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Research Article

Ameliorative Role of *Momordica charantia* in Partial Abdominal Aortic Constriction Induced Experimental Cardiac Hypertrophy

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ABSTRACT

The present study has been designed to explore the beneficial effect of *Momordica charantia* in partial abdominal aortic constriction (PAAC) induced cardiac hypertrophy in rats. The male wistar rats were anaesthetized with thiopentone sodium and were subjected to Partial Abdominal Aortic Constriction (PAAC) for 4 weeks. The treatment with ethanolic extract of *Momordica Charantia* (EMC) (200 mg/kg and 400 mg/kg) was started three days before surgery and it was continued for 4 weeks after surgery. The development of left ventricular (LV) hypertrophy was assessed by measuring ratio of LV weight to body weight (LVW/BW), LV wall thickness (LVWT), LV protein content, LV collagen content and LV RNA concentration. Further mean arterial blood pressure (MABP) was recorded. Moreover, DNA gel electrophoresis was employed to assess the myocardial cell death. The PAAC significantly increased the ratio of LV weight to body weight, LV wall thickness, LV protein content, LV collagen content and LV RNA concentration. Further PAAC significantly increased MABP and myocardial cell death. The EMC (400mg/kg) markedly attenuated PAAC induced increase in LV hypertrophy, MABP and LV necrotic cell death. These results implicate ameliorative role of *Momordica Charantia* in PAAC induced myocardial cell death and pathological cardiac hypertrophy.

Keywords: Aortic constriction, Cardiac hypertrophy, *Momordica Charantia*

INTRODUCTION

Cardiac hypertrophy is a major predictor of progressive heart disease and an adverse prognosis. It is recognized as an adaptive process to a variety of physiological and pathological conditions like ischemic heart disease, hypertension and heart failure. Hence, it is a well established risk factor for cardiovascular mortality in patients [1,2]. The induction, progression, and subsequent detrimental effects of cardiac hypertrophy is characterized by an increment in cardiomyocyte size, increased protein synthesis and changes in the organization of sarcomeric structure [2].

Momordica Charantia fruit is usually oblong and resembles a small cucumber. The published reports indicate that the plant is having anthelmintic, antibacterial, antibiotic, antidiabetic, antiinflammatory, antileukemic, antimicrobial, antimutagenic, antimycobacterial, antioxidant and antitumor activities. Moreover, *Momordica Charantia* fruit is a PPAR (Peroxisome proliferator activated receptor) dual agonist and its role in cardiac dysfunction still remains unexplored [3,4].

Peroxisome proliferator activated receptor (PPAR) belongs to a class of nuclear receptors and is involved in regulation of genes responsible for glucose and lipid metabolism. The role of PPAR agonists in various cardiovascular complications such as vascular endothelial dysfunction, myocardial ischemia reperfusion-induced injury, hypertension, and hypertension-induced cardiac hypertrophy has been explored[15]. Moreover, PPAR-dual agonists such as *momordica charantia* recently have been reported to exhibit beneficial effect in diabetes and vascular endothelial dysfunction[5,6]. However, the effect of PPAR dual agonists in cardiac hypertrophy still remains unexplored. Hence, the present study has been designed to investigate the role of *momordica charantia* in partial abdominal aortic constriction-induced cardiac hypertrophy in rats.

MATERIALS AND METHODS

Male Wistar albino rats weighing 200 to 230 g were used in the present study. They were maintained on rat feed and water ad libitum and were exposed to a 12-hour light and 12-hour dark cycle. The Institutional Animal Ethics Committee approved the experimental protocol and care of the animals was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Reg. No. 10/2010/CPCSEA).

