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CARDIOPROTECTIVE EFFECT OF PERGULARIA DAEMIA

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ABSTRACT

The bioactive components and antioxidants in Pergularia Daemia (PD) function as an effective anti-inflammatory agent as reported in our earlier studies. In the present study, the cardio-protective effect of ethanol extract of PD against acute and chronic noise stress was assessed by evaluating the level of stress index viz., plasma corticosterone and the cardiac enzymes viz., serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and lactate dehydrogenase (LDH) in serum which were the indicators of the extent of damage done by the noise stress. The levels of antioxidants in myocardium viz. superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), malonaldialdehyde (MDA) (lipid peroxidation) and vitamin C were also estimated as supporting evidence. It was further confirmed by histological sections of cardiac tissues in albino rats. The results revealed that acute noise stress (45 minutes once) significantly increased plasma corticosterone but did not alter the levels of cardiac enzyme and antioxidant levels. Chronic noise stress (21 days) significantly decreased plasma corticosterone ($p < 0.05$) and antioxidant levels and increased SGOT, SGPT, LDH and MDA levels revealing the damage done to the cardiac tissue. Histological

examination of the cardiac tissue confirmed the damage done by chronic noise exposure through the multiple hemorrhages with infiltration of inflammatory cells. PD extract (200 mg/kg) effectively prevented all these changes in acute and chronic noise stress proving the antistressor and cardio-protective effects of PD.

KEYWORDS: Pergularia Daemia, Cardio-protective effect, Noise stress, Cardiac injury markers, Antioxidants

INTRODUCTION

Though allopathic medicines have gained wide applications in the modern world, herbal plants are still in recognition as folk medicine among wide range of people. About 80% of the people in developing countries rely upon plant-based herbal formulations for their basic health care needs even today and the herbal medicines in the world market was estimated to be of about US $ 60 million.[1] Many herbs are being screened for their medicinal values and curative effects by scientists so that a common man can be benefitted. Pergularia Daemia (PD) is emerging as one of such herbal plant.

Its bioactive components, anti-oxidant properties and anti-inflammatory capability of the ethanol extract of aerial parts of PD were already reported by us.[2, 3] The various parts of the plants viz. leaves, stems, shoots, roots, seeds and fruit have been phytochemically evaluated by Bhaskar and Balakrishnan (2009) for the presence of flavonoids, terpenoids, sterols and cardenolides.[4, 5] The whole plant is used as an effective curative agent for many ailments. Studies were done to show the hepatoprotective capacity[6], antifertility effect[7], antidiabetic effect[8], antibacterial activity[9] and wound healing properties[10] of PD. To the best of our knowledge, the cardio-protective ability of PD against noise stress is not explored so far. The present study was taken up to fill this lacuna.

We are exposed to various types of stress throughout the day. Among all stresses, exposure to the oxidative stress is the common one and it occurs due to excessive production of reactive oxygen species (ROS). Noise is one of such stressors known to induce oxidative stress in animals as well as humans.[11, 12] Noise exposure is a stressful experience for any individual and it is inevitable in many work places like factories, industries, entertainment places and road traffics. Depending upon the duration and intensity of noise exposure, many physiological and psychological changes start appearing in the body which may end up with pathological changes if not taken care in the initial stages.

Noise is known to cause detrimental effects on health, especially on auditory system with hearing loss which was attributed to the over production of ROS.[13] The damaging effects of noise are not limited to the auditory system, but extend to other systems also like cardiorespiratory systems with increased heart rate, myocardial ischemia and increased respiratory rate. It may end up with some psychological problems also.[14, 15] Epidemiologic studies demonstrate that environmental noise is associated with an increased incidence of arterial hypertension, myocardial infarction, and stroke.[16, 14, 15]

The current accepted modern medicine of Allopathy that had been developed gradually over years is concentrating on oxidative stress while developing the medicines. Many antioxidant-based drugs and formulations have been developed in the Indian traditional medicine (Ayurveda) also for the prevention and treatment of oxidative stress. All medical searches are common in one thing i.e., search for a herbal plant product that is rich in antioxidant compounds such as polyphenols, terpenes, flavanoid, tannins and saponins that can be a better choice of medicine as an antistressor.

PD belongs to that category and we have confirmed it by our previous reports.[2, 3] We are moving a step forward by evaluating the cardio-protective activity of PD in an experimental stress model.

MATERIALS AND METHODS

Adult male wistar albino rats weighing 150-180 g were used for the present study. Institutional Animal Ethical Committee (03/007/2014) approved the project. The rats were maintained in standard laboratory conditions with food and water ad libitum. Pure tone noise (sine wave) of fixed frequency (100 dB and 10,000 cps) was produced by functional noise generator, amplified by 40 w amplifier, connected to 2 loudspeakers located 30 cm above the cage and a sound level meter was used to measure the intensity of noise. The rats were divided into 8 groups each containing 6 animals.

Group 1 - Control animals kept in the home cage and not subjected to any experimental procedure.

Group 2 - Experimental animals subjected to acute noise stress for 45 min duration.

Group 3 - Acute noise stress-exposed animals with pretreatment of PD extract at a dose of 200 mg/kg bwt for 7 days and subjected to noise stress for 45 min on the 8th day.

