AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Protective role of fenugreek promotes mitochondrial protection in isoproterenol induced myocardial infarction in rats

Murugesan Madhesh[1]* Balan Kannan[2], Preethi Rajesh[3], Rajmohan Mahalingam Sivaraj[1], Manju Vaiyapuri[3]

1. Department of Biochemistry, Sivaraj Medical College, Salem, Tamil Nadu, 636 011, India.
2.key lab of natural drug and Immune Engineering, Henan University, Kaifeng- 475001, People's Republic of China
3.Department of Biochemistry, Periyar University, Salem, Tamil Nadu, 636 011, India.

ABSTRACT

Lipids and mitochondrial oxidative stress plays a crucial role in the development of cardiovascular disease, and mitochondria compartment is presumed as the main source and susceptible target of intracellular Reactive Oxygen Species (ROS). The present study, an attempt has been made to evaluate the mitochondrial protection in isoproterenol (ISO)-induced myocardial-infarction (MI) in male Wistar rats. The rats were divided into four groups (n=6). Group I received 0.5% CMC treated as normal control group. Group II received isoproterenol (85 mg/kg body weight) intraperitoneal (i.p.) for two consecutive days (14^{th} and 15^{th} days). Group III received fenugreek (250 mg/kg b.wt) intragastric intubation for 15 days. Group IV rats received fenugreek as in Group III and additionally isoproterenol was given for two consecutive days (14^{th} and 15^{th} days).The isoproterenol-induced rats indicated increase in the level of TBARS and decreased in the activities of mitochondrial antioxidants in MI rats, decrease in the levels of mitochondrial phospholipids and increase the levels of mitochondrial cholesterol, free fatty acids (FFAs), triglycerides (TGs) and the activities of mitochondrial enzymes like Isocitrate dehydrogenase (ICDH), Succinate dehydrogenase (SDH), Malate dehydrogenase (MDH) and α-Ketoglutarate dehydrogenase (α-KGDH) were increased in isoproterenol-induced rats. On treatments with fenugreek at a daily dose of (250 mg/kg b.wt) showed significantly decrease in the levels of mitochondrial lipid peroxidation, raise in the mitochondrial antioxidant levels and also diminish in the mitochondrial enzymes. The present study exposed that fenugreek ameliorates the mitochondrial damage in isoproterenol induced myocardial infarction by maintaining lipid peroxidation metabolism due to its free radical scavenging, mitochondrial lipids, antioxidants and mitochondrial enzymes. Histopathological study was also in correlation with the biochemical parameters.

Key notes: fenugreek, isoproterenol, mitochondrial enzymes, antioxidants.

*Corresponding Author Email: biomsv87@gmail.com
Received 19 October 2016, Accepted 01 November 2016

Please cite this article as: Murugesan. M *et al*., A Comparative Study of Timolol Maleate 0.5% v/s Latanoprost 0.005% In the Treatment of Primary Open Angle Glaucoma. American Journal of PharmTech Research 2016.

INTRODUCTION

Human life is always cardinal for human being beginning from his birth to the end of life. Many diseases, minor to major, that plays an important role in troubling the healthy life. Due to the modernization as well as sophistication in the life, human health directly or indirectly faces challenges from various diseases[1]. Myocardial infarction (MI) is a clinical trouble caused by acute necrosis of the myocardium that result of imbalance between coronary blood supply and myocardial demand[2]. The incidence of myocardial infarction is also high among people with Indian origins who are now living abroad[3]. A better understanding of the processes involved in myocardial infarction has stimulated the search for biomolecules which could limit myocardial injury.

