International Journal of Pharmaceutical Research and Development (IJPRD)
Platform for Pharmaceutical Researches & Ideas
www.ijprd.com

EVALUATION OF CARDIOPROTECTIVE ACTIVITY OF DIVYA METHIPACHAK AGAINTS ISOPROTERENOL INDUCED MYOCARDIAL DAMAGE IN RATS

Manodeep Chakraborty*[1],
Seema K. Setty[1], Jignasa S. Patel[1], Jagadish V. Kamath[1]

[1]Department of Pharmacology, Shree Devi College of Pharmacy, Mangalore-574142

ABSTRACT
The present study was designed to investigate the cardioprotective activity of the DivyaMethipachak apolyherbal formulationin isoproterenol (ISO)-induced myocardial necrosis in rats. The study was conducted by administering oral doses of DivyaMethipachak(150 and 300 mg/kg body weight) for 21 days and the standard group rats were administered with Carvedilol (10mg/kg) for last 7 days. All the prophylactic groups apart from normal control subjected to isoproterenol(85 mg/kg, sc for two consecutive days) induced myocardial necrosis. The degree of protection was determined by measuring levels of serum Lactate dehydrogenase (LDH), Creatine kinase iso enzyme-MB (CK-MB) andCreatine kinase iso enzyme-NAC(CK- NAC) and also by changes in ECG pattern.The prophylactic treatment with both high and low dose of DivyaMethipachakand Carvedilol group showed significant decrease in the above serum cardiac marker enzymes level and restoration of ECGchanges compared to ISO induced rats. To conclude DivyaMethipachak in both high and low dosewas found to be cardioprotective against ISO induced myocardial damage in rats.

KEYWORDS *: Anti-hyperlipidemic, Isoproterenol, DivyaMethipachak, Electrocardiographic parameters, Carvedilol.*

Correspondence Author

Manodeep Chakraborty

Shree Devi College of Pharmacy, Airport road, Kenjar, Mangalore-574142, INDIA

Email: manodeep.chakraborty@gmail.com

INTRODUCTION

Heart failure (HF) is a complex, progressive disorder in which the heart is unable to pump sufficient blood to meet the demand of the body.Underlying causes of HF include arteriosclerotic heart disease, myocardial infarction

(MI), hypertension, valvular heart disease and congenital heart disease. Coronary artery disease is the most common cause of HF and accounts for more than 70 % of all cases[1].

Cardiac myocytes rely on aerobic metabolism. MI occurs if the supply of oxygen remains below a critical value, which leads to cell death (by necrosis or apoptosis)[2]. Isoproterenol (ISO) is a synthetic catecholamine and β-adrenergic agonist has been documented to produce myocardial infarction in the heart muscles at larger doses. On autooxidation, ISO generates highly cytotoxic free radicals which bring about the loss of functions and integrity of myocardial membrane[3].

Multiple clinical trials witnessed the therapeutic potency of β-blockers as it improves clinical outcomes in a variety of pathological settings, including chronic heart failure and post-myocardial infarction.Carvedilol is one of the lipophilic, nonspecific ß-blocker with selective α1-adrenergic receptor blocker. It has been used in hypertension and cardiac disorders[4].With the evident therapeutic potential of Carvedilol, some of the side effects have been reported mainly chest pain and arrhythmia. Apart from that carvedilol is also responsible for sweating, shaking, or extreme hunger, signs of allergic reaction[5].