Group 5 - Animal treated with PD extract alone for 7 days

Group 6 - Vehicle control group treated with 5% tween 80 alone for 7 days

Group 7 - Chronic noise stress group exposed to noise stress for 3 hrs/day for 21 days.

Group 7 - Chronic noise stress group treated with PD extract at a dose of 200 mg/kg bwt for 7 days and subjected to noise stress for 3 hrs/day for next 21 days and sacrificed on 28th day.

Group 8 – Animals treated with PD extract for 21 days

Group 9 - Vehicle control group treated with 5% tween 80 for 21 days

In control animals and experimental animals, blood samples were collected at the end of experimental procedures between 9:00 A.M. and 10:00 A.M (in order to avoid the circadian interference) by using the technique of Feldman and Conforti (1980); 3 ml of blood was collected into a heparinized syringe from jugular vein and plasma was separated. Then samples were transferred to micro-centrifuge tubes and centrifuged at 5000 rpm for 20 min at 4° C. The plasma was transferred to a clean micro-centrifuge tubes and stored at −80°C until assayed for plasma corticosterone, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and lactate dehydrogenase (LDH). Plasma corticosterone was assayed by the method of Mattingly et al (1962)[15] SGOT and SGPT were assessed by the method of Reitman and Frankel (1957)[16] and LDH was assessed by King (1965b).[17]

The rats were killed by decapitation (Pentobarbitone sodium anaesthesia – 60 mg/kg) and the heart was immediately dissected out and washed in ice cold saline to remove the blood. 10% tissue homogenate was prepared from it with 0.02M Tris HCl buffer at the pH of 7.5. After centrifugation at 2000 rpm for 10 min, the clear supernatant was collected and used for enzyme assays viz. SOD, catalase and GPx and non-enzymatic antioxidants like Vitamin C and MAD (lipid peroxidation). SOD was assayed by the technique of Marklund (1974)[18], catalase was assayed by the method of Sinha (1972)[19], GPx activity was measured by the method of Rotruck et al. (1973)[20], Lipid peroxidation was assayed by the method of Okhawa et al (1979)[21] and Vitamin C was assayed by the method of Omaye et al (1979).[22]

Histological sectioning of the cardiac tissue was made and was stained with hematoxylin and eosin. Microscopic examination was done to find out the effect of acute and chronic noise exposure and the impact of PD extract on the myocardium.

RESULTS

In the tables, the values were expressed as mean ± SEM**.** The significant level was fixed at $p < 0.05$

Acute noise stress

Effect of PD extract on acute noise stress-induced changes in plasma corticosterone levels: Animals exposed to acute noise stress for 45 minutes showed a significant increase in plasma corticosterone level ($p < 0.05$). After treating the animals with oral administration of PD extract for 7 days, the change was prevented (Table 1).

Table 1. Effect of PD extract on acute noise stress-induced changes in plasma corticosterone

Groups (6 each)	Plasma Corticosterone (µg / dl)
Control	36.52 ± 0.36
Acute noise stress	92.09 ± 0.44
Acute noise stress with PD treatment	65.99 ± 0.39
PD treated only	36.92 ±0.31
Vehicle control	37.09 ±0.30

Table 2- Effect of PD extract on acute noise stress-induced changes in plasma corticosterone - significance between groups

Corticosterone (µg / dl)	Control	Acute noise stress	Aute noise stress with PD	Only PD treated	Vehicle control
Control	-----	-55.57 ± 2.87	-29.48 ± 2.87	0.403 ± 2.87	-0.57 ± 2.87
		p < 0.001*	p <0 .001*	p < 1.000	p < 1.000
Acute noise stress	55.57 ± 2.87	-----	26.09 ± 2.87	55.17 ± 2.87	55.01 ± 2.87
	p < 0.001*		p < 0.001*	p < 0.001*	p < 0.001*
Acute noise stress with PD	29.48 ± 2.87	-26.10 ± 2.87	-----	29.07 ± 2.87	28.91 ± 2.87
	p < 0.001*	p < 0.001*		p < 0.001*	p < 0.001*
PD treated only	0.403 ± 2.87	-55.17 ± 2.87	-29.07± 2.87	-----	0.163 ± 2.87
	p < 1.000	p < 0.001*	p < 0.001*		p < 1.000
Vehicle control	0.567 ± 2.87	-55.01 ± 2.87	-28.91 ± 2.87	0.163 ± 2.87	-----
	p < 1.000	p < 0.001*	p < 0.001*	p < 1.000	

Effect of PD extract on acute noise stress-induced changes on cardiac injury markers There was no significant change in cardiac injury markers after exposure to acute noise stress (Table 3)

Effect of PD extract on acute noise stress-induced changes on antioxidant levels in myocardium: After exposure to acute noise stress, antioxidant levels also did not show any significant change (Table 4).

