

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

Cardioprotective Effect of Fisetin on Lipid Peroxide Metabolism in Isoproterenol Induced Myocardial Infarction in Male Wistar Rats

Mythily Panneerselvam¹, P.T.Devika²

Research Scholar, PG and Research Department of Biochemistry, Mohamed Sathak College of Arts & Science, Sholinganallur, Tamil Nadu, India¹.

Assistant Professor, PG and Research Department of Biochemistry, Mohamed Sathak College of Arts & Science, Sholinganallur, Tamil Nadu, India².

ABSTRACT: The present study aims to evaluate the cardioprotective effect of Fisetin on lipid peroxides and antioxidant enzymes (enzymatic and non-enzymatic antioxidants) in isoproterenol induced Myocardial infarction in wistar rats. Cardiac toxicity is induced by Isoproterenol in rats, administered for an interval of 24 hrs for 2 days through subcutaneous injection. Rats induced by Isoproterenol have shown a significant increase in the activities of thiobarbituric acid reactive substances, lipid hydroperoxides in heart tissue and plasma and a significant decrease in the levels of circulatory enzymatic antioxidants like catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase in heart and the activities of reduced glutathione, Vitamin C and Vitamin E in plasma and heart tissue and ceruloplasmin in plasma. Pre-treatment of fisetin (10, 20 and 30mg/kg) orally for a period of 30 days have showed a significant decrease the level of lipid peroxidation products and increased the activities by improving the enzymatic and non-enzymatic antioxidants. Thus, the study exposes that Fisetin prevents cardioprotective effect against isoproterenol induced Myocardial infarction for its free radical scavenging and antioxidant effects, which keep up the tissue protection system against cardiac damage.

KEYWORDS: Fisetin, Myocardial infarction, Isoproterenol, Lipid peroxides and Antioxidants

I. INTRODUCTION

Myocardial infarction (MI) is one of the most death causing disease for both men and women [1]. Myocardial infarction makes an increase contribution to mortality and morbidity statistics in developing countries due to their lifestyle changes mostly in urban areas [2]. MI occurs as a outcome of prolonged imbalance between coronary supply of blood and demand of myocardium. There is a strong evidence that indicates ischemic tissue produces oxygen free radicals, it contains odd number of electrons, that's makes chemically reactive and often leading to chain reaction which contributes to cell death [3,4]. The model of isoproterenol-induced myocardial infarction is considered as most widely used experimental method to study the beneficial effects of many drugs and cardiac function [5]. Isoproterenol, a β -adrenergic agonist and catecholamine has been reported to show many metabolic and morphological abnormalities in the heart tissue of the experimental animals similar to those in human myocardial infarction [6]. It is also well known that Isoproterenol generate free radicals and stimulate lipid peroxidation, which may cause irreversible damage to membrane of myocardium [7]. Epidemiological studies have shown that intake of diet rich fruits, herbs and spices are associated with low risk of cardiovascular disease [8]. Recently, attention has been focused on non-nutrient phytochemicals and flavonoids, derived from different plant species as effective therapeutic agents in the prevention and management of cardiovascular disease due to their antioxidant nature [9]. Fisetin (3,3',4',7-tetrahydroxyflavone,

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

Figure 1), a dietary phenolic flavonoid rich in strawberry, onion, persimmon and cucumber. It has a wide variety of pharmacological activities such as antioxidant and anti-inflammatory activity mainly acting as a free radical scavenger [10]. Fisetin is able to scavenge free radicals produced by Isoproterenol, as a result of its electron donating capacity, due to the presence of two hydroxyl groups on one ring and a hydroxyl group in another ring.

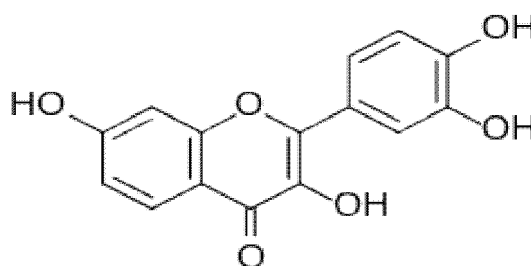


Fig.1. Structure of the flavonoid fisetin

However no systematic studies were found to be done on effect of Fisetin on experimental animal model of Myocardial infarction. Hence the present study was aimed to evaluate the cardioprotective effect of Fisetin on lipid peroxide metabolism and antioxidant enzyme activity on Isoproterenol induced Myocardial infarction in male albino wistar rats.

