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Cardioprotective and antioxidant effects of *Opuntia dillenii* haw fruit on Isoprenaline hydrochloride induced myocardial infarction in rats

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ABSTRACT

The aim of the present study is to investigate the protective effect of *Opuntia dillenii (*ker-gawl) Haw fruit extract against isoprenaline hydrochloride (ISPH) induced myocardial infarction (MI) in rats. Pretreatment with the fruit extract of *Opuntia dillenii* at 2.5 and 5.0 ml/kg body weight for 30 days significantly prevented the elevation of serum marker enzymes namely SGOT, SGPT, lactate dehydrogenase (LDH), troponin and increased the cardiac enzymatic antioxidant, TBARS and lipid profile levels in myocardial injured rats. The effect was more prominent at 5.0 ml/kg body weight. The study results thus demonstrated the cardioprotective potential of *Opuntia dillenii* against ISPH-induced myocardial infarction and associated oxidative stress.

Introduction

Myocardial infarction is a clinical syndrome arising from sudden and persistent curtailment of myocardial blood supply which results in the necrosis of the myocardium (Anversa and Sonnerblick, 1990). It has also been suggested that heart failure subsequent to myocardial infarction may be associated with antioxidant deficit as well as increased myocardial oxidative stress (Hill and Singal, 1996). It is a complex phenomenon affecting the mechanical, electrical, structural and biochemical properties of the heart (Upaganlawar and Balaraman, 2010).

Opuntia dillenii (Ker–Gawl) Haw. (Cactaceae), commonly known as pear bush, prickly pear, malrachette or tuna, is a succulent shrub growing in semi desert regions in the tropics and subtropics (Liu et al., 2009). It is widely distributed throughout India. These plants contain high levels of important nutrients, such as polysaccharides, betalains, phenolic compounds, organic acids, lipids, minerals, vitamins and amino acids, including taurine. Its reddish fruit is commonly used as a colouring agent for foods, drinks and ice cream (Chang et al., 2008). This paper therefore, reports the protective effect of Opuntia dillenii pretreatment on ISPH-induced myocardial injury with reference to serum cardiac marker enzymes, antioxidant parameters and lipid profile.

Materials and methods

Chemicals

Isoprenaline hydrochloride was obtained from sigma chemicals. All other chemicals obtained from local sources and were of analytical grade.

Animals used

Male albino rats of Wistar strain with a mean weight of 100 to 150g were obtained from the small animal's breeding center, Mannuthy, Thrissur, Kerala, India were used for the study. The animals were maintained under standard conditions of humidity, temperature (25±2°C) and light (12hrs light/dark). They were acclimatized to animal house conditions and were fed on a commercial pelleted rats chow (AVM cattle feeds, Coimbatore, Tamilnadu) and water ad libitum.

Extract preparation

Fresh ripe fruits of Opuntia dillenii were collected during the month of December from Kondichettipatty, Namakkal (District), Tamilnadu, India. The fruit was authenticated by Taxonomist Dr.K.Arumugasamy, Associate professor, Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu. Opuntia dillenii haw ripe fruits were extracted within two days after collection. The fruits were peeled (free from cuticle and epidermis) and the pulp was separated from seeds and weighed 1000g of samples were blend in mixer grinder without using water. The palatable dense red juice carefully filtered and then stored at -4°C and it's for further analysis.

Treatment schedule

Myocardial infarction was induced in experimental rats by intraperitoneally (i.p.) injecting with Isoprenaline hydrochloride (11mg dissolved in physiological saline/100g body weight/ day), for two consecutive days (Ganesan et al., 2010).

Experimental procedure

Thrity male rats were completely randomized into five groups of six animals. Group 1: Normal control (distilled water p.o.), Group 2: Isoprenaline hydrochloride (11mg dissolved in physiological saline /100 g body weight /day), i.p. for two consecutive days for the induction of myocardial infarction (Ganesan et al., 2010). Groups 3 and 4 were administered with 2.5 and 5.0ml/kg body weight/day p.o. of Opuntia dillenii fruit extract, respectively for 30 days followed by ISPH (11mg dissolved in physiological saline /100g body weight/day) at an interval of 24h for two days. Group 5 were administered with 5.0ml/kg body weight/ day p.o. of Opuntia dillenii fruit extract alone for a period of 30 days.

