction was allowed to proceed for 60 min at 37°C.

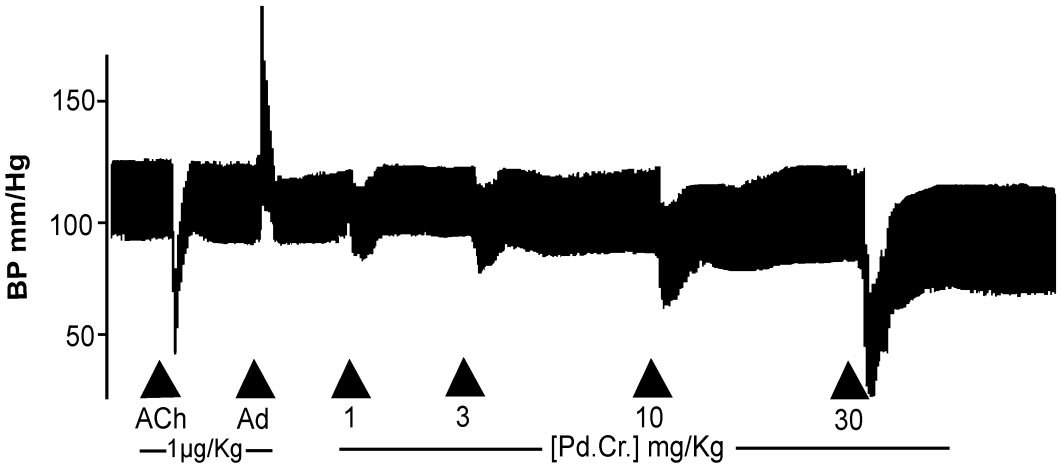

Figure 1. A typical tracing showing effects of acetylcholine (ACh), adrenaline (Ad) and different doses of the aqueous-methanolic extract of prunes (Pd.Cr.) on blood pressure of an anesthetized rat. Triangles indicate the time of administration of drugs.

The reaction was terminated with 0.25 ml of 1 M HCl solution and 30 s later neutralized with same volume of 1 M NaOH solution. Then 1 ml of phosphate buffer and 0.75 ml of colour reagent were added, followed by mixing vigorously using vortex mixer (SeouLin Bioscience, Korea) for 30 s, in bursts of 5 to 10 s, allowed to stand for 5 min, vortex-mixed again, and then centrifuged at 3000 rpm for 10 min in a bench centrifuge to remove denatured proteins and excess cyanuric chloride. Absorbance of the clear supernatant solution was measured by spectrophotometer (Model U2020, IRMECO Germany).

For positive control, 50% DMSO was used instead of extract solution and for negative control; terminating and neutralizing solution were added after the serum before substrate. Captopril (2 µM) was used as standard ACE inhibitor. All determinations were performed in triplicate. The percentage inhibition was calculated by using the formula: ACE Inhibition (%) = [($A_{positive\ control}$ - A_{sample}) × 100] / [($A_{positive\ control}$ -$A_{negative\ control}$)], where "A" is absorbance of respective solution at 382 nm.

Diuretic assay

Rats were randomly assigned into five groups of five animals each. The control received normal saline (10 ml/kg, i.p.). Another group of animals was given frusemide (10 mg/kg, i.p.) as standard diuretic. The other groups of animals were injected with different doses of extract, Immediately after dosing, animals were individually housed in metabolic cages (Techniplast, Italy) and their urine collected for 6 h after the drug administration (Jabeen et al., 2009). After collection, total urine volume was measured and calculated. Na^+ and K^+ concentrations were measured by using clinical flame photometer (Model 410C, Sherwood, UK).

Statistical analysis

The results were analyzed statistically using software GraphPad Prism 5.01. The data was expressed as mean ± SEM. IC_{50} was calculated by nonlinear curve fitting. Student's *t*-test was used to compare an experimental group with saline treated group in diuretic assay. The values at $p<0.05$ were regarded as statistically significant.

