Hardik Savsani. *et al.* / *International Journal of Phytopharmacology*, 5(2), 2014, 95-108.

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

CARDIOPROTECTIVE EFFECT OF FLAVONOIDS RICH FRACTION OF *Premna mucronata* ON ISOPROTERENOL–INDUCED MYOCARDIAL INFARCTION IN WISTAR RATS

Hardik Savsani*[1], Hital Shah[1], Kirti Patel[2], Tejal Gandhi[1]

[1]Department of Pharmacology, Anand Pharmacy college, Nr. Town Hall, Anand-388001, Gujarat, India.
[2]Department of Pharmacy, Faculty of Technology and Engineering,
The Maharaja Sayajirao University, Vadodara-390001, Gujarat, India.

ABSTRACT

Premna mucronata Roxb. is a herbal medicine that has been traditionally used as a natural remedy for myocardial infarction (MI) in india and a previous study revealed that *Premna mucronata* was proven to be cardioprotective in MI. Moreover, very little is known about the molecular mechanisms underlying cardioprotective activities of *Premna mucronata*. Flavonoid fraction is considered as the major active components in *Premna mucronata*. An endeavor has been made to evaluate the cardioprotective effect of flavonoids rich fraction of *Premna mucronata* (FPM) on isoproterenol-induced myocardial infarction in rats. FPM was isolated from *Premna mucronata*. It was identified by various chemical tests and detected by HPTLC. FPM at three dose level, viz 170 µg/kg, 300 µg/kg, 500 µg/kg were selected by dose fixation study and administered once a day p.o. for 28 days in Male Wistar Albino rats (200 ± 30 gm). Nebivolol (5 mg/kg) were used as standard drugs. On the 27th day MI was induced in rats by administration of the first dose of ISO (85 mg/kg, S.C) followed by second dose of ISO after 24 hours. At the end of experiment *i.e* 12 hour after second dose of ISO alteration of ECG pattern and hemodynamic changes measured. Both serum and heart was collected and used for the estimation of various biochemical parameters like CK-MB, LDH, α-HBDH, assay of Na^+/K^+ATPase, Ca^{++}ATPase, Mg^{++}ATPase, Estimation of Na^+, K^+ and Ca^{++}, MDA content, GSH, SOD and Catalase activity. Our result recommend that pretreatment with FPM decreased cardiac marker enzyme level, restoration of antioxidant status and maintained ECG pattern and hemodynamic changes at near normal values. Pretreatment with FPM propound the cardioprotective effect against ISO induced myocardial injury in rat as evidenced by comparing with normal control rats. These salubrious effect observed in the attempts might be due to antioxidant activity.

Key words: Isoproterenol, Myocardial Infarction, *Premna mucronata*, Flavonoids rich fraction.

INTRODUCTION

Cardiovascular disease (CVD) has become a universal cause of morbidity and a leading contributor to mortality in both developed and developing country. By 2020, CVD will be the paramount causes of death in india which include high blood pressure,coronary heart disease, stroke, congestive heart failure (Upaganlawar *et al*., 2011)

Corresponding Author

Hardik H. Savsani
Email: hardik_savsani@rediffmail.com

The identification of major risk factors through epidemiological studies and effective control strategies combining community education and targeted management of high risk individuals have contributed to the fall in CVD mortality rates that has been observed in almost all streamlined countries (Reddy and Yusuf, 1998).

Myocardial infarction (MI) is defined as irreversible injury or subsequent necrosis of myocardial cells due to interruption of blood supply to a part of the heart (Upaganlawar et al., 2012; Patel et al., 2010). Model of myocardial infarction was induced by Isoproterenol [1-(3,4- dihydroxyphenyl) -2- isopropyl aminoethanol

hydrochloride] (ISO), which is a synthetic catecholamine and β-adrenergic agonist. Initially, ISO caused severe stress in the myocardium resulting in necrosis of heart muscles which caused cardiac dysfunction, increased lipid peroxidation along with an increase in the level of myocardial lipids, altered activities of the cardiac enzymes and antioxidants (Wexler, 1978; Karthikeyan *et al*., 2007).

Despite the beneficial effects of modern medical therapy and surgeries, many patients eventually progress to an advanced stage characterized by severely limiting symptoms, marked hemodynamic impairment, frequent hospitalization and high mortality. Current medical therapies for MI are aimed at suppressing neurohormonal activation (e.g., angiotensin converting enzyme inhibitors, angiotensin II receptor antagonists, β-adrenergic receptor antagonists, and aldosterone receptor antagonists) and treating fluid volume overload and hemodynamic symptoms (diuretics, digoxin, inotropic agents). These pharmacotherapies for MI can improve clinical symptoms and slow the progression of contractile dysfunction and expansion of Left ventricular chamber volume, nevertheless, there is still progression, and the prognosis for even the optimally treated patient remains poor (Råmunddal, 2008). Thus there is a need for novel therapies for MI, independent of the neurohormonal axis that can improve cardiac performance and prevent MI.

Global trend of "back to nature", increased use of herbal medicine as Complementary and alternative medicines. The use of herbal medicine has rised over the last 5 years, with out of pocket costs. However, evidence based studies on the efficacy and safety of traditional Indian medicines is limited (Poongothai *et al*., 2002). Therefore, there is a need that experience based empirical knowledge if coupled with elucidation of the exact chemical in plant responsible for therapeutic action could provide a scientific basis to the herbal drugs and increase their acceptability. Such scientifically generated data will project herbal medicine in a proper perspective and help to sustain in global market.

With the above fact on hand, *Premna mucronata* Roxb. Plant was selected for the present study which has both antioxidant and hypocholestremics activities. Furthermore, *Premna mucronata* is also known for improving digestion, Blood purifier, Cardiac stimulant, Cough, expectorant, and also useful in skin disorders (Patel NG, 2012). Polyherbal marketed formulation containing *Premna mucronata* (Colonil Tab) is also prescribed for myocardial infarction by Ayurvedic practitioner.

Previously established the cardioprotective action of the whole extract of *Premna mucronata*, it was decided to establish constituent potency and to control the spectrum of bioactive chemical constituents naturally occurring in the plant. Such a concept was thought of chiefly because the use of plant extract in comparesion to morden medicine have not been taken seriously by many pharmaceutical industry. The main reason for such widespread acceptability of natural word to provide new biologically active constituent/formulation is because of the inadequate scientific data supports the efficacy of these drugs of plant origin.

The experience based empirical knowledge should be coupled with elucidation of the exact chemical in plant extract responsible for therapeutic action to provide a scientific basis to the herbal drug and increase their acceptability. Hence current research must be devoid to the isolation and characterization of the active constituents from the herbal plant source followed by clinical and toxicological studies to obtain a superior therapeutically effective drug and an economical remedy.

In line with above notion, it was found that *Premna mucronata* Roxb. Contain flavonoids like Luteolin, Apigenin, Hispidulin. Luteolin, a flavonoid is capable of protecting the myocardial injury, partly mediated through downregulation of NO production and its own antioxidant properties (Liao et al., 2010). Apigenin, a flavonoid is capable of protecting the myocardial injury, partly mediated through inhibit xanthine oxidase may help prevent formation of superoxide (O_2-) (Akhlaghi and Bandy, 2009). Thus it was decided to prepare a flavonoids rich extract from the previously tested and proved whole extract of *Premna mucronata* supporting the concept of actively graded fractionation.

Hence, the present study was undertaken to evaluate the effect of Flavonoids rich fraction of *Premna mucronata* in experimentally induced myocardial infarction by Isoproterenol.

MATERIAL AND METHOD

Collection and Identification of Plant material

The plant *Premna mucronata* Roxb obtained from a commercial supplier was identified and authenticated by Dr. Geetha K A (Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand, Gujarat, India). A voucher specimen no. APCH-25 has been deposited at the herbarium of M.Pharm department of Anand Pharmacy College.

Isolation of flavonoids rich fraction from *Premna mucronata* Roxb Flavonoids rich fraction of *Premna mucronata* Roxb was prepared according to (Harborne, 1998) with some modification.

Step-1: A small amount (20g) of dried whole plant *Premna mucronata* Roxb was cut into small pieces and blended. It was immersed in 20 ml diethyl ether and was shaken vigorously. Ether layer was discarded. This Powder was immersed in 200 ml of methanol (80%) and heated on a hot water bath for 15 min with continuous stirring at 55°C. The cooled extract was then filtered and the filtrate was reduced to 40ml on a waterbath at 90°C.