Now-a-days herbal products have gained greater acceptance than the allopathic medicines in some of the pathological conditions due to potency and apparent safety profile. In recent trend to achieve the maximum benefit of herbal therapy researcher combining a set of herbs in the form of specific formulation which is expected to deliver maximum potency compared to a single herb. DivyaMethipachak (DMP) is marketed polyherbal formulation, each 100g containing methidana (*Trigonellafoenumgraecum*) - 75mg, sajjikshar - 3mg, trikatu churn (*Zingiberofficinalis, Piper nigrum, Piper longum)* - 3mg, nimbu sat (*Citrus limon*) - 2mg, kalajeera (*Cuminumcymiunm*) - 2mg, sendhanamak (sandhavlavan) - 15mg. The presence of potential phytoconstituents such as alkaloids, flavonoids, saponins, carbohydrates, limonene, gallic acid, ellagic acid, ferulic acid and flavonols, monoterpenes, zingiberol, gingerol, piperine, volatile oil (caryophyllene), piperidine, piperlonguminine, terpinene, bergamotene, citronellal and linalool and this marketed polyherbal formulation claiming to possess cardioprotective activity and anti-obsesity[6]. But till now there is no scientific data has been documented against cardioprotective potential of the formulation. Hence, the present study was carried out to evaluate the cardioprotectiveactivity of DMP a polyherbal formulation on ISO induced MI in rats.

MATERIALS AND METHODS:

Experimental animals:

Laboratory bred Wistar albino rats (180-200 g) of either sex were housed at 25° ± 5°C in a well-ventilated animal house under 12:12 h light dark cycle. The animals had free access to standard food pellets (Amrut Laboratory Animal feed, Maharashtra, India) containing (% w/w) protein 22.10, oil 4.13, fibre 3.15, ash 5.15, sand (silica) 1.12, and water ad libitum. Bedding material was removed and replaced with fresh paddy husk as often as necessary to keep the animals clean and dry. The animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Drug and dosage:

The dried granules of the polyherbal preparation, DivyaMethipachak were supplied by the Patanjalichikitsalaya, manufactured by Divya Pharmacy Ltd., India. The formulation was administered at doses of 150 mg/kg p.o and 300 mg/kg p.o in the form of solution prepared in water. The doses were selected based on the human dose mentioned in the Ayurvedic literature.Isoproterenol hydrochloride was purchased from Sigma Aldrich, Germany. Carvedilol was purchased from Cipla Pharmaceutical Ltd, India. CK-MB,CK-NAC Combi kits were procured from Lab-Care diagnostic, Pvt.Ltd, Mumbai and LDH kits kits were procured from Accurex, India.Other chemicals used were obtained from SD Chemicals Ltd. (Mumbai, India). All chemicals used in the present study were of analytical grade.

Treatment protocol:

Wistar albino rats were divided into five groups of 6 animals each. Group I and II received saline for 21 days and termed as normal control and ISO control respectively. Group III served with standard drug Carvedilol (10 mg/kg, p.o), Group IV and V administered with test drug DMP (150 and 300 mg/kg,p.o) respectively for a period of 21 days.

Isoproterenol (ISO) induced myocardial necrosis in rats[7]:

At the end of the treatment period, Isoproterenol (ISO) (85 mg /kg, s.c) was administered to all the animals (except the normal control) for two consecutive days. 48 h after the first dose of ISO, the animals were anesthetised with ketamine (70mg/kg, i.p) and xylazine. (10mg/kg, i.p). Blood was withdrawn by retro-orbital puncture. Serum was separated by centrifugation for the estimation of biomarkers such as Lactate dehydrgenate (LDH), Creatinine kinase iso enzyme-MB (CK-MB) and Creatinine kinase isoenzyme-NAC (CK-NAC).

Electrocardiogaphic studies[8]:

Under anesthetic conditions induced by combination of ketamine hydrochloride (75 mg/kg, ip) and xylazine (8.0 mg/kg, ip), leads were attached to the dermal layer of both the front paws and hind legs and recording were made with the help of computerized ambulatory ECG system.

Histoarchitectural Studies:

The parts of the hearts stored in 10-percent(w/v) buffered formalin were embedded in paraffin,sections cut at 5 µm, and stained with hematoxylin and eosin. These sections were examined under a lightmicroscope for histoarchitectural changes.