Mitochondria are the important sub cellular organelles for cellular oxidative process and also the main source of reactive oxygen species in the cell. Mitochondria are the location of energy production and electron transport chain and carry out a vital biochemical process called oxidative phosphorylation. It is the main origin of energy, which sustain cellular metabolism and integrity. The diminished in oxygen supply during MI impairs energy production by mitochondria[4]. Normal cardiac function depends on adequate delivery of oxygen and oxidizable substrate to generate sufficient ATP to meet the energy demand of the organ. This process is attained through many metabolic pathways, including TCA cycle and oxidative phosphorylation, which instantly participate in the generation of ATP[5]. An ISO administration lead to the changes in membrane permeability brings about a loss of function and integrity of myocardial membranes[6], alterations of cardiac function and ultrastructure, disruption of mitochondria along with the inactivation of the enzymes concerned with the energy metabolism of myocardium[7].

Isoproterenol (ISO), a synthetic catecholamine and β-adrenergic agonist, are considered to be beneficial for the determination of heart function by exerting a positive isotropic consequences. ISO administration at high doses or excess release of it from the endogenous stores may deplete which are responsible for the development of irreversible damage. On auto oxidation, it can generate highly cytotoxic free radicals known to stimulate peroxidation of membrane phospholipids and induce severe damage to the myocardial membrane. Numerous researches suggests that isoproterenol induced cell death to myocardial cells leads to cause hypoxia, calcium overload and reduction of energy reserves and excessive production of free radicals obtained during oxidative metabolism of catecholamines[3]. However there is a strong literature[8,9] demonstrating that estrogen can protect the heart from ischemic injury and inhibit arteriosclerosis

and myocardial hypertrophy; that means that estrogen would have affected the experimental results if we use the female rats. So in the present study, we only used the male rats to build the acute myocardial infarction induced by isoproterenol to contain the study.

Nature is the lifeline of our health as it supplies all crucial things for survival. Medicinal plants, plants based foods and their constituents have received great attention for their salutary effects to treat many aspects of ischemic heart disease or MI. In parallel, the use of herbs in pharmacotherapy is also rising along with a realization that herbal products can influence the course of heart diseases and may provide an integrated approach of nutritional substances, which helps in restoring and maintaining, the normal body systems [10].

Fenugreek is a leguminous herb, It is commonly cultivated and used as a flavouring for food and as an herbal medicine in India and North African countries. The fenugreek seeds are yellow in colour, bitter to taste[11]. The toxicological studies were carried out in experimental animals and humans[12] demonstrated it to be safe and also it has Hypocholesterolemia[13], Chemopreventive[14], Anti-oxidant[15] and Cardioprotective[6].

Thus, the present study is undertaken to suggest the most therapeutic benefit of fenugreek intake by studying its protective activity against isoproterenol-induced mitochondrial damage in rats. My current research attempted to evidence the molecular mechanism of its therapeutic effect by studying the lipid fractions, antioxidant levels and other biochemical markers in the mitochondrial fractions.

MATERIALS AND METHOD

Chemicals

Isoproterenol hydrochloride were purchased from Sigma Chemical Company, St. Louis, MO, USA. All other chemicals used were of analytical grade.

Formulation and administration of fenugreek

Fenugreek powder was suspended in 0.5% Carboxymethyl Cellulose (CMC) and each animal belonging to three different groups received 1.0 ml of fenugreek suspension at a dose of 250 mg/kg body weight everyday respectively by intragastric intubation[16].

Induction of Myocardial Infarction

Myocardial Infarction was induced by intraperitoneal (i.p.) injection of isoproterenol hydrochloride (85 mg/kg body weight) on 14th and 15th days[16].

Animal Housing and Diets

Male Wistar albino rats aged 6 weeks and weighing about 150g were obtained from Sri Venkateshwara Enterprises Bangalore, India. After one week of acclimatization all animals were housed six per polypropylene plastic cage covered with metal grids and a hygenic bed of husk in a specific-pathogen free animal room under controlled conditions of a 12h light/12 hour dark cycle, and provided with standard food pellets (diet composition, wheat broken-moisture 9.0%, crude protein, 11.5% crude fat, 1.9% crude fibre 4% ash 0.2%, nitrogen-free extract 73.4%) supplied by Hindustan Lever Ltd, Mumbai, India) and tap water *ad libitum*. The study was carried on after getting a clearance from the Institutional Animal Ethical Committee (IAEC) (Reg .no P.Col/52/2010/IAEC/VMCP) of Vinayaka Mission College of Pharmacy, Salem, TamilNadu.