II. MATERIALS AND METHODS

Experimental animals and diet

The experiments were carried out according to the guidelines of the Committee and approved by the Institutional animal ethical committee (IAEC/XLI/03/CLBMCP/2017). All the experiments were done with healthy male albino wistar rats weighing 150 – 180 g. They were housed in polypropylene cages (47 cm x 34 cm x 20 cm) lined with husk, renewed every 24 h under a 12:12 h light dark cycle at around 22°C. The rats had free access to tap water and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Ltd., Maharashtra, India). The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.25% crude fibre, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6% phosphorus, 2.46% glucose, 1.8% vitamins and 56.17% nitrogen free extract (carbohydrates). The diet provided a metabolisable energy of 3000 kcal.

Drug and chemicals

Fisetin was purchased from Sigma Chemical Company, Deisenhofen, Germany. Isoproterenol hydrochloride, nitroblue tetrazolium, phenazine methosulphate, butylated hydroxyl toluene, 1-chloro-2,4-dinitrobenzene, 2,4-dinitrophenylhydrazine, p-phenylenediamine and glutathione were purchased from Sigma Chemical Co., St. Louis, MO, USA. All the other chemicals used in this study were of analytical grade and obtained from E. Merck and HIMEDIA, India.

Induction of experimental myocardial infarction

Isoproterenol (100 mg/kg) was dissolved in normal saline and subcutaneously injected to rats at an interval of 24 hrs for 2 days.

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

Experimental design

The animals were grouped into six rats in each group: Group I: Normal control rats; Groups II, III and IV: Normal rats were treated with Fisetin (10, 20 and 30 mg/kg); Group V: ISO-control rats (100 mg/kg) alone; Groups VI, VII and VIII: rats were pretreated with Fisetin (10, 20 and 30 mg/kg) and then subcutaneously injected with Isoproterenol (100mg/kg). Fisetin was dissolved in 0.5% dimethyl sulfoxide (DMSO) and orally administered using an intragastric tube daily for a period of 30 days. On the 31st day at 9.00 am, the first dose of isoproterenol was subcutaneously induced to rats. Twelve hours after the second dose of ISO-injection, all the rats were anesthetized and then sacrificed by cervical decapitation. Blood was collected and serum and plasma were separated by centrifugation. Heart tissue were excised immediately and rinsed in ice-chilled normal saline and the whole heart was weighed.

Processing of heart sample

Heart tissue were removed immediately and washed in ice cold physiological saline containing 0.9% sodium chloride. Heart tissue sample were homogenized in the appropriate buffer in a homogenizer and used for the following biochemical parameters.

Biochemical estimations

The activity of thiobarbituric acid reactive substances (TBARS) was estimated by the methods of Yagi [12] and Fraga et al., [13]. The values were expressed as nM/ml and mM/100g wet tissue. The levels of lipid hydroperoxides were estimated by the method of Jiang et al., [14]. Values were expressed as $\times 10^{-5}$ mM/dL for plasma and mM/100g wet tissue for heart. The activities of superoxide dismutase (SOD) was assayed according to the procedure of Kakkar et al., [15]. The activity of SOD was expressed as units/mg protein. The activities of catalase (CAT) were assayed by the methods of Sinha., [16], The activity of catalase was expressed as $\mu\text{mol of H}_2\text{O}_2$ consumed/min/mg protein. The activities of glutathione peroxidase (GPx), glutathione reductase (GRx) and glutathione-S-transferase (GST) Rotruck et al., [17], Horn and Burns., [18] and Habig and Jakoby., [19], respectively. The levels of reduced glutathione (GSH), vitamin C, vitamin E and ceruloplasmin were estimated by the methods of Ellman [20], Omaye et al., [21], Baker et al., [22] and Ravin [23], respectively. The content of protein in the heart homogenate was determined by the method of Lowry et al., [24].

Statistical Analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using Statistical Package for the Social Sciences (SPSS) software package version 17.00. Results were expressed as mean \pm SD for six rats in each group. P values < 0.05 were considered significant.

III. RESULTS

Figure- 2(a,b) shows the levels of TBARS in plasma and the heart of normal and experimental rats. Rats induced with Isoproterenol hydrochloride showed a significant ($P < 0.05$) increase in the levels of TBARS in plasma and the heart when compared to normal control rats. Oral pretreatment with Fisetin (10, 20 and 30 mg/kg) to ISO-induced rats daily for a period of 30 days significantly ($P < 0.05$) decreased the levels of TBARS in plasma and the heart when compared with ISO alone induced rats.