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Table 1. Characteristics of HPTLC of standard charantin

Peak	Start Rf	Start Height	Max Rf	Max Height	Max%	End Rf	End Height	Area	Area %	Assigned substance
1	0.34	0.1	0.36	246.8	100.0	0.38	0.4	2738.7	100	Charantin

Table 2. Characteristics of HPTLC of EMC

Peak	Start Rf	Start Height	Max Rf	Max Height	Max%	End Rf	End Height	Area	Area %	Assigned substance
1	0.06	0.1	0.11	94.1	17.51	0.13	0.5	1774.3	12.84	Unknown
2	0.30	0.3	0.38	131.6	24.47	0.41	47.6	3028.8	21.91	Charantin
3	0.41	48.0	0.43	71.7	13.33	0.46	33.2	1554.8	11.25	Unknown
4	0.46	33.4	0.48	42.3	7.87	0.52	20.5	1231.2	8.91	Unknown
5	0.52	20.6	0.53	21.5	3.99	0.56	6.5	356.7	2.58	Unknown
6	0.58	6.8	0.65	57.3	10.66	0.67	45.0	2068.1	14.96	Unknown
7	0.68	45.4	0.71	119.2	22.17	0.77	0.5	3809.3	27.56	Unknown

Figure 1. HPTLC spectra of standard Charantin

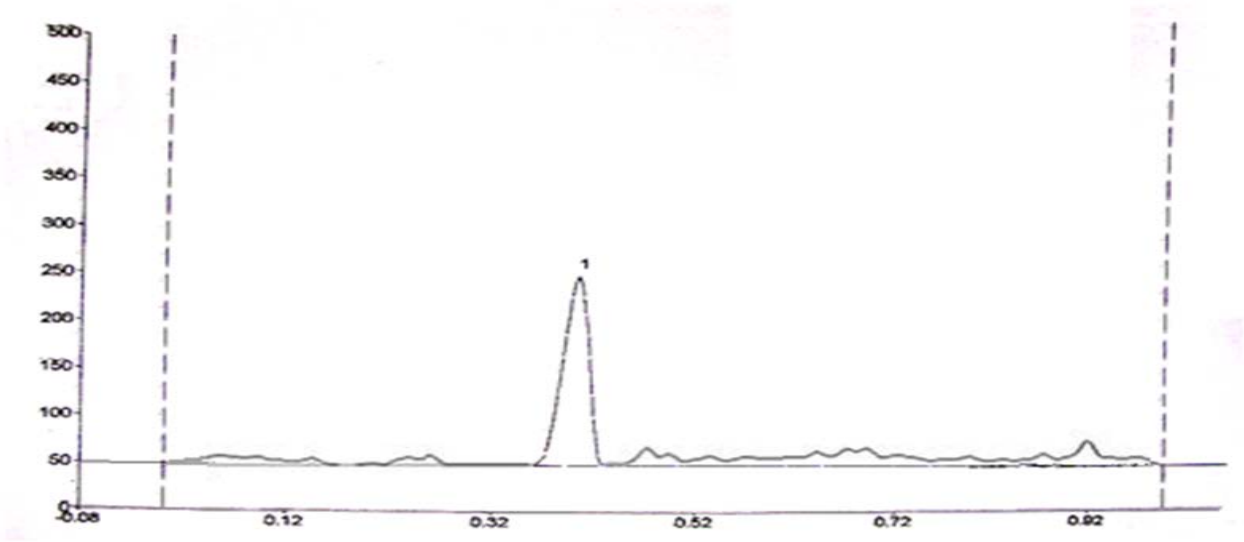

Figure 2. HPTLC spectra of Ethanolic extract of Momordica Charantia

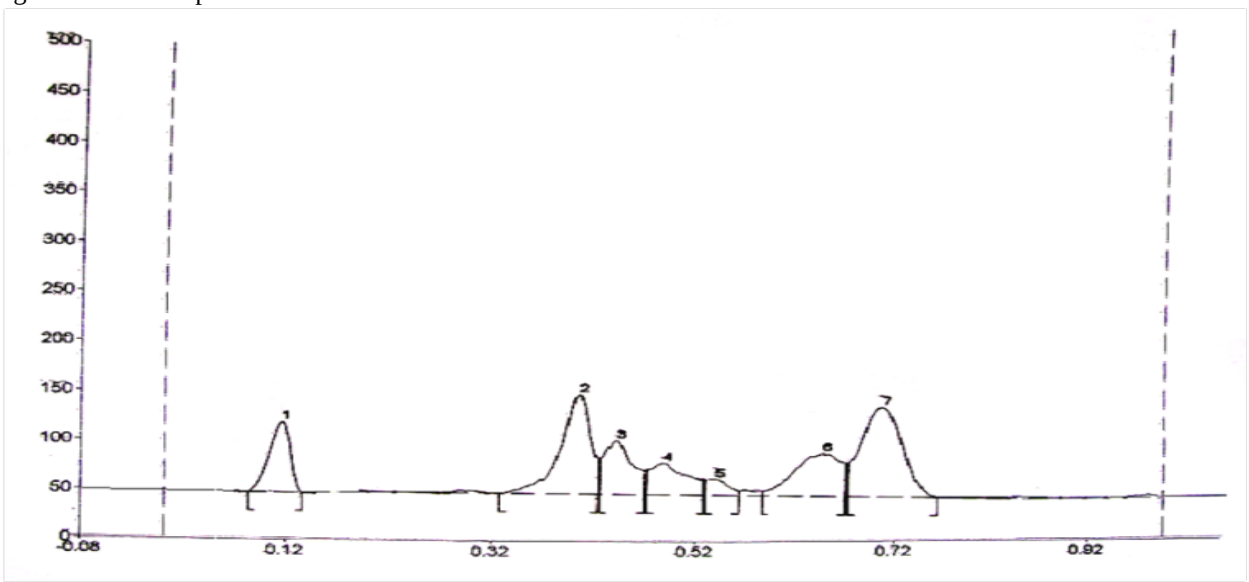

Table 3. Effect of *Momordica Charantia* treatment on morphological, biochemical and haemodynamic parameters

Parameters	Sham Operated	PAAC	PAAC +EMC(200mg/kg)	PAAC+EMC(400mg/kg)
BW (g)	261.45±10.53	263.7 6±11.62[a]	252.4 6±12.24[b]	256.2 6 ±9.62[b]