Experimental Design

The rats in group I obtained 1.0 ml of 0.5% CMC daily via intragastric intubation and served as the untreated control. The rats in group II received fenugreek via intragastric intubation at a daily dose of (250 mg/kg body weight) respectively for a period of 15 days. Group III rats received isoproterenol (85 mg/kg body weight) intraperitoneally twice at an interval of 24h on the 14th and 15th days. Group V rats received fenugreek as in group II for 15 days and at the last of the experimental period on 14th and 15th days rats received isoproterenol (85 mg/kg body weight) injections intraperitoneally twice at an intravel of 24 hr.

At the end of the experimental period, rats were sacrificed by cervical decapitation. The blood was collected and serum obtained after centrifugation were used for various biochemical estimations. Hearts were removed, cleared of blood and immediately transferred to ice cold containers containing 0.9% sodium chloride. Samples of tissues were homogenized in appropriate buffer and used for the determination of the following parameters.

Biochemical Parameters

Heart Mitochondria were isolated by the standard procedure of Takasawa et al.[17] Thiobarbituric Acid Reactive Substances (TBARS) were estimated by the method of Fraga et al.[18] Superoxide Dismutase (SOD) Activity was assayed in the mitochondrial heart by the method of Kakkar et al.[19] Catalse were estimated by the method of Beers RF and Seizer[20]. Glutathione Peroxidase (GPx) was estimated by the method of Rotruck et al.[21] Reduced glutathione (GSH) were estimated by the method of Ellman[22]. From the Mitochondrial Fraction, the lipids were extracted by the method Folch et al.[23] Cholesterol in the mitochondrial lipid fraction was estimated by the method of Zilversmit[24]. The levels of triglycerides in the mitochondrial lipid fraction were estimated by a reagent kit from Accurex Bio Pvt. Ltd, Mumbai. Free Fatty Acid (FFA) in the Mitochondrial Lipid Fraction was estimated by the method of Folholt[25]. Phospholipid content in the Mitochondrial

Lipid Fraction was estimated by the method of Zlatkis[26]. The activities of Isocitrate Dehydrogenase (ICDH) were estimated by the method of king[27]. Succinate Dehydrogenase (SDH) were estimated by the method of Slater and Bonner[28]. Malate Dehydrogenase (MDH) were estimated by the method of Mehler et al.[29] α-Ketoglutarate Dehydrogenase (α-KGDH), were estimated according to the standard procedure of Reed and Mukherjee[30].

Histopathological Examination

After the experimental period, animals were decapitated, and their livers were removed directly, then chopped, and cleaned in saline. For Histopathological Analysis, Liver Specimens fixed in 10% formalin were embedded in paraffin, sliced at 5-mm thickness, and stained with Hematoxylin and Eosin for detection of hepatic damage. The pathological alters were assessed and photographed.

Statistical Analysis

The results presented here are the means ± SD of 6 rats in each group. The results were analyzed using one-way analysis of variance [ANOVA] and the group means were compared using Duncan's Multiple Range Test [DMRT] using SPSS version 12 for Windows. The findings were considered as statistically significant if $P<0.05$[31].

RESULTS AND DISCUSSION

Effect of fenugreek on mitochondrial thiobarbutric acid reactive substances (TBARS) and antioxidant levels in the myocardial infarction rats

Table 1 describes the effect of fenugreek on levels of mitochondrial TBARS in the control and experimental rats. The levels of was significantly (P<0.05) increased in the ISO- induced rats (group 3) as compared with control rats (group 1). Fenugreek administration to the isoproterenol induced rats (group 4) significantly (P<0.05) reduced the levels of mitochondrial TBARS as compared with ISO induced rats (group 3).