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

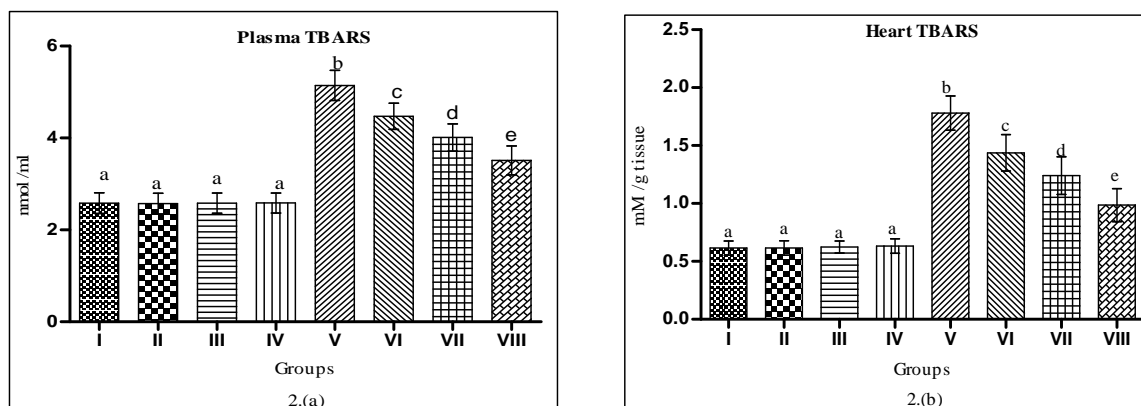


Fig 2(a,b)-Effect of Fisetin on the levels of TBARS in plasma and heart of normal and isoproterenol (ISO)-induced myocardial infarction in rats. Each value is mean \pm SD for six rats in each groups; values not sharing a common superscript (a, b, c, d, e) differ significantly with each other ($P < 0.05$, DMRT)

Figure-3(a,b) shows the levels of lipid hydroperoxide (HP) in plasma and the heart of normal and experimental rats. Rats induced with Isoproterenol hydrochloride showed a significant ($P < 0.05$) increase in the levels of HP in plasma and the heart when compared to normal control rats. Oral pretreatment with Fisetin (10, 20 and 30 mg/kg) to ISO-induced rats daily for a period of 30 days significantly ($P < 0.05$) decreased the levels of HP in plasma and the heart when compared with ISO alone induced rats.

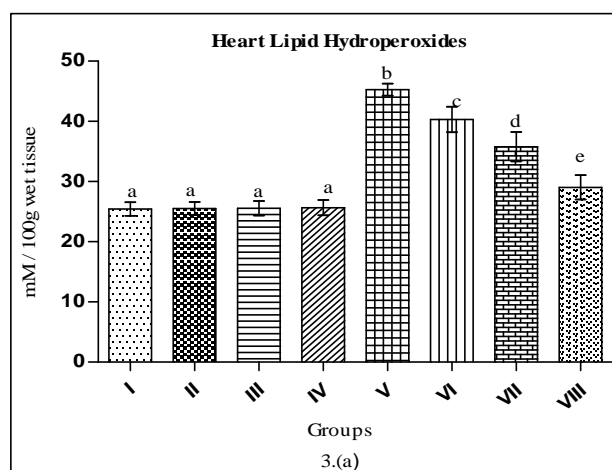


Fig 3(a)-Effect of Fisetin on the levels of lipid hydroperoxides(HP) in heart of normal and isoproterenol (ISO)-induced myocardial infarction in rats. Each value is mean \pm SD for six rats in each groups; values not sharing a common superscript (a, b, c, d, e) differ significantly with each other ($P < 0.05$, DMRT)

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

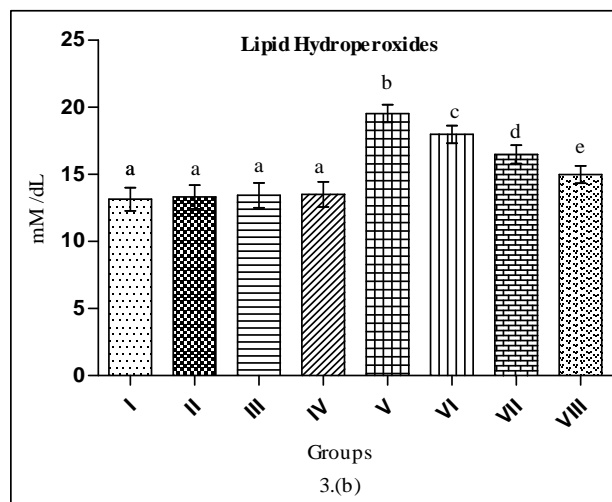


Fig 3.(b)- Effect of Fisetin on the levels of lipid hydroperoxides(HP) in plasma of normal and isoproterenol (ISO)-induced myocardial infarction in rats. Each value is mean \pm SD for six rats in each groups; values not sharing a common superscript (a, b, c, d, e) differ significantly with each other ($P < 0.05$, DMRT)

Table 1- shows the activities of SOD and catalase in the heart of normal and experimental rats. In ISO-treated rats, the activities of these enzymes were declined significantly ($P < 0.05$) in the heart when compared to normal rats. Pretreatment with Fisetin (10, 20 and 30 mg/kg) to ISO-induced rats significantly ($P < 0.05$) increased the activities of these enzymes when compared to ISO-alone induced rats.