LVW/BW (mg/g)	1.87± 0.07	2.93±0.06[a]	2.35±0.06[b]	1.96±0.04[b]
LVWT (mm)	2.18±0.07	3.77± 0.10[a]	2.68±0.07[b]	2.34±0.06[b]
MABP (mmHg)	108.2±2.7	165.1±3.6[a]	142.04±3.1[b]	120.2±2.6[b]
Protein Content	121.5±5.3	175.7±4.2[a]	135.3±3.2[b]	127.5±3.1[b]
Collagen content	1.67±0.06	4.62±0.07[a]	3.17±007[b]	2.52±0.06[b]
RNA Conc.	2.75±0.03	3.42±0.05[a]	3.14±0.02[b]	2.88±0.02[b]

PAAC ,partial abdominal aortic constriction; EMC, Ethanolic extract of Momordica Charantia; MABP, mean arterial blood pressure; BW, body weight; LVW, left ventricular weight; LVWT, left ventricular wall thickness. Values are mean±SEM a: p<0.05 vs sham control; b: p<0.05 vs PAAC control

Phytochemical screening: Chemical tests were carried out on the ethanolic extracts using the standard procedures to identify the presence of glycosides, saponins, flavonoids, alkaloids, tannins, triterpenids, phytosterols, carbohydrates, fats, proteins and volatile oils [7,8]. Futhermore, standardisation of the ethanolic extract was done by HPTLC using Benzene: Methanol (80:20 v/v), detected at 536 nm (Mukherjee, 2002) (Table 1, 2 and Fig. 1, 2). In the Ethanolic extract of *Momordica Charantia*, charantin was detected at Rf 0.38 which showed resemblance to standard charantin.