Isoproterenol induced rats showed significantly (P<0.05) decrease in the activities of Peroxidase Enzyme (SOD, CAT) and other endogenous antioxidant enzymes (GPx, GSH) in the heart mitochondria, when compared to normal control rats (group 1). On treatment with fenugreek (250 mg/kg) to Isoproterenol-induced rats daily for a period of 15 days significantly (P<0.05) increased the activity of SOD and CAT in the heart mitochondria, when compared with isoproterenol induced rats. The activities of endogenous antioxidant enzymes were significantly increased in the heart mitochondria the levels of GPx and GSH also increased significantly in the heart

mitochondria of isoproterenol- induced rats (Table 1) when compared with ISO alone-induced rats (group 3).

Table 1 Effect of fenugreek on heart mitochondrial thiobarbutric acid reactive substances (TBARS) and mitochondrial antioxidants in the control and experimental rats

Groups	Control	fenugreek (250 mg/kg b.wt)	Isoproterenol (85 mg/kg b.wt)	fenugreek (250 mg/kg b.wt)+ isoproterenol (85 mg/kg b.wt)
TBARS (nmoles/mg protein	5.92±1.06[a]	5.77±0.99[a]	9.83±1.03[c]	7.75±1.16[b]
Superoxide dismutase (SOD) (units/mg protein)	19.2±1.58[b]	22.01±1.52[d]	13.97±1.57[a]	17.11±1.16[c]
Catalase (CAT) (nmoles of H_2O_2 consumed / min / mg protein)	2.35±0.15[c]	2.53±0.33[c,d]	0.97±0.37[a]	1.68±0.39[b]
Glutathione peroxidase (GPx) (nmoles of GSH oxidized/min/ mg protein)	1.47±0.34[c,d]	1.36±0.27 [c]	0.96± 0.19[a]	1.11±0.28[b]
Reduced Glutathione (nmoles GSH reduced /mg protein)	6.98±0.54[c]	7.22±1.04[c,d]	4.51± 0.59[a]	5.53±0.46[b]

The results are expressed as mean ± SD of six rats in each group. Values are not sharing a common superscript (a, b, c, d) differ significantly with each other $p<0.05$.

Effect of fenugreek on heart mitochondrial lipids in the myocardial infarction rats

Table 2 depicts the levels of mitochondrial cholesterol, FFA and triglycerides in isoproterenol-induced rats were significantly (P<0.05) increased and the level of phospholipids in the heart mitochondria was significantly (P<0.05) decreased in ISO-induced rats (group 3) as compared to the control rats (group 1). Treatment with fenugreek (250 mg/kg) daily for a period of 15 days significantly (P<0.05) decreased the levels of cholesterol, FFA, triglycerides and significantly (P<0.05) increased the levels of phospholipids in the heart Mitochondrial Fractions of Isoproterenol-induced rats when compared with Isoproterenol-induced untreated rats (Table 2).

Table 2: Effect of fenugreek on the levels of heart mitochondrial lipids in the control and experimental rats

Groups	Control	fenugreek (250 mg/kg b.wt)	Isoproterenol (85 mg/kg b.wt)	fenugreek (250 mg/kg b.wt)+ isoproterenol (85 mg/kg b.wt)
Triglyceride (nmoles/mg protein)	18.01± 1.54[a]	33.12±2.25[c]	17.81±1.39[a]	25.05±1.42[b]
Cholesterol (nmoles/mg protein)	33.11± 2.84[b]	30.91±2.55[a]	58.12±4.39[d]	44.12±3.42[c]

Free fatty acids (nmoles/mg protein)	15.07±1.93[a]	13.52±1.63[a]	26.19±1.25[c]	19.16±1.86[b]
Phospholipids (nmoles/mg protein)	560.13±17.18[c]	574.01±31.15[d]	483.20±21.23[a]	516.99±22.01[b]

The results are expressed as mean ± SD of six rats in each group. Values are not sharing a common superscript (a, b, c, d) differ significantly with each other $p<0.05$.