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Collection of serum and preparation of tissue

After the end of experimental regimen, the rats were sacrificed by mild chloroform anaesthetization and the heart was dissected out. The neck area was quickly cleared of fur to expose the jugular vein. The vein, after being slightly displaced, was sharply cut with sterile surgical blade and an aliquot (5 ml) of the blood was collected and centrifuged at 2500rpm. The serum was collected and diluted in the ratio of 1:10 with saline. Aliquots of the diluted serum were used for the estimation of biochemical analysis. The heart was immediately washed with ice cold 0.9% saline and homogenate was prepared in 0.1 N Tris HCl buffer (pH 7.4). The homogenate was centrifuged and the clear supernatant was collected for the assay.

Analytical procedure

The serum was used for the estimation of cardiac marker enzymes namely serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) (Reitman and Frankel, 1957), lactate dehydrogenase (LDH) (King, 1965) and troponin. Thiobarbituric acid reactive substances (TBARS) (Naehius and Samuelson, 1968), superoxide dismutase (SOD) (Das, 2000), Catalase (Sinha, 1972), glutathione peroxidase (GPX) (Rotruck et al., 1973).

Statistical analysis

Results are expressed as mean ± SD. Multiple comparisons of the significant ANOVA were performed by Duncan's multiple comparison tests. A P value <0.05 was considered as statistically significant. All data were analyzed with the aid of statistical package SPSS 17 version.

Results

Several enzymes in cardiac tissue have long been considered as effective biochemical markers to understand the early injury. In the present study, the levels of transaminases (SGOT & SGPT) and Lactate dehydrogenase (LDH) in normal and experimental rats are listed in Table 1. Isoprenaline hydrochloride treated rats showed significantly (P<0.05) elevated levels of these marker enzymes as compared to control rats. Pretreatment with Opuntia dillenii (2.5ml and 5.0ml / kg body weight) showed a significant (p<0.05) reduction in the activities of all serum cardiac marker enzymes when compared with ISPH treated rats in dose dependent manner. The normal rats receiving Opuntia dillenii alone (5.0ml / kg body weight) did not show any significant change when compared with normal rats, indicating that it does not have any adverse effects.

Cardiac marker enzymes in serum

The cardiac marker enzymes such as aspartate transaminase (AST), alanine transaminase (ALT) and lactate dehydrogenase (LDH) activities in serum of control and experimental rats are presented in the table1.

Table 2 shows the levels of serum cTnT in normal and experimental rats. Rats treated with ISPH-showed a significant (P < 0.05) increase in the levels of cTnT in serum when compared to normal control rats. Pretreatment with *Opuntia dillenii* (2.5 and 5.0ml/kg, respectively) significantly (P<0.05) decreased the levels of cTnT in serum in ISPH-induced rats when compared with ISPH-alone induced rats. However, *Opuntia dillenii* alone (5.0ml/kgbody weight) treated rats did not show any significant change in the level of troponin when compared to control rats.

Cardiac marker - troponin

The serum cardiac troponin levels in control and experimental rats are represented in table 2.

Table 3 shows content of CAT, SOD and GPx in the heart of control and experimental rats. In ISPH control group, the activities of these enzymes declined significantly (p<0.05) when compared to control rats. Pretreatment with the fruit extract of *Opuntia dillenii* (2.5 and 5.0 ml/kg/ day) dose-dependently increased the content of these antioxidants. The animals which were treated with *Opuntia dillenii* alone showed no significant difference in the antioxidant enzyme activity when compared to control animals (Table 3). Generally, the effect of the fruit extract was more prominent at the higher dose.

Values are expressed as mean ± SD of six animals. Statistical comparisons are as in table 1.Units: 50% inhibition of nitrite formed/ min/ mg protein for SOD; µ moles of H_2O_2 decomposed/ min/ mg protein for CAT; µmoles of glutathione oxidized /min/mg protein for GPX.

Fig 1 shows the activity of MDA level in the heart of control and experimental rats. In ISPH- control rats, the activity of MDA level was increased significantly (p<0.05) when compared to control rats. However, pretreatment with fruit extract of *Opuntia dillenii* at both doses, 2.5 and 5.0 ml/kg body weight significantly (p<0.05) decreased the level of MDA when compared to ISPH- control animals. No significant difference was observed in rats treated with *Opuntia dillenii* alone when compared to normal control rats.