Table 3. Effect of PD extract on acute noise stress-induced changes on cardiac injury markers

Groups	SGOT (IU/L)	SGPT (IU/L)	LDH (IU/L)
Control	39.81 ± 0.31	43.08 ± 0.33	43.08 ± 0.33
Acute noise stress	39.68 ± 0.35	44.45 ± 0.32	44.45 ± 0.32
Acute noise stress with PD	38.41 ± 0.30	44.95 ± 0.35	44.95 ± 0.35
PD treated only	38.61 ± 0.30	44.98 ± 0.32	44.98 ± 0.32
Vehicle control	38.27 ± 0.26	42.99 ± 0.25	42.99 ± 0.25

SGOT - Serum glutamic oxaloacetic transaminase

SGPT - Serum glutamic pyruvic transaminase

LDH - Lactate dehydrogenase

Table 4. Effect of PD extract on acute noise stress-induced changes in antioxidant levels in myocardium

Groups (Acute noise stress)	SOD	Cat	GPx	MDA	Vit C
Control	11.48 ± 0.10	7.74 ± 0.10	1.45 ± 0.11	3.19 ± 0.09	1.15 ± 0.09
Acute noise stress	12.09 ± 0.11	7.70 ± 0.10	1.03 ± 0.09	3.27 ± 0.10	10.9 ± 0.07
Acute noise stress with PD	11.92 ± 0.10	7.18 ± 0.08	1.14 ± 0.12	3.28 ± 0.10	1.11 ± 0.10
PD treated only	11.56±0.09	7.18 ± 0.07	1.15 ± 0.12	3.39 ± 0.09	1.11 ± 0.09
Vehicle control	11.57 ± 0.11	7.60 ± 0.08	1.48 ± 0.10	3.30 ± 0.08	1.15 ± 0.10

SOD - Superoxide dismutase (min/mg/ptn), Cat((µmol /min/mg ptn),

GPx - Glutathione peroxidase (mg/min/mg ptn), MDA – Malondialdehyde (nmoles/ in/mg/ptn), Vit C - Vitamin C (µg /mg ptn)

Chronic noise stress

Effect of PD extract on chronic noise stress-induced changes on plasma corticosterone level

Animals exposed to chronic noise stress for 21 days showed a significant decrease ($p < 0.05$) in plasma corticosterone levels and PD treatment with chronic noise stress effectively prevented this change (Table 5, 6).

Table 5. Effect of PD extract on chronic noise stress-induced changes on plasma corticosterone levels

Groups (6 each)	Plasma Corticosterone (µg / dl)
Control	36.52 ± 0.36
Chronic noise stress	57.13 ± 0.3
Chronic noise stress with PD treatment	40.56 ± 0.37
PD treated only	35.03 ± 0.33
Vehicle control	37.09 ± 0.30

Table 6. Effect of PD extract on chronic noise stress-induced changes on plasma corticosterone levels - Significance between groups

Plasma corticosterone	Control	Chronic noise stress	Chronic noise stress with PD	PD treated only	Vehicle control
Control	-----	-20.61 ± 2.45	-4.04 ± 2.45	1.49 ± 2.45	19.38 ± 2.45
		p < 0.001*	p < 0 .481	p < 0.972	p < 0.986
Chronic noise stress	20.61 ± 2.45	-----	16.53 ± 2.45	22.10 ± 2.45	19.38 ± 2.45
	p < 0.001*		p < 0.001*	p < 0.001*	p < 0.001*
Chronic noise stress with PD	4.04 ± 2.872	-16.53 ± 2.45	-----	5.53 ± 2.45	2.81 ± 2.45
	p < 0.481	p < 0.001*		p < 0.191	p < 0.781
PD treated only	-1.49 ± 2.45	-22.10 ± 2.45	-5.53 ± 2.45	-----	-2.73 ± 2.45
	p < 0.972	p < 0.001*	p < 0.191		p < 0.797
Vehicle control	-19.38 ± 2.45	-19.38 ± 2.45	-2.81 ± 2.45	2.73 ± 2.45	-----
	p < 0.986	p < 0.001*	p < 0.781	p < 0.797	

SGOT, SGPT and LDH levels showed significant increase after chronic noise exposure ($p < 0.05$) compared to controls, indicating the damage done to the cardiac tissues by the noise stress. After treating with PD extract for 21 days, exposure to chronic noise stress prevented these changes and were reversed to almost normal (Tables 7, 8, 9 and 10).

Table 7. Effect of PD extract on chronic noise stress-induced changes on cardiac injury markers

Groups	SGOT (IU/L)	SGPT (IU/L)	LDH (IU/L)
Control	39.81 ± 0.31	43.08 ± 0.33	187.83 ± 0.50
Chronic noise stress	68.00 ± 0.41	68.52 ± 0.42	276.83 ± 0.48
Chronic noise stress with PD	41.63 ± 0.35	48.89 ± 0.36	192.67 ± 0.48
PD treated only	34.86 ± 0.35	38.42 ± 0.35	192.83 ± 0.50
Vehicle control	38.43 ± 0.29	44.15 ± 0.24	186.00 ± 0.56

SOD, catalase, GPx and Vitamin C levels showed significant decrease after chronic noise exposure ($p < 0.05$) and MDA level increased significantly compared to that of controls, vehicle controls and PD treated groups. PD extract for 21 days prevented these changes significantly, indicating the protective effect of PD against noise-induced damages in cardiac tissue (Table 11, 12, 13, 14, 15 and 16).