Groups	Normal control	Normal + Fisetin (10mg/kg)	Normal + Fisetin (20mg/kg)	Normal + Fisetin (30mg/kg)	ISO (100mg/kg) alone	Fisetin (10mg/kg) + ISO	Fisetin (20mg/kg) + ISO	Fisetin (30mg/kg) +ISO
SOD (U/mg protein)	2.59 \pm 0.2 ^a	2.63 \pm 0.2 ^a	2.64 \pm 0.2 ^a	2.64 \pm 0.2 ^a	0.91 \pm 0.08 ^b	1.22 \pm 0.1 ^c	1.82 \pm 0.1 ^d	2.31 \pm 0.1 ^e
CAT (μ mol of H ₂ O ₂ consumed/min/mg protein)	2.17 \pm 0.2 ^a	2.20 \pm 0.2 ^a	2.22 \pm 0.2 ^a	2.23 \pm 0.2 ^a	0.82 \pm 0.1 ^b	1.46 \pm 0.1 ^c	1.75 \pm 0.1 ^d	1.99 \pm 0.1 ^e

Table 1-Effect of Fisetin on the activities of superoxide dismutase (SOD) and catalase in the heart of normal and isoproterenol (ISO)-induced myocardial infarction in rats. Each value is mean \pm SD for six rats in each groups; values not sharing a common superscript (a, b, c, d, e) differ significantly with each other ($P < 0.05$, DMRT).

Table-2 depicts the levels of GSH in Plasma and heart, GSH-Px, GST and GSH-Rx in heart. Rats were induced with Isoproterenol(100mg/kg) shows a significantly($P < 0.05$) decrease in the activities of enzymatic antioxidants when compared with normal control rats. Pretreatment with Fisetin (10, 20 and 30 mg/kg) to ISO-induced rats shows significant ($P < 0.05$) increase the activities of the enzymatic antioxidants, when compared to Isoproterenol induced rats.

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

Groups	Normal control	Normal + Fisetin (10mg/kg)	Normal + Fisetin (20mg/kg)	Normal + Fisetin (30mg/kg)	ISO (100mg/kg)	Fisetin (10mg/kg) + ISO	Fisetin (20mg/kg) + ISO	Fisetin (30mg/kg) + ISO
GPx (μ g of GSH Consumed/min/mg protein)	3.78 \pm 0.2 ^a	3.80 \pm 0.2 ^a	3.82 \pm 0.2 ^a	3.83 \pm 0.2 ^a	1.69 \pm 0.1 ^b	1.96 \pm 0.1 ^c	2.41 \pm 0.1 ^d	3.09 \pm 0.1 ^e
GRx (n mol of NADPH oxidized/min/100mg protein)	4.68 \pm 0.4 ^a	4.71 \pm 0.4 ^a	4.74 \pm 0.4 ^a	4.76 \pm 0.4 ^a	2.78 \pm 0.2 ^b	3.22 \pm 0.3 ^c	3.64 \pm 0.3 ^d	3.99 \pm 0.3 ^e
GST(nmol of CDNB conjugated/min/mg protein)	756.2 \pm 70.1 ^a	758.3 \pm 70.4 ^a	759.1 \pm 70.4 ^a	762.2 \pm 70.6 ^a	521.3 \pm 40.1 ^b	598.1 \pm 49.8 ^c	656.3 \pm 54.3 ^d	712.2 \pm 67.1 ^e
Plasma GSH (mg/dL)	9.68 \pm 1.4 ^a	9.70 \pm 1.4 ^a	9.73 \pm 1.3 ^a	9.76 \pm 1.3 ^a	6.20 \pm 0.9 ^b	6.72 \pm 0.8 ^c	7.52 \pm 0.7 ^d	8.06 \pm 0.8 ^e
Heart GSH (mmol/gwet tissue)	5.01 \pm 0.3 ^a	5.06 \pm 0.4 ^a	5.07 \pm 0.4 ^a	5.10 \pm 0.4 ^a	2.53 \pm 0.2 ^b	3.41 \pm 0.3 ^c	3.89 \pm 0.3 ^d	4.64 \pm 0.3 ^e

Table-2 Effect of Fisetin on the activities of glutathione peroxidase (GPx), glutathione reductase (GRx) and glutathione-S-transferase (GST) in the heart and the levels of reduced glutathione (GSH) in plasma and the heart of normal and isoproterenol (ISO)-induced myocardial infarction in rats. Each value is mean \pm SD for six rats in each groups; values not sharing a common superscript (a, b, c, d, e) differ significantly with each other ($P < 0.05$, DMRT).