The methanolic aqueous extract was then transferred into 250ml separating funnel with 60 ml n-butanol was added to the combined methanolic aqueous extract.
Step-2: Butanolic fraction was discarded and methanolic aqueous fraction (40ml) was collected and evaporated to 20ml. To this was added 100 ml of 2M HCl and heated for 30-40 min at 100°C. The cooled extract was then extracted with 30 ml of ethylacetate. The ethylacetate layer was then separated and concentrated to dryness by evaporation at 55°C on a water bath to collect flavonoid rich precipitate (40mg, 0.2% w/w). The collected flavonoid rich fraction of *Premna mucronata* was stored in room temperature and identified by Shinoda test, alkaline reagent test and Vanillin HCL test.

Quantitative analysis of flavonoids rich fraction

Preparation of calibration curve of luteolin and apigenin

The luteolin and apigenin content in FPM was determined by HPTLC (Shinde *et al.*, 2011). A Standard stock solution (5mg/10ml) of luteolin and apigenin (Sigma Aldrich., USA) was prepared in methanol. Working solution of luteolin (200 µg/ml) and apigenin (50 µg/ml) was prepared by appropriate dilutions of the stock solution with methanol. Working solution of luteolin and apigenin 4, 8, 12, 16 and 20 µl were applied on a pre-coated silica gel 60 F_{254} TLC plate (E. Merck) of uniform thickness 0.2 mm using automatic TLC applicator. The plate was developed in 10×10 cm^2 twin trough glass chamber previously saturated with mobile phase Toluene : Ethyl acetate : Formic acid (6: 4: 0.3) v/v/v to a distance of 8 cm. The developed plates were dried and scanned using CAMAG TLC Scanner-3 at 280 nm and 268 nm for luteolin and apigenin respectively after spraying with Natural products-polyethylene glycol (NP-PEG) reagent and peak areas were recorded for all concentrations. Calibration curve of luteolin and apigenin was plotted as peak area versus concentration of luteolin and apigenin respectively.

Preparation of test sample (FPM)

An accurately weighed 100 mg of FPM was dissolved in 10 ml methanol and methanolic extract was filtered through whatman No. 1 filter paper. An aliquot of 20 µl was applied to the plate and developed as per procedure (Shinde *et al.*, 2011). From the calibration curve correlation coefficient (R^2) of luteolin and apigenin was found to be 0.994 and 0.991 respectively and regression equation of luteolin and apigenin was obtained as Y= 7.950X - 81.70 and Y= 2.768X + 252.7 respectively which was used for the calculation of luteolin and apigenin in the FPM.

Chemicals and Kits

Isoprenaline (Isoproterenol) hydrochloride is purchased from Sigma Chemical Co. (St Louis, MO, USA). Nebivolol was obtained as gift sample from Cadila Pharmaceuticals Ltd, Ahmedabad. All the chemicals used in this project were of analytical grade and were obtained from Astron chemicals, Ahmedabad and SD fine chemicals, Mumbai. All the biochemical tests were performed using the standard reagent kits purchased from Coral clinical systems, Goa.

Animals

Healthy albino wistar rats (Male) weighing 200 ± 30 gm were used in the present study (generously provided by Zydus Cadila, Ahmedabad, India). The animals were housed in a group of 3 rats per polypropylene cage (47 cm×26 cm×20 cm) lined with husk, renewed every 24 h under well-controlled conditions of temperature (22 ± 2°C), relative humidity (55 ± 5%) and 12:12 h light-dark cycle. The Animals were fed with standard pellet diet (VRK Nutritional Solutions, Sangli, Maharashtra, India) and tap water *ad libitum*. The experimental protocol was approved by Institutional Animal Ethical Committee in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, New Delhi (Protocol No: 1210, Dated: 24th November 2012). The experiment was conducted in accordance with accepted standard guidelines for the care and use of animals in scientific research.

Dose Fixation studies

Literature study revealed that the human dose for *Premna mucronata* was 1-3 gm. (Patel NG, 2012) Based on this dose range and extractive value, human dose are converted into animal (rat) dose by using factor 6.2 (FDA, 2005). Based on these 3 doses of FPM selected were 170 µg/kg, 300 µg/kg and 500 µg/kg and it was dissolved in distilled water.

Induction of experimental myocardial infarction

Isoproterenol was dissolved in normal saline and injected to rats (85 mg/kg, s.c.) at an interval of 24 h for 2 days to induce experimental MI (Rajadurai *et al.*, 2006).

Experimental design

After acclimatization, the animals were randomly allocated to 6 groups containing 6 animals each:
Group-1 (normal control): Animals received standard laboratory diet and drinking water *ad- libitum* and served as a normal control group.
Group-2 (ISO control): Animals were injected with isoproterenol (85 mg/kg, s.c.) at an interval of 24 h on 27th and 28th day and served as ISO control group.

Group-3 (nebivolol): Animals received nebivolol treatment (5 mg/kg, p.o.) for 28 days and were injected with isoproterenol (85 mg/kg, s.c.) on 27^{th} and 28^{th} day and served as Standard control group.
Group-4 (FPM-1): Animals received FPM-1 treatment (170 µg/kg, p.o.) for 28 days and were injected with isoproterenol (85 mg/kg, s.c.) on 27^{th} and 28^{th} day.
Group-5 (FPM-2): Animals received FPM-2 treatment (300 µg/kg, p.o.) for 28 days and were injected with isoproterenol (85 mg/kg, s.c.) on 27^{th} and 28^{th} day.
Group-6 (FPM-3): Animals received FPM-3 treatment (500 µg/kg, p.o.) for 28 days and were injected with isoproterenol (85 mg/kg, s.c.) on 27^{th} and 28^{th} day.

Non invasive blood pressure (NIBP) determination (indirect method)

Rats were trained for at least one week until the blood pressure was recorded with minimal stress and constraint. Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP) were measured at the end of treatment period using NIBP200A system with 11mm Tail Cuff Sensor (BIOPAC, CA, USA).

Electrocardiography

After recording the NIBP, Lead II ECG was measured in anaesthetized animals (Phenobarbitone 15 mg/kg, i.p) using needle electrodes. Electrodes were inserted under the skin in the right upper limb (-ve), right lower limb (neutral) and the left upper limb (+ve). ECG was recorded using MP36 data acquisition system (BIOPAC, CA, USA) and changes in different parameters were analyzed.

Biochemical analysis

After recording the ECG, the animals were sacrificed and blood samples were collected. Serum was separated from each sample and used for the biochemical analysis. The hearts were immediately isolated, washed with ice-cold phosphate-buffered saline (pH = 7.4), blotted, weighed and subjected to freezing at −80°C (CryoScientific, India) until further use.

Biochemical parameters in serum

Activities of cardiac marker enzymes creatinine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) were measured in the serum using commercial kits based on IFCC methods. The activity of alpha-hydroxybutyric dehydrogenase (α-HBDH) was assayed in serum using colorimetric method (Rosalki, 1962).

Biochemical parameters in heart homogenate

A known weight of heart tissue was homogenized in chilled Tris-HCl buffer (0.1 M, pH 7.4) using a Teflon pestle (EIE Instruments Pvt Ltd, 0603121) and centrifuged at 10,000 rpm at 0°C for 15 min (Plasto Craft Industries Pvt. Ltd, R4R-V/FA). The clear supernatant obtained was used for determination of Na^+/K^+ATPase, Ca^{++}ATPase and Mg^{++}ATPase activity by the method of (Bonting *et al.*, 1962), (Hjertén and Pan, 1983) and (Ohnishi *et al.*, 1982) respectively. The concentrations of Na^+ and K^+ in the heart were estimated using commercial kits. Ca^{++} in the heart was measured by the O-cresolphthalein complexone method using a reagent kit. The levels of reduced glutathione (GSH) and malondialdehyde (MDA) content were estimated by the method of (Moron *et al.*, 1979) and (Botsoglou *et al.*, 1994) respectively. The activities of superoxide dismutase (SOD) and catalase (CAT) were measured by the method of (Misra and Fridovich, 1976) and (Weydert and Cullen, 2009) respectively.

12 TTC staining

Myocardial necrosis was determined by a macroscopic enzyme technique of (Ertl *et al.*, 1982) which is performed by a pathologist Dr. Brahmbhatt (M.D. Pathologist).

Histopathology

Hearts were isolated immediately after sacrifice, washed with cold phosphate buffered saline (pH = 7.4) and fixed in 10% buffered formalin. Histopathology of heart was determined by the method of (Mohanty *et al.*, 2004) which is performed and examined by a pathologist Dr. Brahmbhatt (M.D. Pathologist).

Statistical analysis

Results were presented as Mean ± SEM. Statistical analysis of various biochemical parameters were carried out using the ANOVA followed by Dunnett's post hoc test with the help of computer based fitting program (Graph pad, Prism software, Ver.05, San Diego, CA). Data were considered statistically significant at $P<0.05$.

RESULTS

Concentration of different flavonoids in FPM

The content of luteolin and apigenin in FPM was found to be 0.1% and 31.1% respectively by HPTLC which is illustrated in Fig.2.