Table 8. Effect of PD extract on chronic noise stress-induced changes on SGOT level in myocardium - Significance between groups

SGOT in myocardium	Control	Chronic noise stress	Chronic noise stress with PD	PD treated only	Vehicle control
Control	-----	-24.19 ± 2.52	-1.82 ± 2.52	4.96 ± 2.52	1.54 ± 2.52
		p < 0.001*	p < 0.949	p < 0.309	p < 0.971
Chronic noise stress	24.19 ± 2.52	-----	22.37 ± 2.52	29.15 ± 2.52	25.74 ± 2.52
	p < 0.001*		p < 0.001*	p < 0.001*	p < 0.001*
Chronic noise stress with PD	1.82 ± 2.52	-22.37 ± 2.52	-----	6.78 ± 2.52	3.37 ± 2.52
	p < 0.949	p < 0.001*		p < 0.083	p < 0.670
PD treated only	-4.96 ± 2.52	-29.15 ± 2.52	-6.78 ± 2.52	-----	-3.41 ± 2.52
	p < 0.309	p < 0.001*	p < 0.083		p < 0.661
Vehicle control	-1.54 ± 2.52	- 25.74 ± 2.52	-3.37 ± 2.52	3.41 ± 2.52	-----
	p < 0.971	p < 0.001*	p < 0.670	p < 0.661	

Table 9. Effect of PD extract on chronic noise stress-induced changes on SGPT level in myocardium - Significance between groups

SGPT in myocardium	Control	Chronic noise stress	Chronic noise stress with PD	PD treated only	Vehicle control
Control	-----	-25.45 ± 2.61	-5.82 ± 2.61	4.66 ± 2.61	0.09 ± 2.61
		p < 0.001*	p < 0.202	p < 0.404	p < 1.000
Chronic noise stress	25.45 ± 2.61	-----	19.63 ± 2.61	30.11 ± 2.61	25.54 ± 2.61
	p < 0.001*		p < 0.001*	p < 0.001*	p < 0.001*
Chronic noise stress with PD	5.82 ± 2.61	-19.63 ± 2.61	-----	10.48 ± 2.61	5.91 ± 2.61
	p < 0.202	p < 0.001*		p < 0.004*	p < 0.190
PD treated only	-4.66 ± 2.61	-30.11 ± 2.61	10.48 ± 2.61	-----	-4.57 ± 2.61
	p < 0.404	p < 0.001*	p < 0.004*		p < 0.423
Vehicle control	-0.09 ± 2.61	- 25.54 ± 2.61	-5.91 ± 2.61	4.57 ± 2.61	-----
	p < 1.000	p < 0.001*	p < 0.190	p < 0.423	

Table 10. Effect of PD extract on chronic noise stress-induced changes on LDH level in myocardium - Significance between groups

LDH in myocardium	Control	Chronic noise stress	Chronic noise stress with PD	PD treated only	Vehicle control
Control	-----	-89.00 ± 5.17	-4.83 ± 5.17	-5.0 ± 5.17	0.33 ± 5.17
		p < 0.001*	p < 1.000	p < 0.999	p < 1.000
Chronic noise stress	89.00 ± 5.17	-----	84.16 ± 5.17	84.00 ± 5.17	89.33 ± 5.17
	p < 0.001*		p < 0.001*	p < 0.001*	p < 0.001*
Chronic noise stress with PD	4.83 ± 5.17	-84.16 ± 5.17	-----	-0.17 ± 5.17	5.17 ± 5.17
	p < 1.000	p < 0.001*		p < 1.000	p < 0.853
PD treated only	5.0 ± 5.17	-84.00 ± 5.17	0.17 ± 5.17	-----	5.33 ± 5.17
	p < 0.999	p < 0.001*	p < 1.000		p < 0.838
Vehicle control	-0.33 ± 5.17	-89.33 ± 5.17	-5.17 ± 5.17	-.5.33 ± 5.17	-----
	p < 1.000	p < 0.001*	p < 0.853	p < 0.838	

Table 11. Effect of PD extract on chronic noise stress-induced changes on antioxidant levels in myocardium

Groups (Chronic noise stress)	SOD	Cat	GPx	MDA	Vit C
Control	11.48 ± 0.10	7.74 ± 0.10	1.45 ± 0.11	3.19 ± 0.09	1.15 ± 0.09
Chronic noise stress	9.33 ± 0.09	5.17 ± 0.11	0.74 ± 0.09	6.78 ±0.12	2.06 ± 0.12
Chronic noise stress with PD	11.02 ± 0.09	6.10 ± 0.10	1.45 ± 0.11	4.37 ± 0.11	1.29 ± 0.12
PD treated only	11.28 ± 0.16	7.41 ± 0.09	1.40 ± 0.11	3.23 ± 0.09	1.28 ± 0.11
Vehicle control	11.54 ± 0.12	7.54 ± 0.10	1.45 ± 0.08	3.30 ± 0.08	1.18 ± 0.12

Table 12. Effect of PD extract on chronic noise stress-induced changes on superoxide dismutase in myocardium - Significance between groups

SOD (Chronic noise stress)	Control	Chronic noise stress	Chronic noise stress with PD	PD treated only	Vehicle control
Control	-----	2.15 ± 0.30	0.46 ± 0.30	0.21 ± 0.30	-0.09 ± 0.30
		p < 0.001*	p < 0.548	p < 0.957	p < 0.998
Chronic noise stress	-2.15 ± 0.30	-----	0.17 ± 0.30	-1.94 ± 0.30	-2.24 ± 0.30
	p < 0.001*		p < 0.001*	p < 0.001*	p < 0.001*
Chronic noise stress with PD	-0.46 ± 0.30	-0.17 ± 0.30	-----	-0.26 ± 0.30	-0.55 ± 0.30
	p < 0.548	p < 0.001*		p < 0.912	p < 0.369
PD treated only	-0.21 ± 0.30	1.94 ± 0.30	0.26 ± 0.30	-----	-0.30 ± 0.30
	p < 0.957	p < 0.001*	p < 0.912		p < 0.853
Vehicle control	0.09 ± 0.30	2.24 ± 0.30	0.55 ± 0.30	0.30 ± 0.30	-----
	p < 0.998	p < 0.001*	p < 0.369	p < 0.853	