The myocardial damage was determined by scoring method depending on the severity as follows[9], no change=00 score; mild=01 score (focal myocytes damage or small multifocal degeneration with slight degree of inflammation); moderate=02 score (extensive myofibrillar degeneration) and marked=03 score (necrosis with diffuse inflammation).

Statistical analysis:

Results are expressed as mean ± SE. Statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey-Karmer multiple comparison tests. $P<0.05$ was considered significant.

RESULT:

Effect on LDH and CK-MB and CK-NAC activities (Table 1) - The effects of DMP oral treatments on serum marker enzymes LDH, CK-MB and CK-NAC for 21 days are outlined in table 1. Rats treated with ISO showed a significant increase in activities of serum marker enzymes compared with the normal rat group. Pretreatment of DMP 150 and 300 mg/kg to rats for 21 days, followed by ISO subcutaneous injection on the 22nd and 23rd days, elicited a significant ($p<0.001$) reduction in the ISO-induced increased activities of LDH, CK-MB and CK-NAC.

Effect on histological score (figure 1) - Myocardial integrity was disturbed by administration of isoproterenol for two consecutive days that was evident with significant rise in histological score compared to normal control. Isoproterenol injections caused necrosis of cells with degeneration of myofibril and increased interstitial space. Prior treatment of animals before subjected to isoproterenol induced myocardial damage with CLOLandDMP showed significant fall in histological scores compared to ISO control. High dose of DMP found to cause mild multifocal degeneration with slight inflammation effect of combined therapy of CLOL with high dose of DMP showed least multifocal degeneration, mild inflammation with reduction in interstitial space.

Effect on electocardiographic parameters (Table 2)- Electrocardiographic determination revealed a significant increase in heart rate of ISO control compared to normal control. Prior treatment of animals with Carvedilol and graded doses of DMP resulted in significant fall in elevated chronotropic values compared to ISO control. Subcutaneous administration of isoproterenol for two consecutive days showed enlargement of QRS duration and QT interval compared to normal control. Prior treatment of animals with Carvedilol, DMP-150 and DMP-300 caused restoration of QRS duration and QT interval to normal conditions

compared to ISO control. Increase in PR and RR intervals were noted in ISO control animals compared to normal control. Prophylactic treatmentwithCarvediloland DMP-150/300 showed recovery from abnormal PR and RR interval.

Table 1- Effects of DMP and carvedilol on LDH, CK-MB, CK-NAC level in serum against isoproterenol induced myocardial infarction.

Group No.	Treatment	Blood serum level mg/dl		
		CK-MB	CK-NAC	LDH
1	Normal control	101.54±5.96	165.53±5.71	220.97±5.92
2	ISO control	438.53±2.86$^{\bullet\bullet\bullet}$	390.28±11.58$^{\bullet\bullet\bullet}$	949.85±2.70$^{\bullet\bullet\bullet}$
3	Carvedilol	180.49±2.39$^{\bullet\bullet***}$	203.71±7.09$^{\bullet\bullet***}$	252.66±3.84$^{\bullet\bullet***}$
4	LD DMP 150 mg/kg	337.74±6.60$^{\bullet\bullet\bullet***}$	301.74±5.67$^{\bullet\bullet\bullet***}$	371.75±2.26$^{\bullet\bullet\bullet***}$
5	HD DMP 300 mg/kg	284.05±6.66$^{\bullet\bullet\bullet***}$	247.74±1.51$^{\bullet\bullet\bullet***}$	315.24±3.65$^{\bullet\bullet\bullet***}$