Effect of FPM on heart weight

Fig.3 shows the heart weight of each group mentioned in experimental design 2.8. ISO injected rats significantly ($P<0.05$) increased in the heart weight as compared to normal control rats. Nebivolol treatment significantly ($P<0.05$) decreased in the heart weight as compared to ISO injected rats. Similarly, pretreatment of FPM-1, FPM-2 and FPM-3 significantly ($P<0.05$) decreased in the heart weight as compared to ISO injected rats.

Effect of FPM on ECG pattern

Fig.4. shows the representative ECG tracings of normal and experimental animal. Changes in ST-segment and R-amplitude are depicted in Fig.5. Different ECG parameters like P wave, QRS complex, QT interval, R-R interval and QTc interval are summarized in Table 1. ISO injected rats significantly (P<0.05) increased in ST-segment, QT interval and QTc interval along with significantly (P<0.05) decreased in R-amplitude, P wave, QRS complex and R–R interval as compared to normal control rats. Nebivolol treatment significantly (P<0.05) prevented ISO induced changed in ECG pattern. Pretreatment of FPM-1 significantly (P<0.05) decreased in QT interval and QTc interval along with significantly (P<0.05) increased in R-amplitude and P wave as compared to ISO injected rats. Pretreatment of FPM-2 and FPM-3 significantly (P<0.05) decreased in ST-segment, QT interval and QTc interval along with significantly (P<0.05) increased in R-amplitude, P wave, QRS complex and R–R interval as compared to ISO injected rats.

Effect of FPM on hemodynamic changes

Representative hemodynamic changes in each group are depicted in Fig.6. Different hemodynamic parameters like SBP, DBP, MBP and heart rate are summarized in Table 2. ISO injected rats significantly (P<0.05) decreased in DBP and MBP along with significantly (P<0.05) increased in heart rate as compared to normal control rats. Nebivolol treatment significantly (P<0.05) increased in MBP along with significantly (P<0.05) decreased in heart rate as compared to ISO injected rats. Pretreatment of FPM-2 significantly (P<0.05) increased in MBP along with significantly (P<0.05) decreased in heart rate as compared to ISO injected rats. Pretreatment of FPM-3 significantly (P<0.05) increased in DBP and MBP along with significantly (P<0.05) decreased in heart rate as compared to ISO injected rats.

Effects of FPM on cardiac marker enzymes

Table 3. represents the effects of FPM treatment on cardiac marker enzymes including CK-MB, LDH and α-HBDH. ISO injected rats significantly (P<0.05) increased in CK-MB, LDH and α-HBDH as compared to normal control rats. Nebivolol treatment significantly (P<0.05) decreased in CK-MB and LDH as compared to ISO injected rats. Pretreatment of FPM-1, FPM-2 and FPM-3 significantly (P<0.05) decreased in CK-MB, LDH and α-HBDH as compared to ISO injected rats.

Effect of FPM on levels of electrolytes and activities of membrane bound enzymes

Table 4. represents the effects of FPM treatment on levels of electrolytes such as Na^+, K^+, Ca^{++} and activities of membrane bound enzymes including Na^+/K^+ATPase, Ca^{++}ATPase and Mg^{++}ATPase. ISO injected rats significantly (P<0.05) increased in Na^+, Ca^{++}, Ca^{++}ATPase and Mg^{++}ATPase along with significantly (P<0.05) decreased in K^+ and Na^+/K^+ATPase as compared to normal control rats. Nebivolol treatment significantly (P<0.05) prevented ISO induced changed in levels of electrolytes and activities of membrane bound enzymes. Pretreatment of FPM-1 significantly (P<0.05) decreased in Na^+ and Ca^{++}ATPase as along with significantly (P<0.05) increased in K^+ compared to ISO injected rats. Pretreatment of FPM-2 and FPM-3 significantly (P<0.05) decreased in Na^+, Ca^{++}, Ca^{++}ATPase and Mg^{++}ATPase as along with significantly (P<0.05) increased in K^+ and Na^+/K^+ATPase compared to ISO injected rats.

Effect of FPM on lipid peroxidation and activities of antioxidant enzymes

Lipid peroxidation such as MDA content and Different antioxidant enzymes like GSH, SOD and CAT are summarized in Table 5. ISO injected rats significantly (P<0.05) increased in MDA content along with significantly (P<0.05) decreased in GSH, SOD and CAT as compared to normal control rats. Nebivolol treatment significantly (P<0.05) prevented ISO induced changed in MDA content and activities of GSH, SOD and CAT. Pretreatment of FPM-1 significantly (P<0.05) increased in GSH and CAT compared to ISO injected rats. Pretreatment of FPM-2 and FPM-3 significantly (P<0.05) decreased in MDA content along with significantly (P<0.05) increased in GSH, SOD and CAT as compared to ISO injected rats.

Effect of FPM on microscopic enzyme assay (TTC)

Fig.7. Shows representative TTC staining in each group. ISO injected rats showed large regions of damaged tissue (unstained) as compared to normal control rats. Pretreatment with nebivolol, FPM-1, FPM-2 and FPM-3 increased intensity of staining in myocardium as compared to ISO injected rats.

Effect of FPM on histopathology

Fig.8. illustrates the histopathological photographs of heart tissues of normal and experimental rats. Histopathological examination of myocardial tissue obtained from normal control rats exhibited clear integrity of myocardial membrane, normal cardiac fibers without any infarction and infiltration of inflammatory cells was not seen in this group. ISO injected rats showed widespread myocardial structure disorder and subendocardial necrosis with capillary dilatation and leukocyte infiltration as compared to normal control rats. Pretreatment with nebivolol and FPM depicted decreased infiltration of inflammatory cells and the morphology of cardiac muscle fibers was relatively well preserved with no evidence of focal necrosis.

Table 1. Effect of FPM on ECG parameter of ISO induced myocardial infracted rats (Mean ± SEM)

Groups (n=6)	P wave (s)	QRS complex (s)	QT interval (s)	R–R interval (s)	QTc interval (s)
Normal control	0.073±0.002	0.089±0.004	0.099±0.001	0.203±0.000	0.217±0.037
ISO control	0.038±0.006#	0.047±0.007#	0.148±0.009#	0.169±0.004#	0.269±0.048#
Nebivolol	0.069±0.005*	0.074±0.000*	0.105±0.003*	0.196±0.009*	0.225±0.083*
FPM-1	0.042±0.006*	0.055±0.009	0.139±0.006*	0.178±0.006	0.254±0.072*
FPM-2	0.057±0.009*	0.069±0.001*	0.123±0.001*	0.184±0.004*	0.238±0.022*
FPM-3	0.064±0.007*	0.075±0.003*	0.110±0.000*	0.197±0.007*	0.225±0.018*

Indicate significantly different from Normal control group at P<0.05.
* Indicate significantly different from Model control group at P<0.05.

Table 2. Effect of FPM on hemodynamic changes and heart rate of ISO induced myocardial infracted rats (Mean ± SEM)

Groups (n=6)	SBP (mmHg)	DBP (mmHg)	MBP (mmHg)	Heart rate (BPM)
Normal control	125.4±6.56	98.3±8.32	112.0±9.23	367.2±11.94
ISO control	115.5±12.78	70.2±9.32#	82.3±7.34#	478.8±13.50#
Nebivolol	120.9±9.23	85.4±15.56	110.6±12.53*	398.5±17.66*
FPM-1	115.0±10.20	69.2±12.42	85.1±14.53	444.8±10.57
FPM-2	117.4±14.36	78.4±18.32	97.9±9.42*	420.2±4.14*
FPM-3	120.6 ±8.55	87.7±13.48*	107.8±10.43*	392.8±14.06*

Indicate significantly different from Normal control group at P<0.05.
* Indicate significantly different from Model control group at P<0.05.

Table 3. Effect of FPM on activities of cardiac marker enzymes in serum samples of ISO induced myocardial infracted rats (Mean ± SEM)

Groups (n=6)	CK-MB (U/L)	LDH (U/L)	α-HBDH (U/L)
Normal control	18.51±1.48	297.6±9.44	57.88±7.30
ISO control	270.5±26.64#	688.6±12.81#	133.5±21.38#
Nebivolol	94.09±2.59*	307.0±5.15*	101.9±4.44
FPM-1	115.3±20.70*	571.90±10.91*	94.7±36.18*
FPM-2	71.26±7.68*	459.50±14.64*	69.23±25.12*
FPM-3	29.68±6.77*	368.9±9.07*	55.77±17.92*

Indicate significantly different from Normal control group at P<0.05.
* Indicate significantly different from Model control group at P<0.05.