Table 3- shows the levels of vitamin C and vitamin E in plasma and the heart and plasma ceruloplasmin in normal and ISO-induced rats. Rats induced with ISO exhibited significantly ($P < 0.05$) decrease in the levels of vitamin C and E in plasma and the heart and plasma ceruloplasmin when compared with normal control rats. Oral administration of Fisetin (10, 20 and 30 mg/kg) to ISO-induced rats significantly ($P < 0.05$) increased the levels of vitamin C and E in plasma and the heart and ceruloplasmin in plasma when compared to ISO-alone induced rats.

Groups	Normal control	Normal + Fisetin (10mg/kg)	Normal + Fisetin (20mg/kg)	Normal + Fisetin (30mg/kg)	ISO (100mg/kg) alone	Fisetin (10mg/kg) + ISO	Fisetin (20mg/kg) + ISO	Fisetin (30mg/kg) + ISO
Plasma vitamin C (mg/dL)	2.36 \pm 0.1 ^a	2.41 \pm 0.1 ^a	2.42 \pm 0.1 ^a	2.44 \pm 0.1 ^a	0.98 \pm 0.08 ^b	1.40 \pm 0.1 ^c	1.88 \pm 0.1 ^d	2.06 \pm 0.1 ^e
Heart vitamin C (mmol/mg protein)	2.20 \pm 0.2 ^a	2.21 \pm 0.2 ^a	2.23 \pm 0.2 ^a	2.23 \pm 0.2 ^a	0.89 \pm 0.02 ^b	1.28 \pm 0.1 ^c	1.71 \pm 0.1 ^d	2.03 \pm 0.1 ^e
Plasma vitamin E (mg/dL)	2.43 \pm 0.2 ^a	2.44 \pm 0.2 ^a	2.46 \pm 0.2 ^a	2.46 \pm 0.2 ^a	0.82 \pm 0.07 ^b	1.04 \pm 0.1 ^c	1.46 \pm 0.1 ^d	2.15 \pm 0.2 ^e
Heart vitamin E (mmol/mg protein)	0.95 \pm 0.1 ^a	0.96 \pm 0.1 ^a	0.98 \pm 0.1 ^a	1.0 \pm 0.1 ^a	0.44 \pm 0.09 ^b	0.54 \pm 0.09 ^c	0.63 \pm 0.08 ^d	0.78 \pm 0.09 ^e
Plasma ceruloplasmin (mg/dL)	25.2 \pm 2.1 ^a	25.4 \pm 2.3 ^a	25.5 \pm 2.0 ^a	25.6 \pm 2.4 ^a	16.2 \pm 1.6 ^b	18.8 \pm 1.7 ^c	20.6 \pm 1.9 ^d	23.2 \pm 1.9 ^e

Table-3 Effect of Fisetin on the levels vitamin C and vitamin E in plasma and the heart and ceruloplasmin in plasma of normal and isoproterenol (ISO)-induced myocardial infarction in rats. Each value is mean \pm SD for six rats in each groups; values not sharing a common superscript (a, b, c, d, e) differ significantly with each other ($P < 0.05$, DMRT).