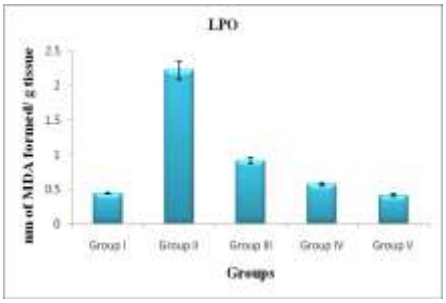

Figure .1. Effect of *Opuntia dillenii* on lipid peroxidation in serum of normal and experimental rats.

The levels of total cholesterol, triglycerides, free fatty acids, phospholipids, HDL, LDL, and VLDL levels in serum of control and experimental groups of rats are shown in Table 4. Rats treated with ISPH showed a significant increase (p<0.05) in these levels with the levels of HDL being an exception where there was a significant decrease. The prior administration of *Opuntia dillenii* significantly decreased the levels of cholesterol, lipoproteins, and lipids with subsequent increase in the levels of HDL cholesterol at close to control levels in the treatment groups (*Opuntia dillenii* 2.5 and 5.0 ml/kg) as compared to group ISPH. In *Opuntia dillenii* alone treated group (5.0 ml/kg) there were no significant alterations in the serum levels of total cholesterol, triglycerides, free fatty acids, phospholipids, HDL, LDL and VLDL in comparison to those of control rats.

Discussion

Many of today's diseases including cardiac diseases have been linked to oxidative stress which is initiated by the reaction of free radicals with biological macromolecules such as proteins, lipids and DNA (Sunmonu, 2010). Generally antioxidants, preferably from natural sources, have been considered as effective treatments (Cunningham, 1998). The present study has clearly demonstrated that the aqueous extract of *Opuntia dillenii* has antioxidant activity which could prevent the occurrence of heart related diseases.

Serum SGOT, SGPT and LDH are well known markers of myocardial infarction. When myocardial cells are damaged or destroyed due to deficient oxygen supply or glucose, the cardiac membrane becomes permeable or may rupture which results in leakage of enzymes. These enzymes enter into the blood stream thus increasing their concentration in the serum (Mathew et al., 1985). Our results showed significant elevation of serum levels

of aspartate transaminase, alanine transaminase, and lactate dehydrogenase in isoprenaline hydrochloride treated rats, which was in line with the previous reports Mali and Bodhankar. (2010).

Activities of these enzymes in serum decreased in *Opuntia dillenii* pretreated group probably due to the protective effect of *Opuntia dillenii* on myocardium, which had reduced the extent of myocardial damage induced by ISPH and thereby restricting the leakage of these enzymes from myocardium.

Cardiac troponin T has been shown to be highly specific and a sensitive marker in the determination of myocardial cell injury. O'Brien et al. (1997) have shown that cTnT is a powerful biomarker in laboratory animals for sensitive and specific detection of cardiac injury arising from various causes. In our study, we have observed an increase in the levels of cTnT in serum of ISPH induced rats. Elevated troponin levels predict the risk of both cardiac death and subsequent infarction. Our results are consistent with a previous report by Acikel et al. (2005). Pretreatment with *Opuntia dillenii* significantly decreased the levels of cTnT in serum of ISPH-induced rats. This could be due to the reduction of the degree of damage in the myocardium by *Opuntia dillenii*.

Lipid peroxide metabolism plays an important role in the pathogenesis of MI. A significant increase in the levels of lipid peroxidation products such as TBARS and LOOH in the heart clearly indicates increased oxidative stress in ISPH induced rats. Alterations in the metabolism of lipid peroxides are closely associated with myocardial damage due to free radicals produced by ISPH (Lekse et al., 2001, Sroka and Cisowski, 2003). Prior treatment with *Opuntia dillenii* decreased the levels of lipid peroxidation products in ISPH induced rats. Thus, *Opuntia dillenii* scavenges the lipid peroxidation products produced excessively by ISPH protected the cardiac tissue because of its anti-lipid peroxidation effect.