Table 4. Effect of FPM on levels of electrolytes and activities of membrane-bound ATPase enzymes in heart homogenate of ISO induced myocardial infracted rats (Mean ± SEM)

Groups (n=6)	Na^+ (mmol/l)	K^+ (mmol/l)	Ca^{++} (mg/dl)	Na^+/K^+ ATPase (mmol/l)	Ca^{++} ATPase (mmol/l)	Mg^{++} ATPase (mmol/l)
Normal control	147.40±2.39	5.38±0.51	7.35±0.58	5.69±0.19	4.65±0.19	2.75±0.12
ISO control	213.8±21.41#	3.09±0.38#	14.19±2.71#	2.79±0.17#	7.71±0.18#	5.02±0.13#
Nebivolol	159.1±1.23*	4.76±0.24*	8.42±0.62*	4.75±0.19*	4.84±0.16*	3.03±0.13*
FPM-1	109.7±23.95*	8.23±2.45*	11.04±2.38	3.28±0.19	6.84±0.18*	4.74±0.15
FPM-2	92.27±13.91*	8.92±0.47*	9.33±2.56*	4.30±0.10*	5.72±0.19*	3.48±0.15*
FPM-3	87.45±7.73*	9.22±0.76*	4.65±0.91*	5.18±0.16*	3.99±0.31*	2.59±0.30*

Indicate significantly different from Normal control group at P<0.05.
* Indicate significantly different from Model control group at P<0.05.

Table 5. Effect of FPM on lipid peroxidation and activities of antioxidant enzymes in heart homogenate of ISO induced myocardial infracted rats (Mean ± SEM)

Groups (n=6)	MDA (µg/ml)	GSH (µg/ml)	SOD (µg/ml)	CAT (mmol of H_2O_2/min/gm of tissue)
Normal control	0.16±0.01	10.08±0.64	17.49±0.79	69.52±2.57
ISO control	0.31±0.02#	3.94±0.67#	7.862±1.68#	26.65±3.57#
Nebivolol	0.13±0.01*	10.1±0.56*	14.73±1.21*	68.52±2.40*
FPM-1	0.22±0.02	9.72±1.80*	13.25±3.34	68.09±7.69*
FPM-2	0.11±0.02*	10.06±2.34*	15.38±3.04*	71.93±9.90*
FPM-3	0.10±0.03*	10.42±1.88*	19.39±4.03*	79.30±19.48*

(Units: MDA, GSH and SOD - µg/ml; CAT- mmol of H_2O_2/min/gm of tissue)
Indicate significantly different from Normal control group at P<0.05.
*Indicate significantly different from Model control group at P<0.05.

Fig 1. Photograph of (A) *Premna mucronata* Roxb. Plant (B) Flavonoid rich fraction of *Premna mucronata* Roxb.

A

B

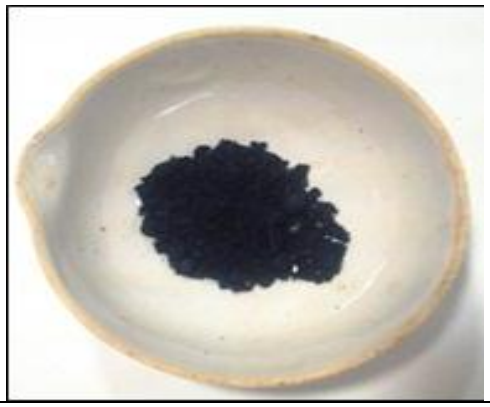
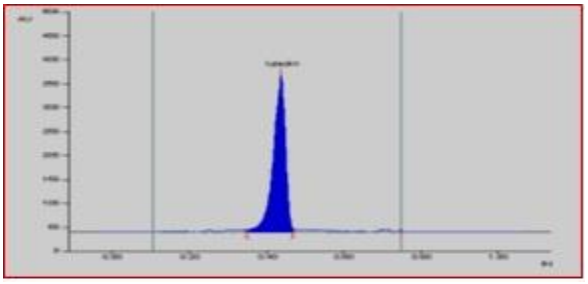
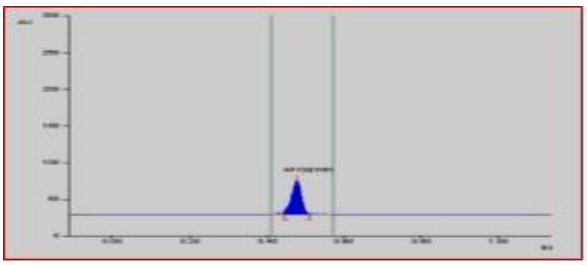


**Fig 2. (A) HPTLC chromatogram of (1) standard luteolin and (2) standard apigenin.
(B) Calibration curve of (1) standard luteolin and (2) standard apigenin.
(C) Flavonoid rich fraction of *Premna mucronata* (1) R_f value of 0.45 indicate luteolin and (2) R_f value of 0.49 indicate apigenin**

A

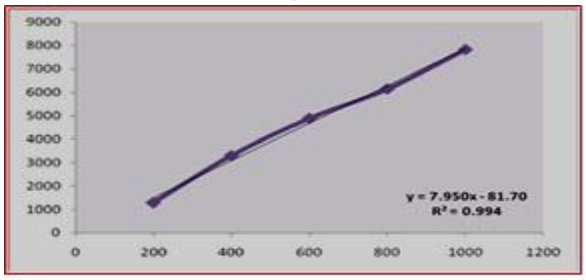


1

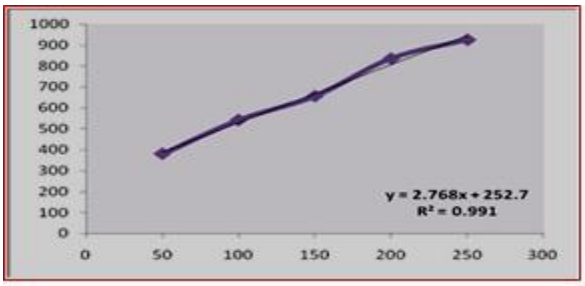


2

B



1



2

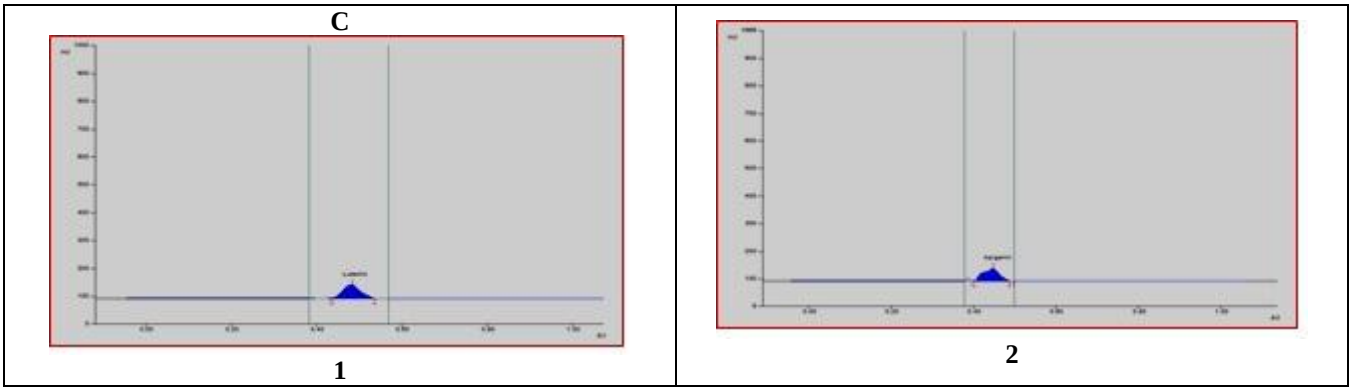
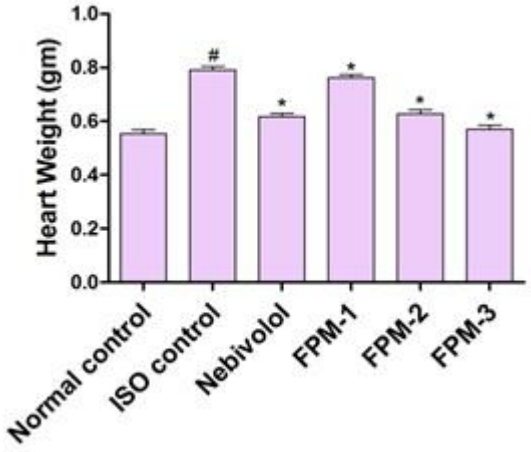


Fig 3. Effect of FPM on heart weight of ISO induced myocardial infracted rats (Mean ± SEM)



Indicate significantly different from Normal control group at P<0.05.
* Indicate significantly different from Model control group at P<0.05.