Table 13. Effect of PD extract on chronic noise stress-induced changes on catalase in myocardium - Significance between groups

Catalase	Control	Chronic noise stress	Chronic noise stress with PD	PD treated only	Vehicle control
Control		2.57 ± 0.22	1.64 ± 0.22	0.33 ± 0.22	-0.002 ± 0.22
		p < 0.001*	p < 0.001*	p < 0.572	p < 1.000
Chronic noise stress	-2.57 ± 0.22	-----	-0.93 ± 0.22	-2.24 ± 0.22	-2.57 ± 0.20
	p < 0.001*		p < 0.003*	p < 0.001*	p < 0.001*
Chronic noise stress with PD	-1.64 ± 0.22	0.93 ± 0.22	-----	-1.31 ± 0.22	-1.64 ± 0.22
	p < 0.001*	p < 0.003*		p < 0.001*	p < 0.001*
PD treated only	-0.33 ± 0.22	2.24 ± 0.22	1.31 ± 0.22	-----	-0.34 ± 0.22
	p < 0.572	p < 0.001*	p < 0.001*		p < 0.566
Vehicle control	0.002 ± 0.22	2.57 ± 0.20	1.64 ± 0.22	0.34 ± 0.22	-----
	p < 1.000	p < 0.001*	p < 0.001*	p < 0.566	

Table 14. Effect of PD extract on chronic noise stress-induced changes on GPx in myocardium - significance between groups

GPx	Control	Chronic noise stress	Chronic noise stress with PD	PD treated only	Vehicle control
Control	-----	0.71 ± 0.23	0.005 ± 0.23	0.183 ± 0.23	-0.03 ±0.23
		p < 0.040*	p < 1.000	p < 0.934	p < 1.000
Chronic noise stress	-0.71 ± 0.23	-----	-0.71 ± 0.23	-0.53 ± 0.23	-0.74 ± 0.23
	p < 0.040*		p < 0.042*	p < 0.191	p < 0.031*
Chronic noise stress with PD	-0.005 ± 0.23	0.71 ± 0.23	-----	0.18 ± 0.23	-0.03 ± 0.23
	p < 1.000	p < 0.042*		p < 0.940	p < 1.000
PD treated only	-0.183 ± 0.23	-0.53 ± 0.23	-0.18 ± 0.23	-----	-0.21 ± 0.23
	p < 0.934	p < 0.191	p < 0.940		p < 0.895
Vehicle control	0.03 ± 0.23	0.74 ± 0.23	0.03 ± 0.23	0.21 ± 0.23	-----
	p < 1.000	p < 0.031*	p < 1.000	p < 0.895	

Table 15. Effect of PD extract on chronic noise stress-induced changes on (Lipid peroxidation) MDA in myocardium - Significance between groups

MDA	Control	Chronic noise stress	Chronic noise stress with PD	PD treated only	Vehicle control
Control	-----	-3.42 ± 0.20	-1.009 ± 0.20	-0.20 ± 0.20	-0.008 ± 0.20
		p < 0.001*	p < 0.001*	p < 0.845	p < 1.000
Chronic noise stress	3.42 ± 0.20	-----	2.41 ± 0.20	3.21 ± 0.20	3.42 ± 0.20
	p < 0.001*		p < 0.001*	p < 0.001*	p < 0.001*
Chronic noise stress with PD	1.009 ± 0.20	-2.41 ± 0.20	-----	0.81 ± 0.20	1.02 ± 0.20
	p < 0.001*	p < 0.001*		p < 0.004*	p < 0.001*
PD treated only	0.20 ± 0.20	-3.21 ± 0.20	-0.81 ± 0.20	-----	0.21 ± 0.20
	p < 0.845	p < 0.001*	p < 0.004*		p < 0.824
Vehicle control	0.008 ± 0.20	-3.42 ± 0.20	-1.02 ± 0.20	-0.21 ± 0.20	-----
	p < 1.000	p < 0.001*	p < 0.001*	p < 0.824	

Table 16. Effect of PD extract on chronic noise stress-induced changes on Vitamin C in myocardium - Significance between groups

Vitamin C	Control	Chronic noise stress	Chronic noise stress with PD	PD treated only	Vehicle control
Control	-----	-0.92 ± 0.25	-0.14 ± 0.25	-0.13 ± 0.25	0.003 ± 0.25
		p < 0.010	p < 0.979	p < 0.984	p < 1.000
Chronic noise stress	0.92 ± 0.25	-----	0.77 ± 0.25	0.78 ± 0.25	0.92± 0.25
	p < 0.010		p < 0.037	p < 0.034	p <0.010
Chronic noise stress with PD	0.14 ± 0.25	-0.77 ± 0.25	-----	0.01 ± 0.25	0.15 ± 0.25
	p < 0.979	p < 0.037		p < 1.000	p < 0.977
PD treated only	0.13 ± 0.25	-0.78 ± 0.25	-0.01 ± 0.25	-----	0.14 ± 0.25
	p < 0.984	p < 0.034	p < 1.000		p < 0.034
Vehicle control	-0.003 ± 0.25	-0.92 ± 0.25	-0.15 ± 0.25	-0.14 ± 0.25	-----
	p < 1.000	p <0.010	p < 0.977	p < 0.034	