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

IV. DISCUSSION

Epidemiological studies illustrates that CVD, especially Myocardial infarction, will contribute to the major death causing disease worldwide in the year 2020[25]. MI is characterized by cardiac damaged, lipid peroxidation increases and altered the activities of myocardium injury markers and reduction of endogenous antioxidant [26-27] Cardiac injury caused by isoproterenol (ISO) at a high dosage in an experimental animal model shows a better discrimination of myocardial damage and clearly shows the involvement of oxidative stress[28]. Dietary natural Flavonoids exert their antioxidant activities and are well reported to prevent CVD[29]. Moreover, a variety of studies have proposed that Fisetin exhibits considerable antioxidant activity. Mutually, on basis of these findings, we proposed to investigate the cardioprotective effects of Fisetin, a flavonol on isoproterenol-induced cardiac injury in rats. The current investigation evaluate that Fisetin exerts potential cardioprotective effects in Isoproterenol-induced oxidative myocardial damage as indicated by decreased levels of lipid peroxidation and by reestablishing endogenous antioxidant activities. In our study, the results reveals that Isoproterenol significantly increased the production of lipid peroxidation along with excessive generation of free radicals. These occurs due to autooxidation of ISO. The increased level of TBARS and lipid peroxides in plasma and heart tissue could be due to Isoproterenol induced free radical generations, damage of membrane and finally cell lysis; On the other hand, amelioration of lipid peroxidation metabolism is observed in fisetin treated MI rats due to its antioxidant activity. The decreased level of TBARS in Fisetin pretreated rats (10,20,30mg/kg) when compared to normal and isoproterenol induced rats (100mg/kg). This could be due to enhanced activities of antioxidant. The low molecular weight and the hydrophobic nature of Fisetin easily pass through the cell membrane and to accumulate intracellularly, which protects the myocardial cells from oxidative stress. The formation of free radicals and lipid peroxides accumulation is one of the most important mechanisms for the cardiac damage caused by Isoproterenol[30]. Free radical scavenging circulatory enzymes such as catalase, superoxide dismutase, glutathione peroxidase are the first line cellular defense against oxidative stress[31], decomposes O_2 and H_2O_2 before the formation of more reactive hydroxyl radical ($OH\bullet$). The equilibrium between these enzymes has the effective removal of oxidative injury in intracellular organelles. However, in pathological conditions like MI, the ROS generation can intensely upset this balance with an increased antioxidant demand. The circulatory antioxidant enzymes such as SOD and CAT are involved in direct removal of reactive oxygen metabolites, which is undoubtedly one of the most effective defense system of the living body against diseases. In the present study, there is decreased levels of SOD and CAT in the heart tissue as observed at ISO-induced rats. This could be due to increased production of ROS which are capable of reducing the activities of these enzymes[32] and its loss of function of myocardial membrane, which is usually noticed during myocardial necrosis[33]. Its leads to the increase of superoxide anion which damages the myocardium[34]. A decrease level of SOD activity can result in the decreased removal of superoxide anions which could be harmful to the myocardial membrane. The activities of CAT and GPx were also decreased significantly. Fisetin pre-treatment prevented the myocardial SOD and CAT activities when compared to normal control and ISO-induction rats. Present findings are also strongly supports of its antioxidant activity against ISO-induced Myocardial damage. Fisetin was reported to scavenge superoxide radicals and hydrogen peroxide. Because of its most powerful antioxidant activity it maintains the level of circulatory enzymes[35]. Thus the study reveals that Fisetin scavenge the superoxide and hydrogen peroxide produced by isoproterenol and reduce cardiac damage. Reactive oxygen species (ROS) are generated from the leakage of electrons into oxygen from various systems in our body and create oxygen stress, for this, the antioxidant enzymatic defense is a very important source to deactivate the oxygen free radical mediated tissue injury[36]. The activities of GSH-dependent antioxidant enzymes (GPx, GST and GRx) were shows decrease level in ISO-treated rats when compared to normal rats. GPx protects the cellular and subcellular membranes from peroxidative damage by elimination of hydrogen peroxide and lipid peroxides. The lowest activities of GPx and GST in heart in ISO-treated rats are due to reduced availability of GSH [37]. Decreased activities of these enzymes can leads to accumulation of oxidants which is responsible to myocardial damage [38]. Fisetin pretreatment maintains the activities of GSH-dependent antioxidant enzymes, which indicates the protective antioxidant nature of the Fisetin against oxidative stress-induced injury.

Vitamin E is the lipid-soluble antioxidant present in cell membranes and lipoproteins that protects against oxidative modification. The major antioxidant of the aqueous phase is vitamin C, which acts as defense during

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

oxidative stress. Thus, scavenger antioxidants are important, because they react with free radicals directly [39,40]. In our study, we observed decrease in vitamin E and vitamin C could be due to increased utilization of ISO-mediated free radicals and lipid peroxidation. Pretreatment of Fisetin showed a marked increase in vitamin C and vitamin E levels in ISO-induced rats. The extracellular antioxidant ceruloplasmin scavenges superoxide radicals and inhibits ferritin dependent lipid peroxidation by catalyzing the oxidative reincorporation of released iron into ferritin [41]. Since ceruloplasmin has ferroxidase and copper binding capacity, it could have been used more to deactivate the excess amount of free radicals and hence ISO-induced MI rats showed decreased level of ceruloplasmin [42]. Fisetin pretreatment prevented the loss of ceruloplasmin, by reducing the iron- and copper-mediated myocardial injury.