Fig 4. Representative electrocardiographic pattern in each group

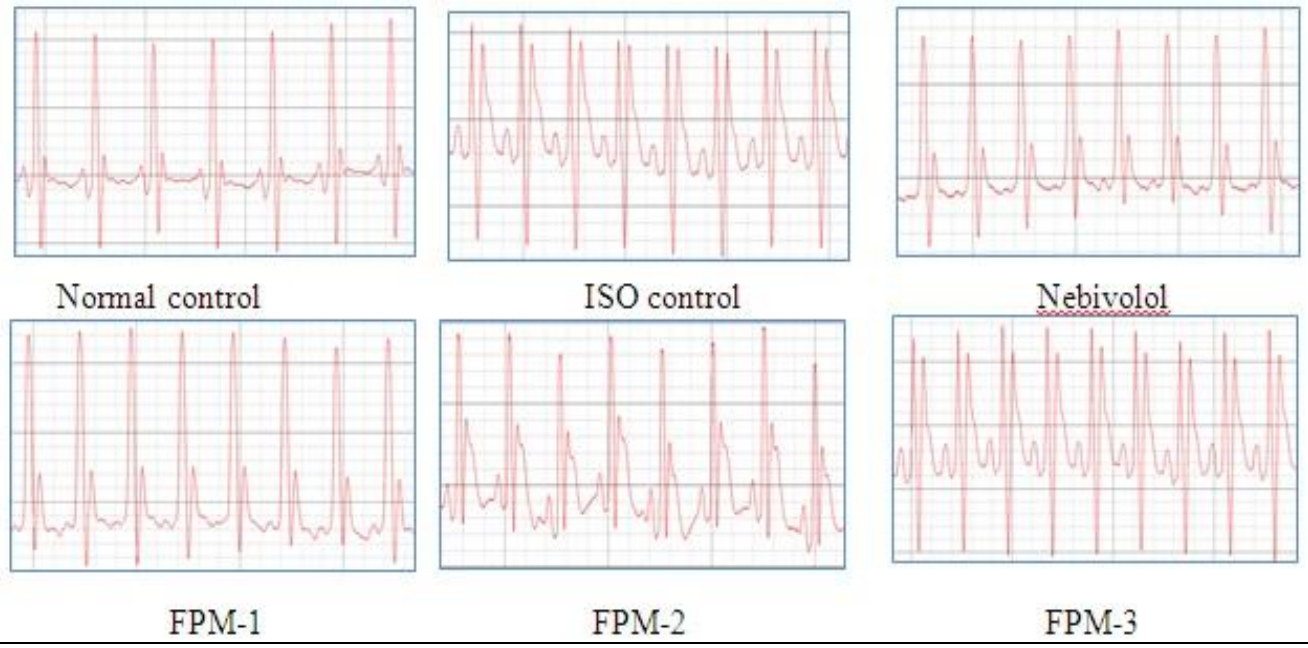


Fig 5. Effect of FPM on ST-segment and R-amplitude of ISO induced myocardial infracted rats (Mean ± SEM)

Indicate significantly different from Normal control group at P<0.05.

* Indicate significantly different from Model control group at P<0.05.

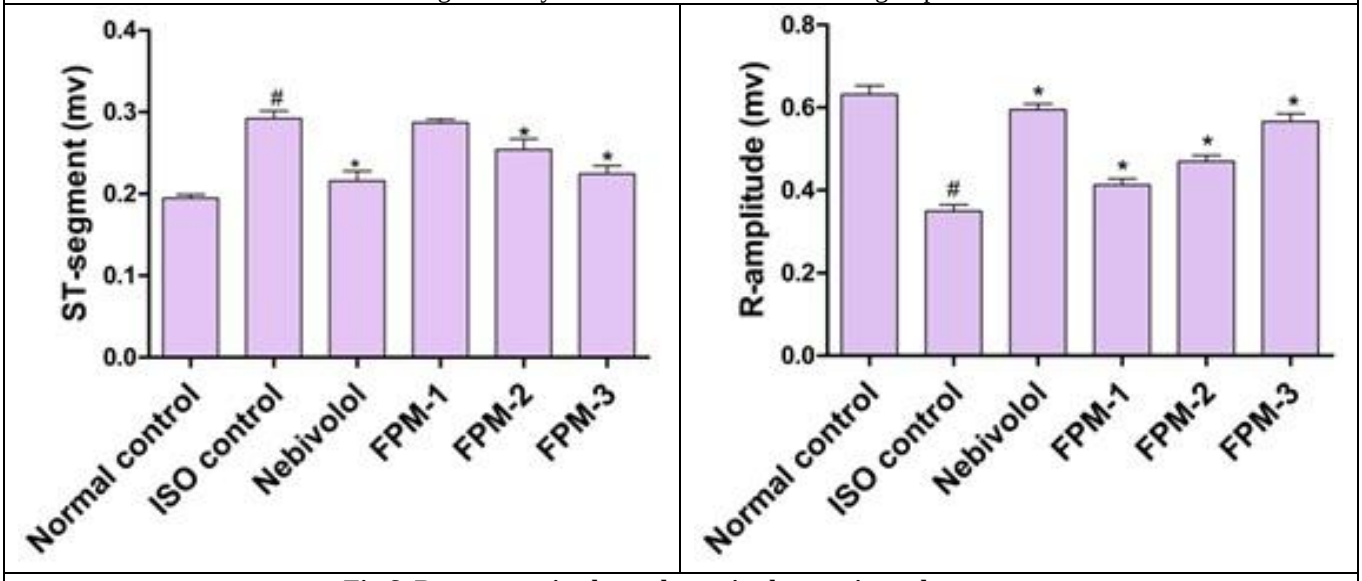


Fig 6. Representative hemodynamic changes in each group.

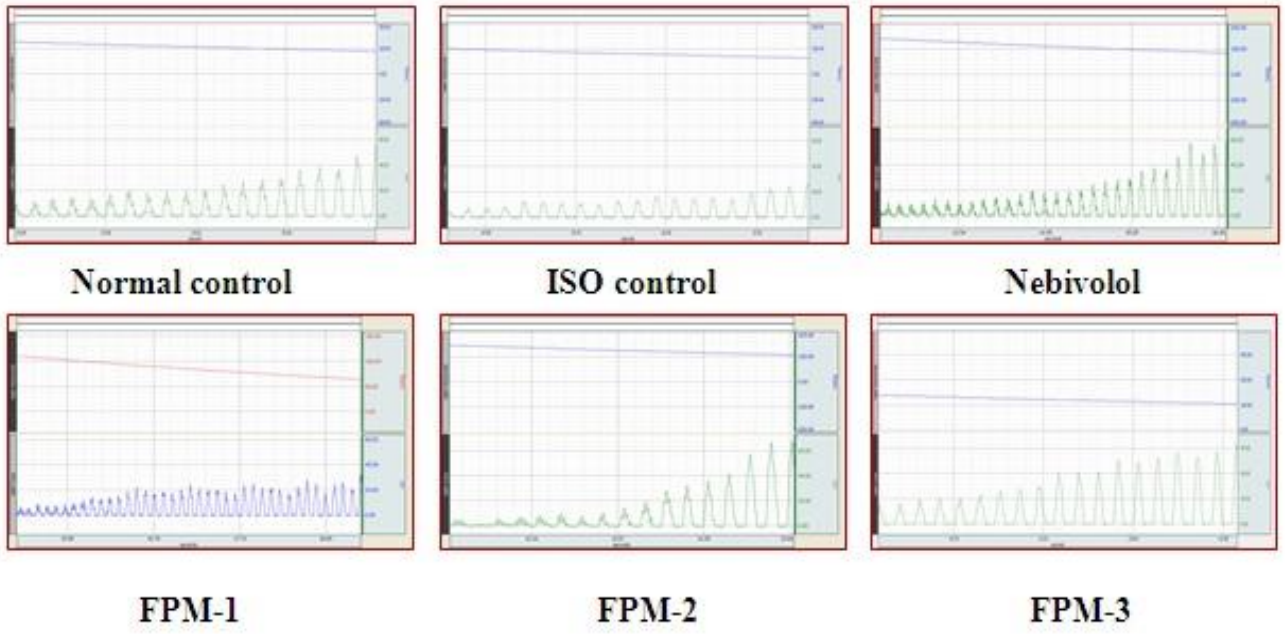


Fig 7. Representative TTC staining in each group.