Histological examination of the cardiac tissues in different groups

In control animals, the histological section of the myocardium showed normally placed nucleus and few congested blood vessels (Fig 1). Acute noise stress (Fig 2), acute noise stress with PD treatment (Fig 3), PD treatment alone (Fig 4) and vehicle control treated for 7 days and 21 days (Fig 5 and 8) did not show any marked change in the histological section of cardiac tissues. In rats exposed to chronic noise stress, the cardiac tissue showed multiple foci of hemorrhagic necrosis with few inflammatory cells (shown by the yellow arrows in Fig 6). Treatment with PD extract for 21 days, reduced the ischemic changes and the number of

inflammatory cells caused by exposure to chronic noise stress. This indicates cardio-protective effect of PD extract against chronic noise stress (Fig 7). Cardiac tissues in vehicle control samples also did not show any damaging effects (Fig 8).

Histology of cardiac tissues in different groups

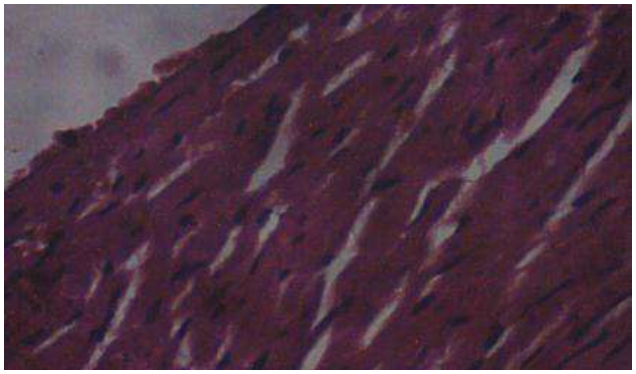

Fig 1. Cardiac tissue in control animals

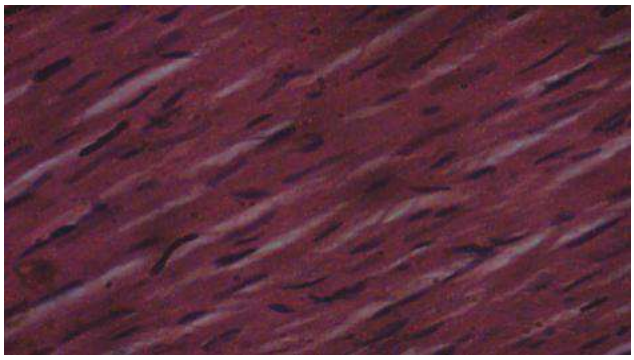

Fig 2. Cardiac tissue after acute noise stress

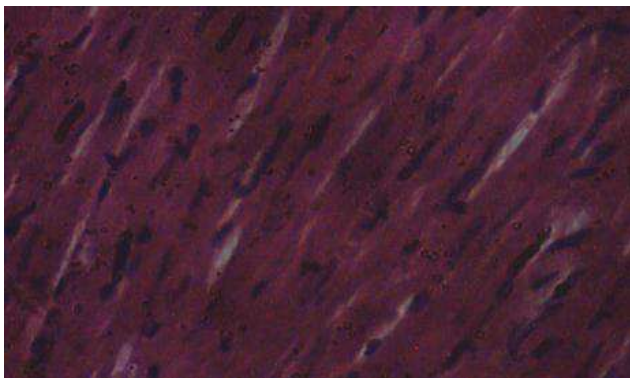

Fig 3. Cardiac tissue in acute noise stress after PD treatment

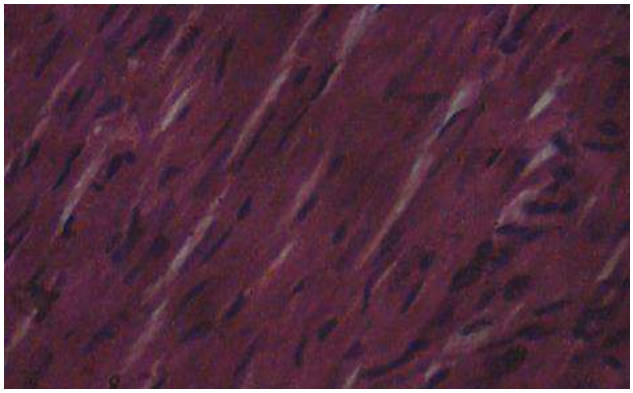

Fig 4. Cardiac tissue with PD treatment alone for 7 days

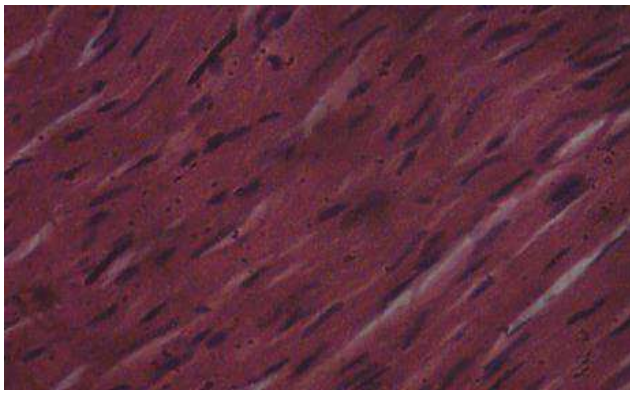

Fig 5. Cardiac tissue in vehicle control (7 days) control animals after 7 days

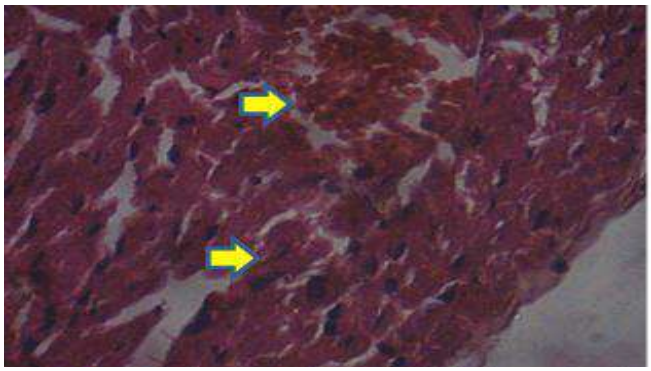

Fig 6. Cardiac tissue after chronic noise stress