Fisetin has effective scavenger of free radicals under *in vivo* conditions. Antioxidant activities are mainly due to its hydroxyl groups at C-3, C-3', C-4' and C-7 positions and are enriched by carbonyl group at C-4. The presence of double bond between C-2 and C-3 conjugated with the 4-oxo group also eases higher electron delocalization. Pretreatment with fisetin has been previously reported to reduce H₂O₂ induced cell death and it protects cells against cell damage even at a low to high concentration. The potent free radical scavenging activity of Fisetin was attributed to the presence of the C ring group. The observation was also made that the more hydroxyl groups in the Fisetin possesses, more effective free radical scavenger the Fisetin becomes [43]. Thus, Fisetin may act as an antioxidant by scavenging ROS and also improved the endogenous antioxidant system in ISO-treated rats. Thus, the antioxidant property of fisetin might be protect the myocardium against ISO-induced oxidative damage.

V. CONCLUSION

In our study, we have observed that Fisetin was safe and highly effective in preventing cardiovascular disease in Isoproterenol induced myocardial infarction in rats, due to both anti-oxidative and anti-lipid peroxidation effects.

REFERENCES

- [1] Aronow WS. Epidemiology, pathophysiology, prognosis and treatment of systolic and diastolic heart failure. *Cardiol* 2006;14:108-24.
- [2] Boudin S, Laclau M N, Tariosse, et al. Alteration of mitochondrial function in a model of chronic ischemia *in vivo* in rat heart. *Amer J Physiol Heart Circ Physiology* 2002; 282: H821.
- [3] Muruganandan S, Gupta S, Kataria M, et al. Mangiferin protects the streptozotocin-induced oxidative damage to cardiac and renal tissue in rats. *Toxicol* 2002; 176: 165.
- [4] De-Jian J, Gui-Shan T, Feng Y, et al. Protective effect of xanthones against myocardial ischemia-reperfusion injury in rats. *Acta Pharmacol Sin* 2003; 24: 175.
- [5] Wexler BC. Myocardial infarction in young vs old male rats: pathophysiological changes. *Amer Heart J* 1978; 96:70-80.
- [6] Nirmala C, Puvanakrishnan R. Effect of curcumin on certain lysosomal hydrolases in isoproterenol-induced myocardial infarction in rats. *Biochem. Pharmacol* 1990; 51:47-51.
- [7] Subhash D, Narinder KK, Nityanan S. Effect of isoprenaline on lipid profile cardiac enzymes in rats. *J Exp Biol* 1978; 16:376-8.
- [8] Sathish V, Ebenezer KK, Devaki T. Synergistic effect of nicorandil and amlodipine on tissue defense system during experimental myocardial infarction in rats. *Mol Cell Biochem* 2006; 243(1-2):133-8.
- [9] Banerjee SK, Maulik SK. Effect of garlic on cardiovascular disorders: a review. *Nutr J* 2002; 1: 4.
- [10] Pauletti PM, Castro-Gamboa I, Siqueria SDH, Tomazela DM, Eberlin MN, Da Silva BV. New antioxidant action C-glycosylxanthones from the stems of *arrabidaea* amygdaloides. *J Nat Prod* 2003; 10:1284.
- [11] Hertog MGL, Hollman PCH, van de Putte B, Content of potentially anticarcinogenic flavonoids of tea infusions, wines and fruit juices, *Journal of Agricultural and Food Chemistry*, 41,1993,1242– 1246.
- [12] Yagi K. Lipid peroxides and human disease. *Chem Phys Lipids* 1987;45:337e51.
- [13] Fraga CG, Leibovitz BF, Toppel AL. Lipid peroxidation measured as thiobarbituric acid reactive substances in tissue slices. Characterization and comparison with homogenate and microsomes. *Free Radical Biol Med* 1988;4:155e61.
- [14] Jiang ZY, Hunt JV, Wolff SP. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxides in low-density lipoprotein. *Anal Biochem* 1992;202:384e9.
- [15] Kakkur P, Das B, Viswanathan PN. A modified spectrophotometric assay of SOD. *Indian J Biochem Biophys* 1984;21:130e2.
- [16] Sinha KA. Colorimetric assay of catalase. *Anal Biochem* 1972;47:389e94.
- [17] Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WB. Selenium: biochemical role as a component of glutathione peroxidase. *Science* 1973;179:588e90.
- [18] Horn HD, Burns GH. Assay of glutathione reductase activity. In: Bergmeyer HV, editor. *Methods of enzymatic analysis*. New York: Academic Press; 1978. p. 142.