RESULTS AND DISCUSSION

Phytochemical analysis

The aqueous-methanolic extract of *P. domestica* dried fruits (Pd.Cr.) was positive for flavanoids and tannins but was negative for alkaloids, saponins, anthraquanones and cyanogenic glycosides. Total phenolic contents as determined by Follin-Ciocaltue method were 18.2 ± 0.61 mg of gallic acid equivalent (GAE) per gram of the extract, which is in agreement with the earlier studies suggesting prunes as rich source of dietary polyphenols (Wu et al., 2004). Short term oral administration of polyphenols has been shown to decrease blood pressure in rats via improvement in endothelium dependant vasodilatation (Diebolt et al., 2001). Polyphenols are widely distributed in plant kingdom and regular consumption of polyphenol rich fruits, vegetables and beverages is associated with prevention of cardiovascular diseases, cancers, osteoporosis, neurodegenerative diseases and diabetes due to their ability to decrease oxidative stress involved in pathogenesis of such chronic disease (Pandey and Riazi, 2009).

Hypotensive effect in anesthetized rats

The intravenous administration of the crude extract of *P. domestica* (Pd.Cr.) decreased SBP, DBP and MABP in normotensive anesthetized rats. The hypotensive response was dose dependant and was mediated at the dose range of 1 to 30 mg/kg. Figure 1 shows a representative tracing of an experiment and the combined results of different experiments are plotted in Figure 2. At the dose of 1.0 mg/kg, the Pd.Cr.

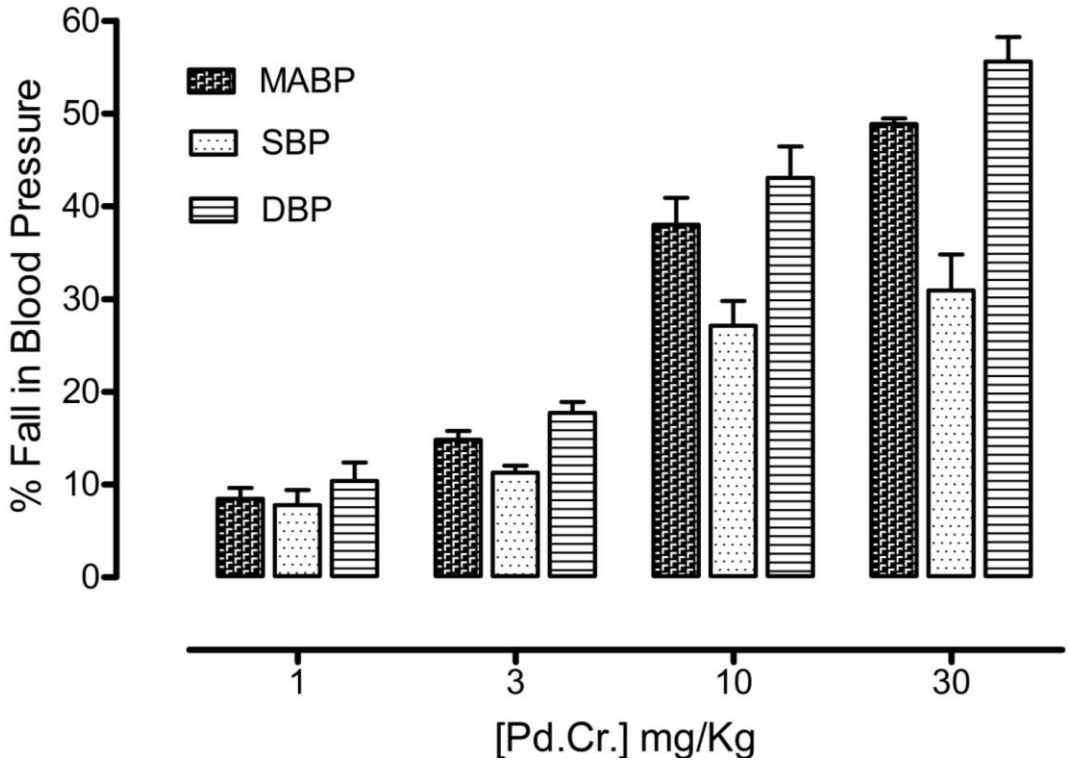

Figure 2. Bar-diagrams showing the effects of aqueous-methanolic extract of prunes (Pd.Cr.) on mean arterial blood pressure (MABP), systolic blood pressure (SBP) and diastolic blood pressure (DBP) of anesthetized rats. The values shown are mean ± SEM of three to four determinations