Experimental Design**:** The present study comprised six groups with each comprising of 12 to 14 animals. In Group 1 (sham operated), surgery was performed to expose the abdominal aorta but it was not constricted. In Group 2 (PAAC group), surgery was performed and rats were subjected to partial abdominal aortic constriction. In Group 3 (sham operated low treatment group), rats were subjected to surgery without aortic banding and were treated with ethanolic extract of *momordica charantia* (200 mg/kg per day orally) for three days before surgery and it was continued for 4 weeks after surgery. In Group 4 (sham operated high treatment group), rats were subjected to surgery without aortic banding and were treated with ethanolic extract of *momordica charantia* (400 mg/kg per day orally) for three days before surgery and it was continued for 4 weeks after surgery.In Group 5 (treatment low dose), rats were administered ethanolic extract of *momordica charantia* (200 mg/kg per day orally) for three days before PAAC and it was continued for 4 weeks after surgery. In Group 6 (treatment high dose), rats were administered ethanolic extract of *momordica charantia* (400 mg/kg per day orally) for three days before PAAC and it was continued for 4 weeks after surgery.

Morphologic Assessment of Cardiac Hypertrophy: At the end of the four weeks, the rats were euthanized and hearts were excised and washed with cold saline. The left ventricular weight including interventricular septum and right ventricular weight was noted separately and expressed as milligrams per gram of body weight. The left ventricle was divided into three parts and wall thickness of each slice was noted at eight different parts using an ocular micrometer. The mean value of all three slices was taken and expressed in millimeters [18].

Estimation of Collagen Content: The left ventricular collagen content was estimated biochemically in terms of hydroxyproline concentration as previously described [5, 14]. The hydroxyproline content was expressed as milligrams per gram dry weight of left ventricle.

Biochemical Assessment: The left ventricle was stored at –80ºC in liquid nitrogen for quantitative estimation of biochemical parameters. The left ventricle was homogenized and protein content was determined spectrophotometrically at 750 nm by Lowry's method [21] and expressed as mg/g of left ventricular weight.

The RNA was extracted from homogenized left ventricular tissues using method of Chomczynski and Sacchi [22]. RNA concentration was estimated spectrophotometrically at 260 nm. One absorbancy unit at 260 nm in a 1 cm light path cuvette was assumed to be equal to 40 µg/mL of RNA. The purity of RNA was assessed by determining the ratio of absorbance at 260 and 280 nm and the ratio was more than 1.8.

The DNA was extracted from homogenized left ventricular tissue using method of Ausubel et al [22]. The concentration of DNA was determined spectrophotometrically at 260 nm.

DNA Gel Electrophoresis: 12 µg of extracted DNA was added to equal volume of loading dye (40% sucrose, 0.1% bromophenol blue, 0.7% sodium dodecyl sulphate) and the mixture was loaded in the well. Electrophoresis was carried out using 1.8% agarose gel in 1 x TBE buffer (Tris HCl 89 mM, boric acid 89 mM, EDTA 2 mM) for 1.15 hr at 400 mA, 50V and 3W in submarine electrophoresis apparatus (Pharmacia Biotech, Freibury, Germany). Ethidium bromide (0.5µg/mL) was added to the gel for DNA detection [18].

Measurement of Mean Arterial Blood Pressure: The mean arterial blood pressure (MABP) in carotid artery of anesthetized rats was recorded using a pressure transducer (BIOPAC System, Goleta, CA) just before morphologic and biochemical studies [18].

Drugs and Chemicals: Atorvastatin was obtained as a kind gift from Ranbaxy Lab. Ltd, Gurgaon, India. Folin-Ciocateu's Phenol Reagent, Tris buffer, agarose and Chloramine T were purchased from Loba Chemie, Coimbatore, India. Sarcosyl and Proteinase K were procured from Sigma-Aldrich, Mumbai, India. All other reagents used in the present study were of analytical grade.