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

- [19] Habig WH, Jakoby WB. Assays for differentiation of glutathione-S-transferases. *Meth Enzymol* 1981;77:398-404.
- [20] Ellman GC. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959;82:70-7.
- [21] Omaye ST, Turnbull TO, Sauberlich HE. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. In:
- [22] McCormic DB, Wright DL, editors. *Methods of enzymology*. New York: Academic Press; 1979. p. 3e11.
- [23] Baker H, Frank O, Angelis B, Feingold S. Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. *Nutr Rep Int* 1980;21:531e6.
- [24] Ravin HA. An improved colorimetric enzymatic assay for ceruloplasmin. *J Lab Clin Med* 1961;58:162e8.
- [25] Lowry OH, Rosebrough MJ, Farr AL, Randall RJ. Protein measurement with Folin-phenol reagent. *J Biol Chem* 1951;193:265-75.
- [26] Aronow WS. Epidemiology, pathophysiology, prognosis, and treatment of systolic and diastolic heart failure. *Cardiol Rev*. 2006;14:108.
- [27] Ojha SK, Nandave M, Arora S, Narang R, Dinda AK, Arya DS. Chronic administration of Tribulus terrestris Linn extract improves cardiac function and attenuates myocardial infarction in rats. *Int J Pharmacol*. 2008;4:1e10.
- [28] Panda VS, Naik SR. Cardioprotective activity of Ginkgo biloba Phytosomes in isoproterenol-induced myocardial necrosis in rats: a biochemical and histoarchitectural evaluation. *Exp Toxicol Pathol*. 2008;60: 397e404.
- [29] Denollet J, Sys SU, Brutsaert DL. Personality and mortality after myocardial infarction. *Psychosom Med*. 1995;57:582-91.
- [30] Pandey KB, Rizvi SI. Current understanding of dietary polyphenols and their role in health and disease. *Curr Nutr Food Sci*. 2009;5:249-263.
- [31] Rossetto M, Vanzani P, Mattivi F, Lunelli M, Scarpa M, Rigo A. Synergistic antioxidant effect of catechin and malvidin 3-glucoside on free radical-initiated peroxidation of linoleic acid in micelles. *Arch Biochem Biophys*. 2002;408:239-45.
- [32] Priscilla DH, Prince PSM. Cardioprotective effect of gallic acid on cardiac troponin-T, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in Wistar rats. *Chem Biol Interact*. 2009;179:118-24.
- [33] Madhesh M, Vaiyapuri M. Luteolin a dietary flavonoid attenuates isoproterenol-induced myocardial oxidative stress in rat myocardium: An *in vivo* study. *Biomed Prev Nutr*. 2013;3:159-64.
- [34] Rathore N, John S, Kale M, Bhatnagar D. Lipid peroxidation and antioxidant enzymes in isoproterenol induced oxidative stress in rat tissues. *Pharmacol Res*. 1998;38:297-303.
- [35] Kornfeld OS, Hwang S, Disatnik M-H, et al. Mitochondrial reactive oxygen species at the heart of the matter: New therapeutic approaches for cardiovascular diseases. *Circ Res*. 2015;116:1783-99.
- [36] Roleira FMF, Tavares-Da-Silva EJ, Varela CL, et al. Plant derived and dietary phenolic antioxidants: Anticancer properties. *Food Chem*. 2015;183:235-58.
- [37] Poliodoro G, Cdi I, Arduini A, Gla R, Federici G. Superoxide dismutase, reduced glutathione and thiobarbituric acid reactive products in erythrocytes of patients with multiple sclerosis. *Indian J Biochem* 1984;16:505e 10.
- [38] Saravanan G, Prakash J. Effect of garlic (*Allium sativum*) on lipid peroxidation in experimental myocardial infarction in rats. *J Ethnopharmacol* 2004;94:155e8.
- [39] Anandhan R, Deepa R, Devaki T. Protective effect of Picrorhiza kurroa on mitochondrial glutathione antioxidant system in D-galactosamine induced hepatitis in rats. *Curr Sci* 1999;76:1543e6.
- [40] Packer JE, Slater TF, Wilson RL. Direct observation of free radical interaction between vitamin E and vitamin C. *Nature* 1979;278:737.
- [41] Freisleben H, Packer L. Free-radical scavenging activities, interactions and recycling of antioxidants. *Biochem Soc Trans* 1993;21:325e30.
- [42] Lekse JM, Xia L, Stark J, Morrow JD, May JM. Plant catechols prevent lipid peroxidation in human plasma and erythrocytes. *Mol Cell Biochem* 2001;24:364e70.
- [43] Jash S. K., and Mondal, S. (2014) Signpost Open Access J. Org. Biomol. Chem., 2, 89-128. Volume 02, Article ID 010314.