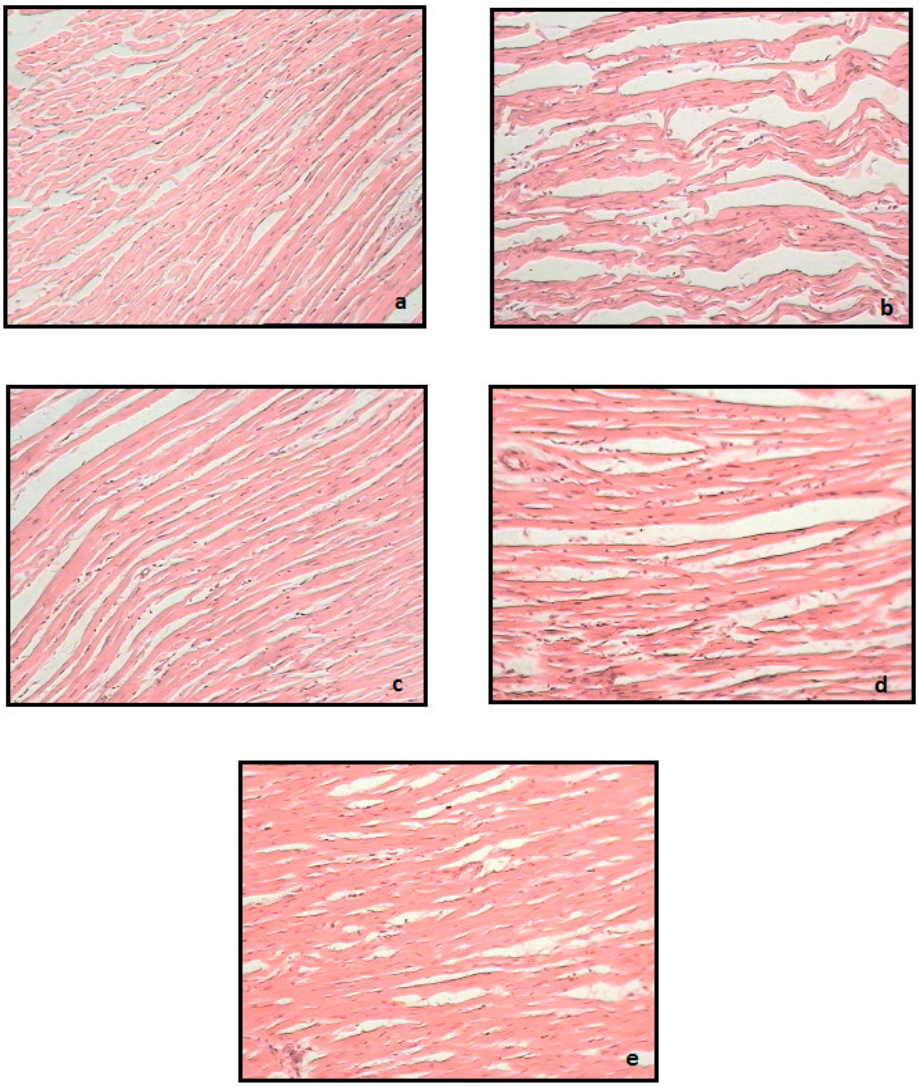
Values are expressed as mg/dl, mean ± SEM; n=6. $^{\bullet}$p<0.05, $^{\bullet\bullet}$p<0.01, $^{\bullet\bullet\bullet}$p<0.001 when compared to control, *p<0.05, **p<0.01, ***p<0.001 when compared to ISO control.

Table 2—Effects DMP and carvedilolon electocardiographic parameters in isoproterenol induced myocardial infarction.

Treatment	Heart rate	QRS duration (ms)	QT segment (ms)	RR interval (ms)	PR interval (ms)
Normal control	225.00±5.0	160.00±0.0	172.50±2.50	210.62±4.67	62.50±2.50
ISO control	435.00±5.0$^{\bullet\bullet\bullet}$	245.00±5.0$^{\bullet\bullet\bullet}$	296.87±3.12$^{\bullet\bullet\bullet}$	325.00±2.50$^{\bullet\bullet\bullet}$	150.00±5.0$^{\bullet\bullet\bullet}$
Carvedilol	227.50±2.5***	180.00±0.0***	217.00±2.00$^{\bullet\bullet\bullet***}$	244.00±4.00$^{\bullet\bullet***}$	85.00±5.00***
LD DMP 150 mg/kg	235.00±5.0***	200.00±0.0$^{\bullet\bullet**}$	277.50±2.00$^{\bullet\bullet\bullet*}$	272.50±2.50$^{\bullet\bullet\bullet***}$	97.50±2.5$^{\bullet***}$
HD DMP 300 mg/kg	215.00±5.0***	190.00±9.0 $^{\bullet***}$	262.50±2.50$^{\bullet\bullet\bullet***}$	257.50±2.50$^{\bullet\bullet\bullet***}$	80.00±0.0***