Abnormalities in lipid profile are associated with increased risk of myocardial infarction. High level of circulating cholesterol and its accumulation in the heart tissue is usually accompanied by cardiovascular damage (Mediene-Benchekor et al., 2001). In the present study, fruit extract of *Opuntia dillenii* restored the total cholesterol, triglycerides, free fatty acids, phospholipids, LDL, HDL and VLDL level to near normal levels, thereby reducing the risk of cardiovascular disease. This observation is in agreement with the findings of Gunjal et al. (2010) who reported a positive correlation between *Moringo oleifera* extract and cholesterol level.

Antioxidants constitute the foremost defense system that limit the toxicity associated with free radicals. The equilibrium between antioxidants and free radicals is an important process for the effective removal of oxidative stress in intracellular organelles. However, in pathological conditions like MI, the generation of ROS can dramatically upset this balance with an increased demand on the antioxidant defense system. The decreased level of antioxidant enzymes like SOD, CAT and GPX in ISPH induced myocardial infarction is due to the release of free radicals that ultimately damage the myocardial tissue. Pretreatment of MI rats with *Opuntia dillenii* increased the level of antioxidant enzymes suggest the free radical scavenging activity of the fruit extract. Similar observation was reported by Fard et al. (2010) using *Lagenaria siceraria* (Molina) standley fruit juice in ISO treated rats.

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Table 1
Effect of *Opuntia dillenii* on cardiac marker enzymes in serum of normal and experimental rats

Groups	SGOT (IU/L)	SGPT (IU/L)	LDH (IU/L)
Group I	39.46±2.66	32.32±1.74	200.31±1.94
Group II	138.19±7.59a*	82.06±6.59a*	929.54±10.27a*
Group III	85.35±3.48b*	69.55±4.02b*	418.62±20.53b*
Group IV	49.93±2.66c*	41.38±4.38c*	231.81±8.49c*
Group V	38.71±1.00dns	31.96±2.66dns	208.18±6.49dns

Values are expressed as mean ± SD of six animals. Asterisk (*) represents statistical significance
[a] compared between group II and I, [b] compared between group III and II, [c] compared between group IV and II, [d] compared between group V and I. *p<0.05, ns- non significant

Table 2
Effect of *Opuntia dillenii* on troponin in serum of control and experimental rats

Groups	Troponin ng/ml
Group I	0.06±0.01
Group II	2.10±0.04a*
Group III	0.32±0.02b*
Group IV	0.13±0.01c*
Group V	0.03±0.01dns

Values are expressed as mean ± SD of six animals. Statistical comparisons are as in table 1.

Table 3
Effect of *Opuntia dillenii* on enzymatic antioxidant in heart of normal and experimental rats

Groups	SOD	CAT	GPX
Group I	0.26±0.01	20.56±1.01	3.75±0.01
Group II	0.12±0.009a*	10.71±0.72a*	1.81±0.006a*
Group III	0.17±0.009b*	13.86±0.39b*	2.92±0.01b*
Group IV	0.23±0.01c*	19.08±0.68c*	3.55±0.06c*
Group V	0.27±0.01dns	21.38±1.01dns	3.92±0.08dns

Table 4
Effect of *Opuntia dillenii* on lipid profiles in serum of normal and experimental rats

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Free fatty acids (mg/dl)	Phospholipids (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)
Group I	96.56±4.98	47.71±1.95	24.17±1.55	115.15±8.54	51.35±2.65	21.62±0.94	9.52±0.36
Group II	131.54±3.73a*	79.83±4.32a*	48.27±3.54a*	149.25±9.18a*	93.5±5.11a*	11.54±0.43a*	21.45±1.06a*
Group III	120.86±3.52b*	62.07±1.81b*	33.49±2.76b*	126.54±8.25b*	67.64±4.96b*	13.52±0.66b*	16.62±0.63b*
Group IV	99.57±6.96c*	51.08±1.34c*	26.51±1.96c*	119.26±5.45c*	54.89±2.98c*	20.46±1.08c*	10.92±0.79c*
Group V	96.18±2.83dns	46.89±1.06dns	24.11±1.31dns	115.08±8.35dns	51.15±3.26dns	21.22±1.46dns	9.65±0.62dns

Values are expressed as mean ± SD of six animals. Statistical comparisons are as in table 1.