Figure 3. Gel Electrophoresis

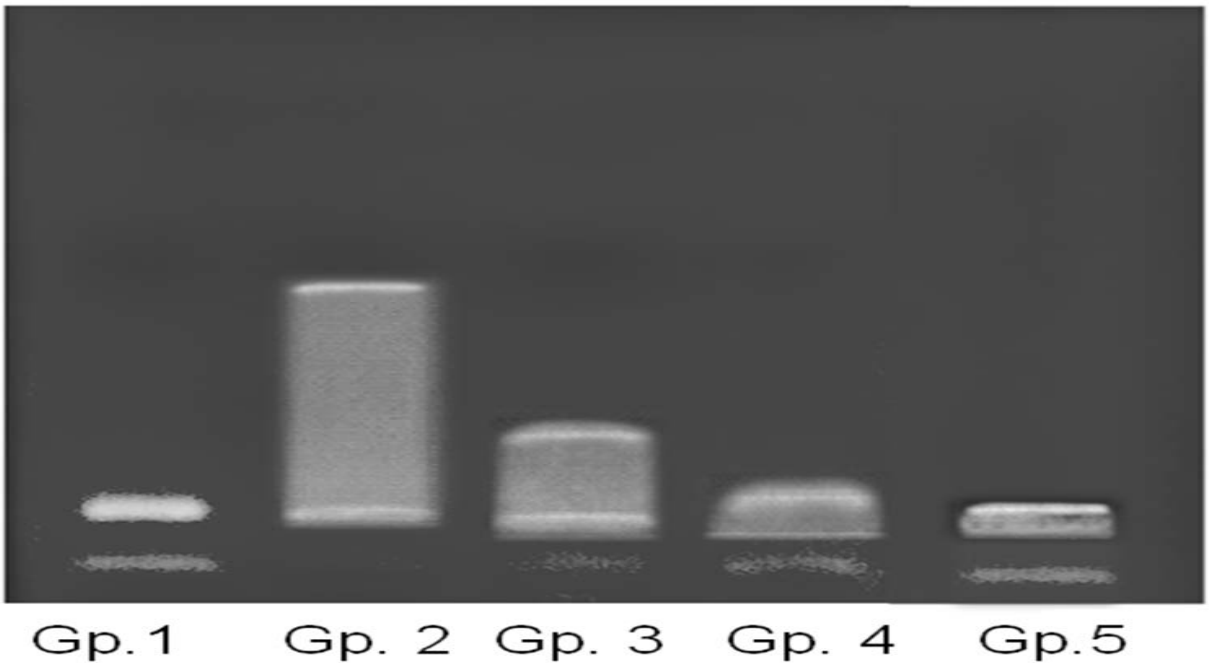

Figure 3. Effect of EMC on gel electrophoretic pattern of DNA. L-1 represents DNA extracted from left ventricle of sham control heart, L-2 represents DNA extracted from left ventricle of PAAC control heart, L-3 represents effect of EMC (100 mg/kg per day orally) on DNA extracted from left ventricle of PAAC control heart, L-4 repre-sents effect of EMC (200 mg/kg per day orally) on DNA extracted from left ventricle of PAAC control heart and L-5 repre-sents effect of EMC (400 mg/kg per day orally) on DNA extracted from left ventricle of PAAC control heart

STATISTICAL ANALYSIS

The results were expressed as mean ± standard deviation. The data obtained from various groups were analyzed using one-way analysis of variance followed by Tukey's multiple range test. The *P* value <0.05 was considered to be statistically significant.

RESULTS

There was no significant change in body weight of rats subjected to sham surgery and partial abdominal aortic constriction with or without the *momordica charantia* treatment.