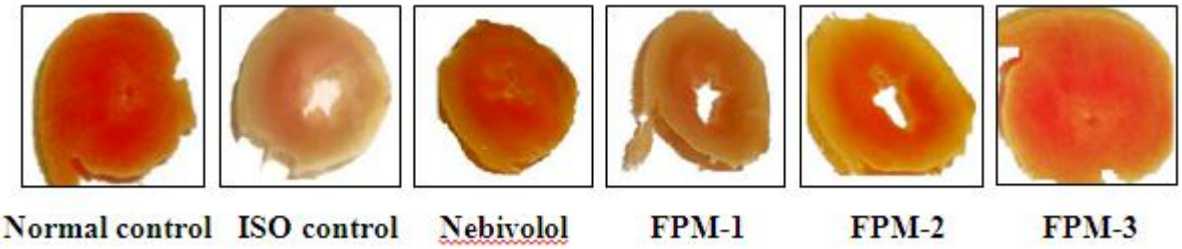
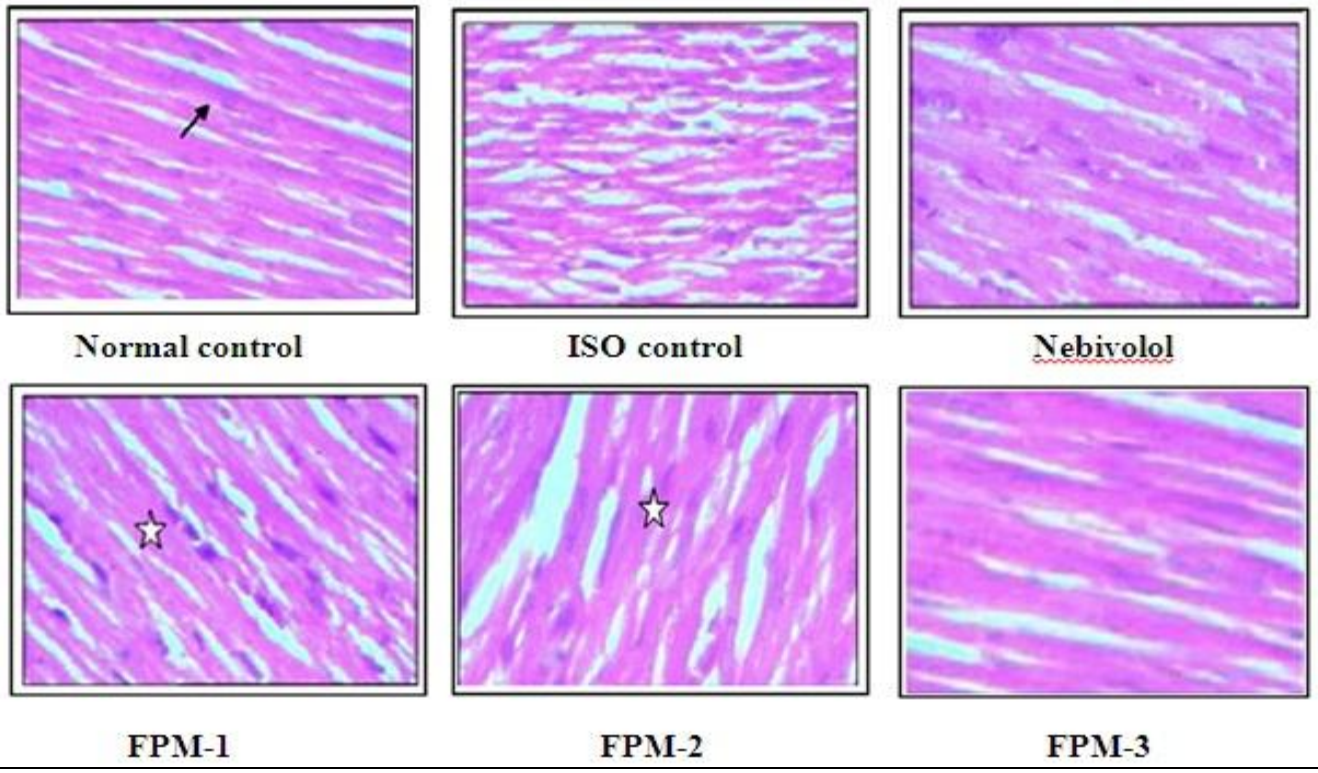


Fig 8. Representative Histopathological changes in each group. (Heart tissues were stained with hematoxylin and eosin and visualized under light microscope at 40× magnification)



DISCUSSION

Historically, there has been tacit agreement as to the meaning of the term ‘‘myocardial infarction’’ is the irreversible injury and subsequent necrosis in a wave front from subendocardium to subepicardium due to severe and prolonged reduction in coronary perfusion (Buja, 2005).

In this project, myocardial infarction model was induced in male wistar rats on 27^{th} and28^{th} day by Isoproterenol (85 mg/kg, S.C) administration. ISO is sympathomimetic β-adrenergic receptor agonist, causes severe stress to the myocardium resulting in an infarct like necrosis of heart muscle. The rat model of isoproterenol induced myocardial necrosis serves as a well accepted standardized model to evaluate several cardiac disfunction (Wexler, 1978) and to study the efficacy of various natural and synthetic cardioprotective agent (Rathore *et al.*, 1998). ISO induced myocardial infarction is widely used experimental model for several reasons. The model is characterized by an extraordinary technical, simplicity, an excellent reproducibility as well as an acceptable low mortality. Myocardial infarction induced by ISO has been reported to show many metabolic and morphologic aberrations in the heart tissue of the experimental animals similar to those observed in human myocardial infarction (Nirmala and Puvanakrishnan, 1996). ISO induced necrosis is maximal in the subendocardial region of the left ventrical and in the interventricular septum (Boluyt *et al.*, 1995). The model for myocardial infarction in wistar rats was successfully established by ISO (85 mg/kg, S.C) as apparent from the myocardial necrosis. In the present study, we found that FPM treatment exerts a strong cardioprotection in ISO induced MI.

Following isoproterenol administration, Increase in heart weight might be attributed to increased water content, edematous intramuscular space (Upaganlawar *et al.*, 2009) and increased protein content. These results are in consistent with the previous report (Judd and Wexler, 1974), which has observed extensive edematous intramuscular space, accumulation of mucopolysaccharides and cellular infiltration after 4 h of induction of myocardial infarction. It has been proposed that a 1% increase in myocardial water content could be expected to result in possibly a 10% reduction in myocardial function (Patel *et al.*, 2010). Pretreatment of Nebivolol and FPM brings down the heart weight. It is indicative of its protection of myocardium against infiltration and it also could be due to the decrease in water content of the myocardium.

Electrocardiograph-abnormalities are the main criteria generally used for the definite diagnosis of myocardial infarction. In the present study ISO administration in rats showed a decrease in P wave

intensity, QRS complex, R–R interval, R-amplitude and elevation of ST-segment, QT interval and QTc interval. These changes could be due to the consecutive loss of cell membrane in injured myocardium (Holland and Brooks, 1977). The consecutive loss of cellular membrane damage due to oxidative stress might be characterized by ST elevation (Koti *et al.*, 2009). The Q wave appears when the infarcted muscle is electrically inert and the loss of forces normally generated by the infarcted area leaves unbalanced forces of variable magnitude in the opposite direction from the remote region. Decreased R-amplitude might be due to the onset of myocardial edema following isoproterenol administration (Patel *et al.*, 2010). Nebivolol treatment in isoproterenol treated rats prevented the altered ECG pattern. Pretreatment with FPM in isoproterenol treated rats prevented the altered ECG pattern towards normal suggesting the cell membrane stabilizing potential of FPM which might be due to its potent antioxidant property.

In the present study ISO administration in rats showed fall in SBP, DBP, MBP and increase in heart rate. Changes in hemodynamic parameter indicated the activation of sympathetic nervous system (Goyal *et al.*, 2010). It has been demonstrated that an increase in heart rate is responsible for increased oxygen consumption leading to accelerated myocardial necrosis (Rona, 1985). The change in hemodynamic parameter in standard control was not significant as compared to ISO control animals. Pretreatment with FPM significantly prevented ISO induced change in hemodynamic parameter and heart rate which is evidence by rise in SBP, DBP, MBP and decrease in heart rate.

Myocardium contains abundant concentrations of diagnostic markers of myocardial infarction. ISO administration in rats showed significant rise in activites of CKMB, LDH and α-HBDH as compared to normal control. It has been reported that myocardial necrosis show membrane permeability alterations which bring about the loss of function and integrity of myocardial membranes (Todd *et al.*, 1980). All the macromolecules to leak from damaged tissues, enzymes (because of their tissue specificity and catalytic activity) are the best markers of tissue damage. Increased activities of these marker enzymes in the serum are indicative of cellular damage, severity of necrotic damage and loss of functional integrity of cell membrane (Bhakta *et al.*, 1999). The levels of these enzymes present in serum are reported to be directly proportional to the number of necrotic cells present in the cardiac tissue (Geetha *et al.*, 1990). CK-MB isoenzyme activity is useful not only as an index of early diagnosis of MI, but any type of myocardial injury. α-HBDH release was observed to start after 1 h of anoxia and to increase to 84% of intracellular α-HBDH activity after 7 h of anoxia (Dissmann *et al.*, 1998). Nebivolol treatment prevented ISO induced change in activities of the cardiac marker enzymes. Pretreatment with FPM significantly lowered activities of the cardiac marker enzymes in serum. It demonstrated that FPM could maintain membrane integrity thereby restricting the leakage of these enzymes.