Values are expressed as mg/dl, mean ± SEM; n=6. $^{\bullet}$p<0.05, $^{\bullet\bullet}$p<0.01, $^{\bullet\bullet\bullet}$p<0.001 when compared to control, *p<0.05, **p<0.01, ***p<0.001 when compared to ISO control.

Figure 1:



Heart tissue from rats. a)-normal control (normal texture of cell); b)-isoproterenol (ISO) control (necrotic cells with degeneration of myofibril and increased interstitial space); c)-carvedilol group (recovery from necrosis with mild inflammation and less interstitial space); d)animals pretreated with LD DMP and isoproterenol (extensive myofibrillar degeneration); e)-animals pretreated with HD DMP and isoproterenol (small multifocal degeneration and slight inflammation).

DISCUSSION:

The aim of the present study was to elucidate the role of the polyherbal formulation DMP during for its cardioprotective activity in Isoproterenol (ISO) induced myocardial necrosis in rats. The result revealed beneficial effect of DMP in Isoproterenol (ISO) induced myocardial necrosis in rats.

Isoproterenol is a well-known cardiotoxic agent and widely used as an agent to evaluate the effect of drugs in the myocardial consequences of myocardial damages. By its positive inotropic and chronotropic actions, increases the myocardial oxygen demand that leads to ischemic necrosis of myocardium in rats. As a result of this, cytosolic enzymes such as LDH, Creatine kinase iso enzyme-MB andCreatine kinase iso enzyme-NACwere released into blood stream and serve as a sensitive index to assess the degree of myocardial necrosis[10].

A number of pathophysiological mechanisms have been proposed to explain the ISO-induced myocardial damage, including altered permeability, increased turnover of norepinephrine, and generation of cytotoxic free radicals on autooxidation of catecholamine[11].

Excessive activation of sympathetic system by isoproterenol accompanied by vagal hypo activityproduces severe myocardial damage[12]. This is evident by disturbances in electrocardiography due to isoproterenol. The cholinergic blockage has a direct impact on ECG by extension of QT interval[13]. The prolongation QT interval at times of myocardial stress is an indication of arrhythmias[14] and sudden cardiac collapse[15,16].

The Carvedilol is nonspecific adrenergic receptor blocker. It reduces blood pressure primarily from beta-adrenoceptor blockade and vasodilation, the latter resulting from alpha 1-adrenoceptor blockade. These multiple actions of carvedilol provide the underlying rationale for the use of the drug in the treatment of coronary artery disease and congestive heart failure[4].

The polyherbal formulation DMP in both low and high dose provides protection against myocardial necrosis by decreasing the LDH, CK-MB and CK-NAC level. The result of experimental data was further supported by histopathological analysis. As mentioned earlier, DMP contains 8 different herbal constituents and the formulation is described in the ancient ayurvedic literature. Apart from the presence of potential phytoconstituents such as steroidal saponins (diosgenin, yamogenin, tigogenin and neotigogenin), alkaloids (mainly trigonelline), zingiberol, piperine, piperlonguminine and free amino acids justify the potential of this herbal formulation. This may be due to the combination and complementary actions of the individual components of the polyherbal formulation.