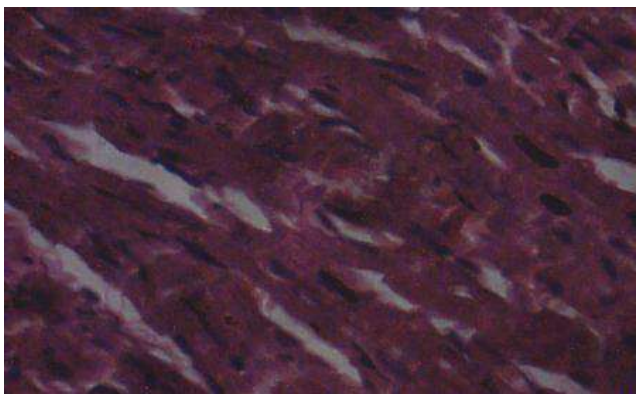

Fig 7. Cardiac tissue in chronic noise stress after PD treatment

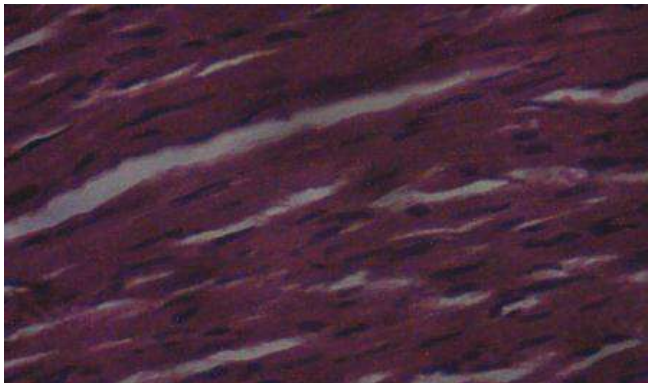

Fig 7. Cardiac tissue with PD treatment alone for 21 days

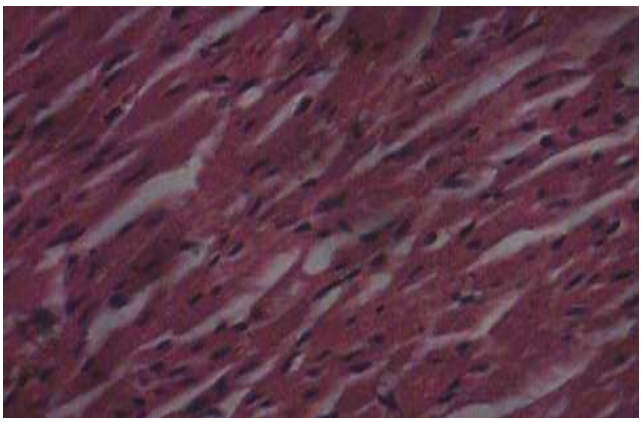

Fig 8. Cardiac tissue in vehicle control (21 days) animals after 21 days

DISCUSSION

The results of the present study form yet another proof to consider PD as one of the herbal plants with curative effect because of its protective effect on the heart against noise stress. Any sound above 80 dB is considered to be a noise stressor. This is proved by the significant elevation in plasma corticosterone level. This is well supported by the reports of Sembulingam et al., and Archana et al 2000.[23, 24]

The noise induced stress is an oxidative stress. Exposure to noise, whatever may be the duration, causes depletion of antioxidants and elevation of ROS leading to an imbalance between the oxidants and antioxidants with an increase in the components of ROS like superoxide, hydroxyl radical and hydrogen peroxide.[25] These ROS are capable of causing damage, dysfunction and death at the cellular level[32] Any sound above 80 dB is considered to be a stressor causing various functional disabilities with biochemical and immunological changes which in turn affects the normal histological architecture of the organs.[26, 27] To

neutralize these noise-induced ROS, the anti-oxidant levels increase. But in chronic noise stress, the anti-oxidants are depleted ending up with oxidative stress.[26]