Effect of fenugreek on the activities of mitochondrial enzymes in the control and experimental rats

Table 3 shows the activities mitochondrial enzymes of ICDH, SDH, MDH and α-KGDH were decreased significantly (P<0.05) in ISO-induced rats (groups 3 and 4) as compared to the control rats (group 1). The results supported the deleterious effect of isoproterenol on TCA cycle enzymes. Treatment with fenugreek (250 mg/kg) daily for a period of 30 days significantly (P<0.05) increased the activities of these enzymes in isoproterenol alone induced rats, when compared to isoproterenol- induced untreated rats (Table 3).

Table 3: Effect of fenugreek on the activities of mitochondrial enzymes in the control and experimental rats

Groups	Control	Fenugreek (250 mg/kg b.wt)	Isoproterenol (85 mg/kg b.wt)	Fenugreek (250 mg/kg b.wt) + isoproterenol (85 mg/kg b.wt)
Isocitrate dehydrogenase (ICDH)	749.6±52.15[c]	759.6±51.35[c,d]	531.2±36.16[a]	676.33±34.16[b]
Succinate dehydrogenase (SDH)	39.15±3.16[b,c]	40.72±3.26[d]	17.97±3.22[a]	34.05±2.91[b]
Malate dehydrogenase (MDH)	330.17±10.95[c]	335.60±11.85[d]	151.61±9.35[a]	250.59±9.32[b]
α-ketoglutarate dehydrogenase (α –KGDH	78.97±7.05[c]	93.12±6.84[d]	61.1± 5.19[a]	75.53±6.26[b]

ICDH units: nmoles of α-ketoglutarate formed/h/mg protein; SDH: nmol of succinate oxidized/min/mg protein; MDH units: nmoles of NADH oxidized/min/mg protein; α-KGDH units: nmoles of ferrocyanide formed/h/mg protein

The results are expressed as mean ± SD of six rats in each group. Values are not sharing a common superscript (a, b, c, d) differ significantly with each other $p<0.05$.