Effects of Ethanolic extract of Momordica Charantia Treatment on Morphologic and Haemodynamic Parameters: No significant change in body weight was observed in rats subjected to partial abdominal aortic constriction (PAAC) (Table 3). PAAC resulted in a significant increase in ratio of left ventricular weight to body weight (LVW/BW) and left ventricular wall thickness (LVWT) as compared to the control group and sham group. However, treatment with *momordica charantia* (200 and 400 mg/kg per day orally) significantly attenuated PAAC-induced increase in LVW/BW and LVWT in a dose-dependent manner (Table 3). PAAC significantly increased Mean arterial blood pressure (MABP) which was markedly attenuated in a dose dependent manner by EMC treatment (Table 3).

Effect of Ethanolic extract of Momordica Charantia Treatment on Biochemical Parameters: PAAC significantly increased protein content and RNA concentration in left ventricle. *Momordica charantia* (200 and 400 mg/kg per day orally) treatment significantly attenuated PAAC induced increase in protein content and RNA concentration. (Table 3)

Effect of Ethanolic extract of Momordica Charantia Treatment on Left Ventricular Collagen Content : A significant increase in left ventricle collagen content was observed in the rats subjected to PAAC as compared with rats in the sham treated group. The treatment with *momordica charantia* significantly attenuated PAAC-induced increase in collagen content in a dose-dependent manner (Table 3).

Effect of Ethanolic extract of Momordica Charantia on Electrophoretic Pattern of DNA: PAAC produced DNA smearing in agarose gel electrophoresis. The DNA smearing is the marker of necrotic cell death. *Momordica charantia* (200 and 400 mg/kg per day orally) significantly reduced PAAC induced DNA smearing (Figure 3).

DISCUSSION

The partial abdominal aortic constriction (PAAC) model used in this study [16, 17] involves placing a suture on the aorta below the diaphragm leading to pressure overload induced left ventricular hypertrophy. PAAC subjected for 4 weeks produced significant cardiac hypertrophy as witnessed by increased ratio of left ventricular (LV) weight to body weight, LV wall thickness, LV protein content, LV collagen deposition and LV RNA

concentration [19- 22]. *Momordica Charantia* treatment significantly attenuated PAAC-induced increase in LVW/BW, LVWT, LV protein content, LV RNA concentration and collagen deposition in a dose-dependent manner.

DNA smearing is an index of necrotic cell death [18, 22]. PAAC induced cardiac hypertrophy has been noted to produce DNA smearing which suggest an increase in necrotic cell death in left ventricle. Moreover, *momordica charantia* has been noted to attenuate PAAC induced increase in necrotic cell death perhaps due to PPAR agonist action.

The abdominal aortic constriction may be initially responsible to increase MABP, which has been observed to return to the normal value after about one and a half-hour of PAAC. However, MABP has been noted to increase gradually and attain peak level after 3-4 wk of PAAC. The marked increase in MABP in PAAC model may be due to pathological cardiac hypertrophy as reported recently [14, 18]. The PAAC induced increase in MABP has been noted to be attenuated by *momordica charantia* treatment. It suggests that PAAC induced cardiac hypertrophy may be responsible to increase MABP.

Momordica Charantia is reported as PPAR dual agonist and is able to inhibit hypertrophy probably through PPARα/γ dual agonistic mechanism. PPARα and PPARγ downregulation is responsible for the progression of cardiac hypertrophy which is observed by increased expression of fetal genes, increase in number of inflammatory cytokines, increased oxidative stress and decreased fatty acid oxidation [15-20]. Treatment with EMC activates PPARα/γ and is able to reverse cardiac hypertrophy which is shown by decreased oxidative stress, cytokines and increased fatty acid oxidation.

Hence, in the present study, we have focused on the effects of momordica charantia on cardiac hypertrophy in rats. To the best of our knowledge, it is the first study to explore the effect of PPAR- α / γ dual agonist in PAAC-induced cardiac hypertrophy.

Hence, on the basis of this discussion, it may be concluded that *Momordica Charantia* exhibits pleiotropic cardiac effects in PAAC-induced cardiac hypertrophy in a dose-dependent manner possibly through its PPAR (Peroxisome proliferator activated receptor) dual agonist action.

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