Prophylactic therapy of DMP-150/300 mg/kg and CLOL (10mg/kg) was potent enough to avoid the prolongation of QT interval indicating absence of arrhythmias and cardioprotective potential. Similarly, abnormally elevated QRS duration, PR and RR intervals are also predictors of myocardial damage. Prior treatment of animals with DMP-150/300 mg/kg and CLOL showed substantial normalisation in electrocardiographic determinations and diminish the permeability of endogenous biomarkers to extracardiac regions. Among both the doses high dose of DMP was found to be more effective.

ACKNOWLEDGEMENTS:

The authors are thankful to the Management of Shree Devi College of Pharmacy for providing necessary facilities to conduct the research work and also thankful to Dr.JnaneshwarNayak, Patanjalichikitsalaya, Mangalore.

REFERENCES

1. Richard AH, Champe PC, Cubeddu LX, Clark MA. Lippincott's illustrated review: Pharmacology. 4th ed. New Delhi: Wolters Kluwer India Private Limited; 2009. p. 229.
2. Prabhu S, Mallika J, Sabitha KE, Shyamaladevi CS. Cardioprotective effect of mangiferin on isoproterenol inducednmyocardial infarction in rats. Indian J ExpBiol 2006; 44:209-15.
3. Panda VS, Naik SR. Evaluation of Cardioprotective Activity of Ginkgo biloba and Ocimum sanctum in Rodents. Altern Med Rev 2009 Jun;14(2):161-71.
4. Ruffolo RR Jr, Feuerstein GZ. Pharmacology of carvedilol: rationale for use in hypertension, coronary artery disease, and congestive heart failure. Cardiovasc Drugs Ther 1997 May;11(1):247-56.
5. http/carvedilol-side-effects.html retrieved on 2.6.2012. at 3.30 PM.
6. http://www.lovenaturalremedies.com/Divya-Yog-Mandir-Trust-Swami-Ramdev/Herbal-/-Ayurvedic/Other-medicines/Divya-Methi-Pachak-100-gm.html retrieved at 2/5/2011 at 7.00p.m
7. Buerke I, Prufer D, Dahm M, Meyer J, Oelert H, Darius H. Blocking of classical complement pathway inhibits endothelial adhesion molecule expression and preserves ischemic

myocardium from reperfusion injury. J PharmacolExpTher. 1998; 286:429-38.
8. Singh PN &Athar MS, Simplified calculation of mean QRS vector (mean electrical axis of heart) of electrocardiogram, Indian J PhysiolPharmacol, 2003; 47: 212.
9. Singh PN &Athar MS, Simplified calculation of mean QRS vector (mean electrical axis of heart) of electrocardiogram. Indian J PhysiolPharmacol, 2003; 47:212.
10. Maheswari C, Umadevi M, AnudeepaJ, Ramya R, Venkatnarayanan R. Cardioprotective effect Of *Orthosiphonstamineu*s on isoproterenol induced myocardial infarction in rat. Intr J Pharm Tech 2011 Sep;3(3):2896-904.
11. Noronha-Dutra AA, Steen EM, Woolf N. The correlation between catecholamine and lipid peroxidation induced damage in heart cells. Basic Res Cardiol 1985;80:133-136.
12. Ahnve S &Vallin H, Influence of heart rate and inhibition of autonomic tone on the QT interval, Cir Res, 65 (1982) 435.
13. Sun DQ, Nguyen N &DeGrado TR, Ischemia induces translocation of the insulin-responsive glucose transporter GLUT 4 to the plasma membrane of cardiac myocytes, Cir Res, 89 (1994) 793.
14. Zuanetti G, De Ferrari GM, Priori SG & Schwartz PJ. Protective effect of vagal stimulation on reperfusion arrhythmias in cats, Cir Res, 61 (1987) 429.
15. Schwartz PJ & Wolf S, QT interval prolongation as predictor of sudden death in patients with myocardial infarction, Cir Res, 57 (1978) 1074.
16. Ahnve S, Is QT interval prolongation a strong or weak predictor for cardiac death?, Cir Res, 84 (1991) 1862.

*****