ATPases are membrane bound enzymes involved in energy mediated translocation of Na^+, Ca^{++} K^+ and Mg^{++}ions (Upasani and Balaraman, 2001). The present study showed decreased activities of Na^+/K^+ATPase and Mg^{++}ATPase and increased activity of Ca^{++}ATPase in isoproterenol injected rats, which is in line with the previous report (Upaganlawar *et al.*, 2009). An increase in sodium and calcium along with decrease in potassium were observed in ISO injected rats. Increased concentration of sodium might be due to decrease in Na^+/K^+ATPase (Jennings *et al.*, 1986). Na^+/K^+ATPase is a lipid-dependant enzyme containing –SH group and increased lipid peroxidation leads to oxidation of the protein and in turn inactivates the enzymes (Jayachandran *et al.*, 2009). Depletion of ATP by ISO leads to opening of K^+ channel leading to the decrease in K^+ ions in the myocardial tissue. Increased level of intracellular Na^+ concentration also operates to depress Ca^{++} effect and augment Ca^{++} influx. The increased activity of Ca^{++}ATPase may be due to activation of adenylate cyclase (Patel *et al.*, 2010). Nebivolol treatment in isoproterenol treated rats maintains the levels of electrolytes and activities of membrane bound enzymes. Pretreatment with FPM significantly maintains the levels of electrolytes towards normal and these effects of FPM could be due to the prevention of 'SH' group of the ATPase from oxidative damage through the inhibition of peroxidation of membrane lipids indicating the membrane stabilizing effect of FPM.

Lipid peroxidation (LPO) is an important pathogenic event in myocardial infarction and the accumulated lipid peroxides reflects the various stages of the disease and its complication (Golikov *et al.*, 1989). MDA is a major lipid peroxidation end product. ISO administration in rats showed increased MDA content may contribute to increased generation of free radicals or decreased activities of antioxidant defense system (Zhou *et al.*, 2006). Increased level of lipid peroxides injures blood vessels, increasing adherence and aggregation of platelets to the injured sites (Gryglewski *et al.*, 1978). Thus, lipid peroxidation in vivo has been identified as one of the basic deteriorative reactions in cellular mechanisms of myocardial ischemia. Amongst the various mechanisms proposed to explain isoprenaline-induced cardiac damage, generation of highly cytotoxic free radicals through auto-oxidation of catecholamines has been implicated as one of the important causative factor (Peer *et al.*, 2008). Nebivolol treatment in isoproterenol treated rats prevented lipid peroxidation. Pretreatment with FPM significantly decrease in the level of

myocardial MDA content which can be attributed to potent antioxidant activity of FPM against isoproterenol auto-oxidation generated free radicals.

In the present study we observed a decreased concentration of GSH in the heart of isoproterenol injected rats. GSH, a tri-peptide, is one of the most abundant non-enzymatic antioxidant bio-molecules present in the body. GSH has a direct antioxidant function by reacting with superoxide radicals, peroxy radicals and singlet oxygen followed by the formation of oxidized GSH and other disulfides (Meister, 1988). Decreased glutathione levels in myocardial infarction may be due to its increased utilization in protecting SH containing proteins from lipid peroxides. Decreased activity of these enzymes lead to accumulation of oxidants and make myocardial cell membranes more susceptible to oxidative damage. Furthermore, inactivation of glutathione reductase, an enzyme responsible for conversion of oxidized glutathione (GSSG) to GSH, leads to accumulation of GSSG which in turn inactivates enzymes containing –SH group and inhibits protein synthesis (Lil *et al.*, 1988). Nebivolol treatment in isoproterenol treated rats increased concentration of endogenous antioxidant. The present study observed a significant rise in the concentration of GSH in the heart of FPM pre-co-treated rats.

Activities of antiperoxidative enzymes (SOD and catalase) were decreased significantly in the heart tissue of isoproterenol injected animals. SOD is a class of enzymes, which catalyses the dismutation of two superoxide radicals to form hydrogen peroxide and molecular oxygen. Thus, generated H_2O_2 is inactivated by catalase (Jayalakshmi *et al.*, 2006). During myocardial infarction, superoxide radicals generated at the site of damage, modulates SOD, resulting in the loss of activity and accumulation of superoxide radical, which damages myocardium. Due to oxidative stress there is increased utilization of antioxidant enzymes like SOD (Manjula *et al.*, 1994). CAT is protected the cellular constituents against oxidative damage. A significant reduction in the activity of CAT with a concomitant increase in LPO observed in ISO group due to excessive generation of free radicals by ISO. Nebivolol treatment prevented ISO induced change in activities of antiperoxidative enzymes. Pretreatment with FPM restored the decreased levels of SOD and CAT in heart tissue which can be attributed to direct free radical scavenging effect of FPM or its ability to protect antioxidant enzymes from oxidative damage.

As discussed earlier ISO induce ischemic injury resulting in necrosis as was identified from TTC-stained myocardial slices. Other researchers have also reported induction of ischemic injury with ISO (Upaganlawar *et al.*, 2012; Prabhu *et al.*, 2006). Area of infarction indicates loss of membrane integrity which might be due to significant leakage of lactate dehydrogenase enzyme. Further increase in nitrosative stress and reactive oxygen species production led to an enlarged infarct size in the ISO induced MI (Upaganlawar *et al.*, 2012). The present study showed unstained of heart section in ISO injected rats. Nebivolol treatment in isoproterenol treated rats significantly increased staining in heart tissue. Pretreatment with FPM significantly increased staining in heart tissue which can be attributed to their potent antioxidant activity which prevents leakage of lactate dehydrogenase enzyme.

Histopathological examination of myocardial tissue in ISO injected rats showed coagulative necrosis, separation of cardiac muscle fibers and infiltration of inflammatory cells. Nebivolol treatment prevented ISO induced change in Histopathology of myocardial tissue. The reduced inflammatory cell infiltration and normal cardiac muscle fiber architecture further confirmed the cardioprotective effect of FPM.

CONCLUSION

In conclusion, FPM (170 µg/kg, 300 µg/kg and 500 µg/kg) p.o. once for 28 days significantly counteracted the effect of ISO induced MI by restoration of antioxidant status, inhibiting lipid peroxidation, attenuation of serum marker enzymes, increased tissue Calcium & Sodium levels, decreased Potassium levels along with normalized ECG and hemodynamic changes. This finding might be a scientific support to understand the beneficial effects of FPM on cardioprotection against myocardial injury, in which oxidative stress has long been known to contribute to the pathogenesis.

ACKNOWLEDGEMENT

The authors are cordially thankful to all co-investigators, his guide Dr Kirti V. Patel and Dr Tejal R. Gandhi, Principal, Anand Pharmacy College for providing all necessary facilities.

REFERENCES

Akhlaghi M & Bandy B. Mechanisms of flavonoid protection against myocardial ischemia–reperfusion injury. *Journal of molecular and cellular cardiology*, 46, 2009, 309-317.

Bhakta T, Mukherjee P K, Mukherjee K, et al. Evaluation of hepatoprotective activity of< i> Cassia</i>< i> fistula</i> leaf extract. *Journal of ethnopharmacology*, 66, 1999, 277-282.

Boluyt M, Long X, Eschenhagen T, et al. Isoproterenol infusion induces alterations in expression of hypertrophy-associated genes in rat heart. *American Journal of Physiology-Heart and Circulatory Physiology*, 269, 1995, H638-H647.

Bonting SL, Caravaggio LL & Hawkins NM. Studies on sodium-potassium-activated adenosinetriphosphatase. IV. Correlation with cation transport sensitive to cardiac glycosides. *Archives of biochemistry and biophysics*, 98, 1962, 413-419.

Botsoglou NA, Fletouris DJ, Papageorgiou GE, et al. Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. *Journal of Agricultural and Food Chemistry*, 42, 1994, 1931-1937.

Buja L M. Myocardial ischemia and reperfusion injury. *Cardiovascular pathology*, 14, 2005, 170-175.

Dissmann R, Linderer T & Schröder R. Estimation of enzymatic infarct size: Direct comparison of the marker enzymes creatine kinase and α-hydroxybutyrate dehydrogenase. *American Heart Journal*, 135, 1998, 1-9.

Ertl G, Kloner RA, Alexander RW, et al. Limitation of experimental infarct size by an angiotensin-converting enzyme inhibitor. *Circulation*, 65, 1982, 40-48.