produced 8.57 ± 1.06% (n = 4) fall in MABP; at 3.0 mg/kg, it produced 14.83 ± 0.93% (n = 4) fall in MABP; at 10.0 mg/kg, it produced 38.03 ± 2.89% (n = 4) fall and at the dose of 30.0 mg/kg, it produced 48.87 ± 0.61% (n = 3) fall in MABP. The extract was found to decrease both systolic and diastolic blood pressures. The extract caused 7.80 ± 17.60%, 11.23 ± 1.02%, 29.80 ± 3.78% and 32.40 ± 7.48% fall in SBP and 10.40 ± 1.97%, 17.73± 1.15%, 43.05 ± 3.40% and 55.70 ± 3.70% fall in DBP at the doses of 1.0, 3.0, 10.0 and 30.0 mg/kg, respectively. The decrease in DBP was more than that of SBP. Saleem et al. (2004) demonstrated that intravenous administration of citric acid and malic acid has hypotensive effects in anesthetized rats. Chlorogenic acids have also been shown to decrease blood pressure in spontaneously hypertensive rats (Mishima et al., 2005; Suzuki et al., 2002). Prunes have been reported to contain citric acid, malic acid and chlorogenic acids (Jabeen and Aslam, 2011). These phytochemicals, at least in-part, may be responsible for observed hypotensive effect the of *P. domestica* extract.

ACE inhibition

ACE is an important enzyme involved in pathogenesis of hypertension. The Pd.Cr. was tested for ACE inhibitory activity at different concentrations. Captopril (2 µM), used as standard ACE inhibitor, was found to inhibit serum ACE activity by 92.00 ± 1.52 %. The Pd. Cr. showed 8.90 ± 2.15%, 16.44 ± 3.6%, 25.67 ± 2.99%, 40.50 ± 3.77%, 73.43 ± 4.99%, 83.67 ± 3.71% and 90.47 ± 2.82% inhibition of ACE activity at doses of 0.01, 0.025, 0.5, 1.0, 2.0, 5.0 and 50.0 mg/ml, respectively. The percentage inhibition of serum ACE activity by the Pd.Cr. is plotted in Figure 3. The IC_{50} of the the Pd.Cr. was found to be 1.102 mg/ml. Several earlier studies have demonstrated ACE inhibitory activity of edibles; for example, regular consumption of pomegranate juice for two weeks has been found to decrease blood pressure in hypertensive individuals. In these patients, *in vivo* ACE activity was found to be decreased by 36% and *in vitro*, the juice inhibited serum ACE activity by 31% (Aviram and Dornfeld, 2001). Aqueous and ethanolic extracts of kiwifruit were reported to inhibit ACE activity by 50% at concentration of 50 mg/ml (Jung et al., 2005). In our study, the same concentration of the Pd.Cr. produced almost double ACE inhibitory activity, suggesting that regular consumption of prunes in diet may also be beneficial in the treatment of hypertension. The ACE inhibitor activity of the Pd.Cr. may be due to its chemical constituents, such as flavonoids and tannins as these