SGOT, SGPT and LDH are cardiac injury markers that indicate damage to the heart. The results of our study showed that acute noise stress did not alter these variables significantly indicating that noise stress for short duration was well tolerated by the cardiac tissues. But these enzymes increased significantly in chronic noise stress. Due to noise-induced steroidal storm (plasma corticosterone), lipid peroxidation was increased causing alteration in the functional status of the myocardial cell membrane and leakage of these enzymes. This was cause for the cardiac tissue injury. PD treatment significantly decreased the SGOT, SGPT and LDH levels which implies that PD is capable of rendering membrane stabilization as reported in our previous study.[3] It was well supported by the results of Bhaskar V.H (2009).[28]

Chronic noise stress significantly increased the levels of ROS also, which in turn increased the activity of related enzymes viz., SOD, catalase and GPx and Vitamin C which counter-acted the increased production of ROS.[29] These three enzymes work together to protect the cells against the harmful effects of ROS.[30] Administration of PD extract effectively prevented all these noise-induced harmful effects of ROS and protected the cardiac tissue against oxidative stress.

Lipid peroxidation is an important biomarker of oxidative stress. In lipid peroxidation process, the bilipid layer of the cell membrane is destroyed by the ROS causing cell death. This seems to be noticed in some clinical conditions like atherosclerosis, ischemic or traumatic brain damage, Alzheimer's disease, diabetes mellitus, coronary artery disease, psoriasis, cancer, motor neuron disease, etc.,[31] Our results also show that exposure to chronic noise stress, significantly increased the lipid peroxidation in myocardial tissues indicating the cell membrane damage due to free radicals. We also noticed that administration of PD extract significantly decreased the lipid peroxidation (lower MDA levels) in myocardium, thereby reducing the impact of noise stress. This beneficial effect of PD extract may be attributed to the presence of flavanoid, tannins, alkaloid, glycosides, terpenoids, steroids and carbohydrates [(2).] Vyas et al (2011) have already reported that PD extract supplementation significantly decreased oxalate-induced kidney lipid peroxidation.[32]

Our study on cardio-protective effect of PD extract is also supported by the plasma and serum analysis of the noise-exposed and PD treated animal models for ROS and antioxidants status. The injury to the cardiac tissue by oxidative stress had been already reported.[33, 34] However, these scientific evidences are mostly indirect; that is, assessed by the decrease in the injury markers. Yet, PD treatment showed significant reduction in these injury markers.

However, in the present study, we could obtain the direct evidence for the noise-induced myocardial injury and its prevention by PD extract by the histological studies of the cardiac tissue in experimental animals. Our results showed that acute noise stress did not affect the architecture of the myocardium compared to that of control, PD treated and vehicle treated groups of animals. However, chronic noise exposure showed multiple foci of hemorrhages in the cardiac tissues which are clear indications of the damages done to the cardiac tissues. Literature also reveals some evidences for the death of cardiac tissue by simulated ischemia-induced oxidative stress and these damaging changes were prevented when treated with antioxidants like metal-chelating agents and thiol donating groups.[35, 36, 37] Based on this evidence, PD extract can be considered as an effective cardio-protective agent because the hemorrhagic spots in myocardium produced by chronic noise exposure were not seen in PD treated group along with chronic noise stress.

The mechanism of chronic noise-induced pathological changes in the myocardial architecture, may be due to increased levels of stress hormones like corticosterone and catecholamines that causes increased peripheral resistance, coronary spasm, hypertension and ischemic heart disease.[38] These changes, in turn, may act at the level of mitochondria, inducing a loss of membrane stabilization, failure of enzymatic pattern and local deficiency of ATP that affects the sodium and calcium channels.[39] The intense biochemical changes occurring at the mitochondrial level in myocardium can also predispose to these structural changes.[40] As PD extract prevented the damaging effects of chronic noise stress, this herbal plant can be considered as an effective cardio-protective agent.

The cardio-protective mechanism exhibited by PD extract through modulation of stress index, serum cardiac injury enzymes and various antioxidants shows the antioxidant defense of the PD extract on myocardial tissue.

Our preliminary studies on ethanolic PD extract revealed the presence of flavonoids, phenols, tannins, alkaloid, glycosides, terpenoids, steroids and carbohydrates by qualitative tests. Gas

mass spectrometric analysis of PD extract identified the bioactive compounds like 2-hydroxy-methyl ester (Benzoic acid compound), 2-Methoxy-4-vinylphenol (Phenolic compound) , Phthalic acid di-(1-hexen-5-yl) ester (Organic acid ester), l-(+)-Ascorbic acid 2,6-dihexadecanoate (Fatty acid) and Methyl (Z)-5,11,14,17-eicosatetraenoate (Fatty acid ester).[2]

Considering the findings of our previous study, the free radical scavenging activity of PD extract on myocardial tissues may be attributed to the presence of phenolic and fatty acid contents in it. It can also be suggested that the observed free radical scavenging potential of PD extract may be assigned to the hydrogen donating capacity of phenolic components present in sizeable amounts in the ethanoic extract.

CONCLUSION

Chronic noise stress showed a significant decrease in antioxidant status of heart and an increase in cardiac injury enzymes with multiple foci of hemorrhagic necrosis in myocardium. Treatment with PD extract effectively protected the myocardium from the damaging effects of chronic noise stress. Thus, results of the present study help to consider PD as an excellent traditional herbal medicine against stress effects and also as a cardio-protective agent.

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