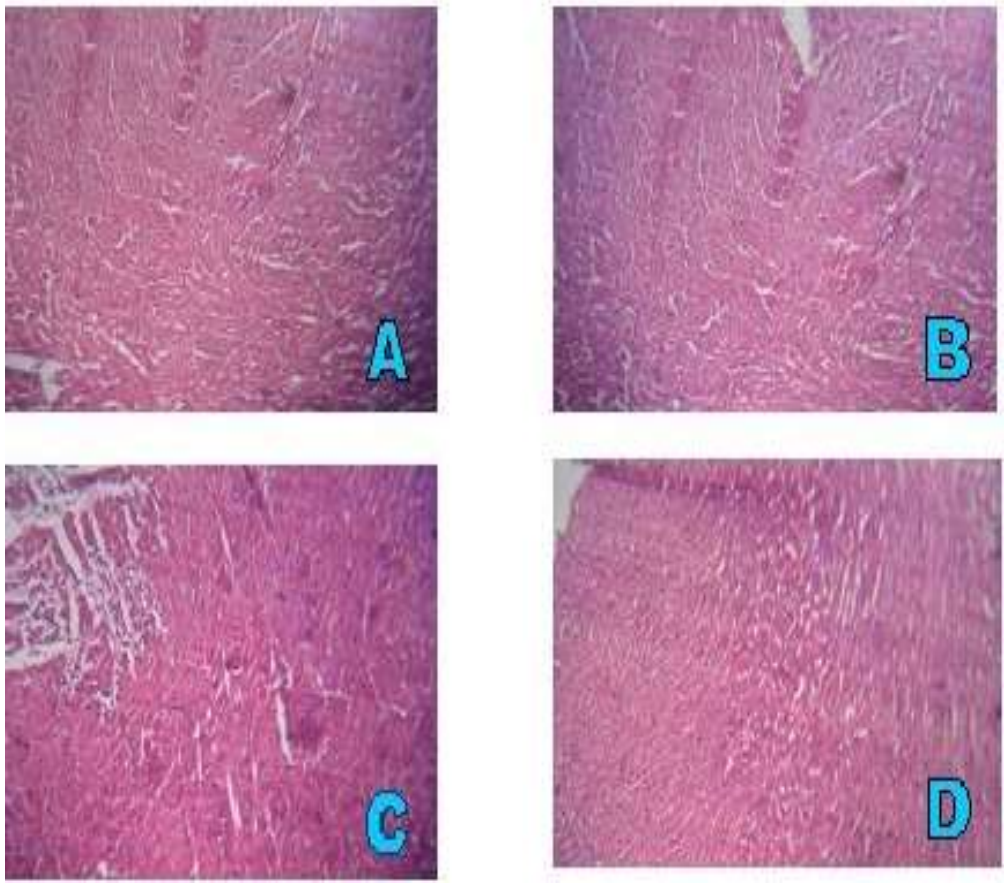

Figure 1: Histopathology changes in the myocardial infarction of control and experimental rats

(A) showing normal cardiac cells and aorta with normal architecture. The muscle fibres appear normal in size and eosinophilic color, nuclei are normal. There is no evidence of atrophy, inflammation or muscle damage. (B) fenugreek treated rats showing normal architecture of cardiac cells. The muscle fibres appear normal in size and eosinophilic color. The nuclei are normal and there is no evidence of atrophy, inflammation or muscle damage. (C) ISO treated rats showing perivascular cuffing of vasa vasorum with intimal fibrosis, disruption of medial elastic fibres with diffuse interstitial fibrosis and myocytolysis. (D) isoproterenol+ fenugreek rats showing heart muscle bundles. The muscle fibres appear normal in size and eosinophilic color. The nuclei are normal. There is no evidence of infiltration and myocytosis.

In the present study, treatment with fenugreek demonstrated significant promotes mitochondrial protection in isoproterenol induced myocardial infarction in rats. Isoproterenol documented that the pathophysiological and morphological alterations induced by isoproterenol in the heart tissues of experimental animals are similar to those observed in human myocardial infarction[32,33]. Mitochondria are the major oxygen deep organelle of the myocardial cell, they are familiar to

reduce oxygen univalently and it serves as a locus in the cell where free radical reactions may originate. Respiratory chain produces a large continuous flux of oxygen radicals including superoxide anion, hydrogen peroxide, and hydroxyl radical, single oxygen attack cellular macromolecules oxidizing membranous phospholipids, destructive protein and DNA[34].

Lipid peroxidation, is a free radical-mediated propagation of oxidative insult to polyunsaturated fatty acids, has been associated with altered membrane structure and enzyme inactivation. Increased levels of lipid peroxidation in heart mitochondria may diminish mitochondrial membrane fluidity, increase the negative surface charge distribution, and change membrane ionic permeability including proton permeability, which uncouples oxidative phosphorylation[35]. In the present study we observed an increase concentration of mitochondrial TBARS in the isoproterenol induced rats suggesting that increase lipid peroxidation, which could be assigned to a deficiency of antioxidant defense mechanism[36]. Our results are line with previous reports[7]. Changes in the metabolism of lipid peroxides are closely associated with mitochondrial damage due to free radicals produced by isoproterenol[37]. Treatment with fenugreek significantly diminished the concentration of TBARS in the isoproterenol-induced rats. The former are known to increase antioxidant activity in lipid peroxidation and protect cells from oxidative damage[38]. Our results supported the anti-lipid peroxidative action of fenugreek against isoproterenol-induced mitochondrial damage in myocardium. Thus, fenugreek protected the heart from myocardial damage by scavenging free radicals and thereby blocking the peroxidation of lipids in mitochondria.