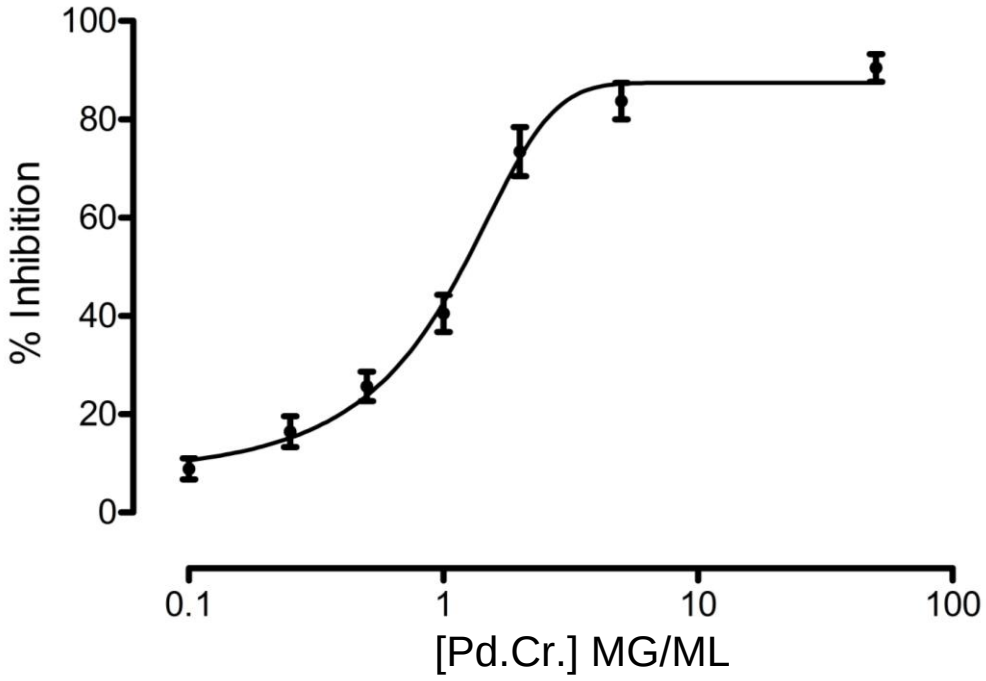

Figure 3. Concentration-dependant change in ACE inhibitory activity of the aqueous-methanolic extract of prunes (Pd.Cr.). The values shown are mean ± SEM of three determinations.

Table 1. Effect of intraperitoneal administration of normal saline, frusemide and crude extract of prunes (Pd.Cr.) on urine output and urinary concentration of electrolytes in Sprague-Dawley rats.

Group	Urine volume (ml/ 100 gm/ 6 h)	Sodium (mmol/L)	Potassium (mmol/L)
Normal saline (10 ml/kg)	1.19 ± 0.34	49.67 ± 3.57	12.22 ± 0.77
Frusemide (10 mg/kg)	4.27 ± 0.047***	107.50 ± 4.31***	26.17 ± 2.57***
Pd.Cr. (100 mg/kg)	1.40 ± 0.23	47.50 ± 3.33	13.48 ± 1.48
Pd.Cr. (300 mg/kg)	1.56 ± 0.31	52.50 ± 3.36	15.07 ± 1.08

The values are mean ± SEM of five determinations. *** $p < 0.001$ compared to normal saline group.

constituents from different sources have been found to inhibit ACE (Liu et al., 2003; Loizzo et al., 2007).

Diuretic activity

The crude extract of prunes was also studied for its diuretic activity. As detailed in Table 1, the volume of urine excreted in rats per 100 g of the body weight in 6 h as well as Na^+ and K^+ concentrations in urine of the frusemide treated group (10 mg/kg) were significantly greater than the normal saline treated control group ($p < 0.001$). But, the Pd.Cr. did not significantly increase the urine and urinary electrolytes output at the doses of 100 and 300 mg/kg compared to the saline treated group. Therefore, we suggest that the Pd.Cr. does not processes diuretic activity.

Conclusion

The results of this study show that intravenous administration of the crude extract of *P. domestica* has dose-dependant blood pressure lowering effect in rats. The extract also inhibited serum ACE activity, *in vitro*, with IC_{50} value of 1.102 mg/ml. The present study justifies the traditional use of prunes as antihypertensive and cardioprotective medicinal food.

ACKNOWLEDGEMENTS

Authors wish to thank Professor Dr. Khalid Husssain Janbaz, Chairman Department of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan for allowing us to use the facility of PowerLab in his research Lab and Mr. Abdul

Hameed, Botanist, Cholistan Institute of Desert Studies, the Islaimia University of Bahawalpur for identification of the plant material.

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