FDA C. Guidance for Industry, Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. *Center for Drug Evaluation and Research, US Department of Health and Human Services*, 2005, 7.

Geetha A, Sankar R, Marar T, et al. Alpha-tocopherol reduces doxorubicin-induced toxicity in rats--histological and biochemical evidences. *Indian journal of physiology and pharmacology*, 34, 1990, 94.

Golikov A, Polumiskov V, Davydov B, et al. Lipid peroxidation and the major factors of its activation in patients with myocardial infarction]. *Kardiologiia*, 29, 1989, 53.

Goyal S, Arora S, Sharma A, et al. Preventive effect of crocin of< i> Crocus sativus</i> on hemodynamic, biochemical, histopathological and ultrastuctural alterations in isoproterenol-induced cardiotoxicity in rats. *Phytomedicine*, 17, 2010, 227-232.

Gryglewski R J, Dembińska-kiéć A, Żmuda A, et al. Prostacyclin and thromboxane A< sub> 2</sub> biosynthesis capacities of heart, arteries and platelets at various stages of experimental atherosclerosis in rabbits. *Atherosclerosis*, 31, 1978, 385-394.

Harborne J B. Phytochemical methods A Guide to modern techniques of plant analysis, springer, City, 1998,

Hjertén S & Pan H. Purification and characterization of two forms of a low-affinity Ca< sup> 2+</sup>-ATPase from erythrocyte membranes. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 728, 1983, 281-288.

Holland RP & Brooks H. TQ-ST segment mapping: critical review and analysis of current concepts. *The American journal of cardiology*, 40, 1977, 110-129.

Jayachandran K, Vasanthi HR & Rajamanickam G. Antilipoperoxidative and membrane stabilizing effect of diosgenin, in experimentally induced myocardial infarction. *Molecular and cellular biochemistry*, 327, 2009, 203-210.

Jayalakshmi R, Thirupurasundari C & Devaraj SN. Pretreatment with alcoholic extract of shape *Crataegus oxycantha* (AEC) activates mitochondrial protection during isoproterenol–induced myocardial infarction in rats. *Molecular and cellular biochemistry*, 292, 2006, 59-67.

Jennings RB, Reimer KA & Steenbergen C. Myocardial ischemia revisited. The osmolar load, membrane damage, and reperfusion. *Journal of molecular and cellular cardiology*, 18, 1986, 769-780.

Judd J T & Wexler B C. Myocardial glycoprotein changes with isoproterenol-induced necrosis and repair in the rat. *American Journal of Physiology--Legacy Content*, 226, 1974, 597-602.

Karthikeyan K, Bai B & Devaraj S N. Cardioprotective effect of grape seed proanthocyanidins on isoproterenol-induced myocardial injury in rats. *International journal of cardiology*, 115, 2007, 326-333.

Koti B, Vishwanathswamy A, Wagawade J, et al. Cardioprotective effect of lipistat against doxorubicin induced myocardial toxicity in albino rats. 2009,

Liao P-H, Hung L-M, Chen Y-H, et al., Cardioprotective effects of luteolin during ischemia-reperfusion injury in rats. *Circulation journal: official journal of the Japanese Circulation Society*, 75, 2010, 443-450.

Lil J, Stantman F & Lardy H. Antioxidant enzyme systems in rat liver and skeletal muscle. *Arch Biochem Biophys*, 263, 1988, 150-160.

Manjula T, Deepa R & Shyamala Devi C. Effect of aspirin on lipid peroxidation in experimental myocardial infarction in rats. *The Journal of Nutritional Biochemistry*, 5, 1994, 95-98.

Meister A. Glutathione metabolism and its selective modification. *J Biol Chem*, 263, 1988, 17205-17208.

Misra H P & Fridovich I. The oxidation of phenylhydrazine: superoxide and mechanism. *Biochemistry*, 15, 1976, 681-687.

Mohanty I, Singh Arya D, Dinda A, et al. Protective effects of< i> Curcuma longa</i> on ischemia-reperfusion induced myocardial injuries and their mechanisms. *Life sciences*, 75, 2004, 1701-1711.

Moron M S, Depierre J W & Mannervik B. Levels of glutathione, glutathione reductase and glutathione< i> S</i>-transferase activities in rat lung and liver. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 582, 1979, 67-78.

Nirmala C & Puvanakrishnan R. Protective role of curcumin against isoproterenol induced myocardial infarction in rats. *Molecular and cellular biochemistry*, 159, 1996, 85-93.

Ohnishi T, Suzuki T, Suzuki Y, et al. A comparative study of plasma membrane Mg< sup> 2+</sup>-ATPase activities in normal, regenerating and malignant cells. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 684, 1982, 67-74.

Patel NG, Patel KV, Gandhi TR, et al. Evaluation of the Cardioprotective effect of *Premna mucronata* Roxb (Verbenaceae) in the experimental model of myocardial ischemia-reperfusion injury. *Int J Mod Pharm Res*, 1, 2012, 1-20.

Patel V, Upaganlawar A, Zalawadia R, et al. Cardioprotective effect of melatonin against isoproterenol induced myocardial infarction in rats: a biochemical, electrocardiographic and histoarchitectural evaluation. *European journal of pharmacology*, 644, 2010, 160-168.

Peer PA, Trivedi PC, Nigade PB, et al. Cardioprotective effect of Azadirachta indica A. Juss. on isoprenaline induced myocardial infarction in rats. *International journal of cardiology*, 126, 2008, 123.

Poongothai S, Karkuzhali K, Sharadha J, et al. Evaluation of safety and efficacy of Hyponidd, an Ayurvedic compound: a double blind, placebo controlled study in type 2 diabetic patients with secondary failure to oral drugs. *Int J Diab Dev Ctries*, 22, 2002, 19-27.

Prabhu S, Jainu M, Sabitha K, et al. Role of mangiferin on biochemical alterations and antioxidant status in isoproterenol-induced myocardial infarction in rats. *Journal of ethnopharmacology*, 107, 2006, 126-133.

Rajadurai M, Prince M & Stanely P. Preventive effect of naringin on lipids, lipoproteins and lipid metabolic enzymes in isoproterenol-induced myocardial infarction in wistar rats. *Journal of Biochemical and Molecular Toxicology*, 20, 2006, 191-197.

Råmunddal TA. Myocardial metabolism in experimental infarction and heart failure. 2008,

Rathore N, John S, Kale M, et al. Lipid peroxidation and antioxidant enzymes in isoproterenol induced oxidative stress in rat tissues. *Pharmacological Research*, 38, 1998, 297-303.

Reddy K S & Yusuf S. Emerging epidemic of cardiovascular disease in developing countries. Circulation, 97, 1998, 596-601.

Rona G. Catecholamine cardiotoxicity. *Journal of molecular and cellular cardiology*, 17, 1985, 291-306.

Rosalki S. A simple colorimetric method for the determination of serum alpha-hydroxybutyric dehydrogenase activity. *Journal of Clinical Pathology*, 15, 1962, 566.

Shinde DB, Chavan MJ & Wakte PS. HPTLC in Herbal Drug Quantification. High-Performance Thin-Layer Chromatography (HPTLC). Springer, 2011.

Todd GL, Cullan GE & Cullan GM. Isoproterenol-induced myocardial necrosis and membrane permeability alterations in the isolated perfused rabbit heart. *Experimental and molecular pathology*, 33, 1980, 43-54.

Upaganlawar A, Gandhi C & Balaraman R. Effect of green tea and vitamin E combination in isoproterenol induced myocardial infarction in rats. *Plant foods for human nutrition*, 64, 2009, 75-80.

Upaganlawar A, Gandhi H & Balaraman R. Isoproterenol induced myocardial infarction: Protective role of natural products. *J. Pharmacol. Toxicol*, 6, 2011, 1-17.

Upaganlawar A, Patel V & Balaraman R. Tomato lycopene attenuates myocardial infarction induced by isoproterenol: Electrocardiographic, biochemical and anti–apoptotic study. *Asian Pacific journal of tropical biomedicine*, 2, 2012, 345-351.

Upasani C & Balaraman R. Effect of vitamin E, vitamin C and spirulina on the levels of membrane bound enzymes and lipids in some organs of rats exposed to lead. *Indian Journal of Pharmacology*, 33, 2001, 185-191.

Wexler B C. Myocardial infarction in young vs old male rats: pathophysiologic changes. *American Heart Journal*, 96, 1978, 70-80.

Weydert CJ & Cullen JJ. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. *Nature protocols*, 5, 2009, 51-66.

Zhou B, Wu L-J, Li L-H et al. Silibinin protects against isoproterenol-induced rat cardiac myocyte injury through mitochondrial pathway after up-regulation of SIRT1. *Journal of pharmacological sciences*, 102, 2006, 387-395.