Antioxidants constitute the foremost defense system that limits the toxicity associated with free radicals. The equilibrium among antioxidants and free radicals is an important process for the effective removal of oxidative stress in intracellular organelles[39,40]. Superoxide dismutase and catalase are anti-peroxidative enzymes that protect the cellular constituents against oxidative damage. In the present study, isoproterenol induction was found to reduce the levels of GSH and antioxidant enzymes in the cardiac mitochondria such as SOD, CAT and GPx, and this observation agrees with earlier finding[7] Reduced activities of these enzymes by ROS may affect the heart mitochondria substrate oxidation, leading to reduced oxidation of substrates, reduced rate of transfer of reducing equivalents to molecular oxygen, and depletion of cellular energy. Reduction in the activities of SOD and catalase in isoproterenol induced myocardial infarction in rats may lead to the generation of O_2- and H_2O_2, which in turn the production hydroxyl radicals (OH•) and bring about a number of reactions harmful to the cellular and subcellular membranes[41]. This was parallelled by reduction in the level of reduced glutathione and the activities of glutathione-

dependent anti-oxidant enzymes in the heart mitochondria of infarction-induced rats[42]. GSH is important in protecting the myocardium against free radical mediated injury and thus reduction in cellular GSH content could impair recovery after ischemia. Decrease in GSH level in ISO-induced rat might be due to its increased utilization in protecting –SH group containing proteins from free radicals or enhanced protective mechanism to oxidative stress in myocardial injury[43] and the decreased availability of GSH might have resulted in the lowered activities of GPx and GST.

The administration of fenugreek significantly enhances glutathione and the activities of anti-oxidant enzymes in the heart mitochondria. The administration of fenugreek protects the rat myocardium from oxidative stress during isoproterenol-induced myocardial infarction is an effect probably related to the normal maintenance of heart glutathione, which protects myocardial cellular and subcellular membranes from oxidative damage by attenuating lipid peroxidation.

In present study, altered levels of lipids were detected in the mitochondrial fraction of the heart. The mitochondrial lipids suggest clear evidence for altered cardiac function and ultrastructure in MI. Elevated levels of mitochondrial cholesterol are well linked with MI[44].We detected an increased level of cholesterol in the mitochondria of heart tissue in isoproterenol-induced rats which suggests redistribution of cholesterol in the mitochondria of ischaemic cells. Increased cholesterol levels in the mitochondrial membrane affect the permeability of ions and the fluidity. Our results are line with previous finding Murugesan[7]. Phospholipids are essential components for integrity of cellular membrane and subcellular organelles[45,46]. The metabolic products of isoproterenol produced more free radicals and the phospholipid rich mitochondrial membrane is vulnerable to free radical attack. This may be the reason for reduced levels of phospholipids in mitochondrial fractions of the heart of isoproterenol-induced rats. Treatment with fenugreek significantly increased the level of phospholipids in the isoproterenol-induced rats. Fenugreek has the ability to reduce the free radical formation it may protect phospholipids in mitochondria.

Accumulation of triglyceride is one of the risk factors of CVD. We detected an increased level of triglycerides and free fatty acids in the isoproterenol-induced rats. Our previous study suggested that the decreased delivery of fatty acids from the cardiomyocytes reverses triglyceride accumulation and leads to contractile dysfunction and it may also due to a decrease in the activity of lipoprotein lipase, leads to decreased uptake of TGs from the circulation[7]. Free fatty acids liberated from adipose tissue also enter into the myocardium and the process is proportional to the free fatty acid concentration in the coronary sinus[48]. Mitochondria utilize FFAs as energy fuel by oxidative metabolism and remaining FFAs are used as precursors for synthesis of fat and triacylglycerol. When the supply of oxygen is reduced as in MI, oxidation of FFAs ceases, leading

to increased synthesis of triacylglycerol due to FFAs accumulation[47]. Increased levels of FFAs inhibit respiratory activities and depress cardiac function in ischemic condition[48].

Treatment with fenugreek reduced the levels of triglycerides and free fatty acids in isoproterenol-induced rats. Fenugreek may channel fatty acids to triacylglycerol synthesis and divert lipids from toxic metabolic pathways. Deposition of free fatty acids is a consequence of changes in myocardial lipid metabolism. Thus, in ischemic heart, hydrolysis of phospholipids prevails in myocardial mitochondria. All the changes in the metabolism of the sub cellular fraction may lead to damage of the membranes of the cardiac myocyte mitochondria, which may be the cause of disorders of electrolyte metabolism and contractile properties of the myocardium.

Mitochondrial membrane lipid peroxidation results in irreversible loss of mitochondrial lipid functions such as mitochondrial respiration, oxidative phosphorylation and iron transport[49]. ICDH is mainly expressed in the heart and skeletal muscle mitochondria. It is NADP/NAD dependent and controls the mitochondrial redox balance and subsequent oxidative damage. SDH is a component of electron chain and is bound to the inner mitochondrial membrane[50]. Mitochondrial enzymes (ICDH, SDH, MDH and α-KGDH) catalyse the oxidation of several substrates through the tricarboxylic acid (TCA) cycle, yielding reducing equivalents, which are channeled through the respiratory chain for the synthesis of ATP by oxidative phosphorylation. Inhibition of these enzymes by reactive oxygen species (ROS) may affect the mitochondrial substrate oxidation, resulting in reduced oxidation of substrate, reduced rate of transfer of reducing equivalents to molecular oxygen and depletion of cellular energy[51,52]. Thus the marker enzymes of TCA cycle are affected by the free radicals produced by ISO.

Isoproterenol administration to rats significantly decreased the activities of mitochondrial TCA cycle enzymes, such as ICDH, SDH, MDH and α-KGDH. During the myocardial infarction, increased lipid peroxidation was seen in the mitochondria. These dehydrogenases are settled in the outer membrane of the mitochondria and are affected by enhanced levels of free radicals produced following isoproterenol administration[53]. Inhibition of these enzymes by ROS may affect the mitochondrial substrate oxidation, resulting in reduced oxidation of substrates, reduced rate of transfer of reducing equivalents of molecular oxygen and depletion of cellular energy[7]. Previous studies have reported that the decrease in mitochondrial enzymes might be due to the marked deficiency in one or more electron transport chain components[54]. Treatment with fenugreek showed significantly improved activities of TCA cycle enzymes in the heart mitochondria fraction in ISO-treated rats due to their free radical scavenging activities.

Histopathological findings also affirm the findings of this study. The ISO-induced myocardium elucidated focal confluent necrosis of muscle fiber with inflammatory call infiltration, edema with fibroblastic proliferation and phagocytosis. Normal control rats treated with fenugreek (250 mg/kg body weight) showed normal cardiac fibers without any pathological changes. This indicates that fenugreek (250 mg/kg body weight) does not possess any adverse effects under normal condition.

CONCLUSION

In the current study, fenugreek intake was observed to exhibit cardioprotective effects as demonstrated by maintaining the integrity of the lipid peroxidation, mitochondrial membranes restoring the activities of the antioxidants and mitochondrial enzymes to near normal levels of rats induced with myocardial infarction and histopathological analysis suggesting its cardioprotective action. Moreover, fenugreek enhanced the mitochondrial energy status and anti-oxidant defence of the myocardium, suggesting that the activation of ATP production and reduction of oxidative stress is likely to play a role in the mechanism of its cardioprotective effects. The cardioprotective effect of fenugreek can be correlated directly with its ability to activate the energy status of the anti-oxidant defence system. Thus fenugreek may be useful as a safe and effective diet containing agent in the management of cardiovascular diseases.

ACKNOWLEDGEMENTS

Dr. M. Murugesan is thankful to the Sivaraj Siddha Medical College, Salem-636 307, Tamil Nadu, and India for providing the necessary infrastructural facilities.

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