Research Article

Mitochondrial Protection of Galangin on Isoproterenol Induced Myocardial Infarction In Rats

M. Murugesan[1]*, R. Swetha[2], S. Sowmiya[2] and MS. Rajmohan[2]

[1]Department of Biochemistry, Sivaraj Medical College, Salem, Tamil Nadu, 636 011, India.
[2]Department of Siddha, Sivaraj Medical College, Salem, Tamil Nadu, 636 011, India.

ABSTRACT

Objective and Methods: Myocardial mitochondrial damage is considered to be an important trigger for the pathogenesis of heart disease and is particularly susceptible to oxidative stress. 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 (14th and 15th days). Group III received galangin (20 mg/kg b.wt) intragastric intubation for 15 days. Group IV rats received galangin as in Group III and additionally isoproterenol was given for two consecutive days (14th and 15th days).

Results: The isoproterenol-induced rats indicated increase in the level of mitochondrial 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 decreased in isoproterenol-induced rats. On treatments with galangin at a daily dose of (20 mg/kg b.wt) showed significantly decrease in the levels of mitochondrial lipid peroxidation, raise in the mitochondrial antioxidant levels and also increased level of mitochondrial enzymes.

Conclusion: The present study exposed that galangin 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. Transmission Electron Microscopy (TEM) studies was also in correlation with the biochemical parameters.

Key words: galangin, isoproterenol, mitochondrial enzymes, antioxidants.

INTRODUCTION

Cardiovascular diseases (CVDs) will be the superior cause of mortality in India by the year 2030, 23.3 million people will die annually from CVDs and it will continue as the most common threats to human life in both developing and developed countries.[1] The data expresses the seriousness of the burden from CVDs which need serious attention, particularly for myocardial infarction (MI). It occurs as a output of increased myocardial metabolic demand and decreased supply of oxygen and nutrients via the coronary circulation to the myocardium, resulting in cell injury.[2] Increased production of catecholamines due to adrenergic over stimulation is considered to be a notable cause of stress-induced cardiac dysfunction.[3]

Mitochondria holds about 30% of the heart cell's mass and it takes part in the production and regulation of cellular bioenergetic supply in the form of adenosine triphosphate (ATP) which is needed for cardiac contraction and relaxation. Mitochondrial dysfunction act as one of the eminent sources of reactive oxygen species (ROS) production in the heart and it is usually correlated with heart failure.[4] Excessive ROS production induce damage of electron transport complexes, deteriorates respiration and dysfunction of cells. The role of mitochondria in energy metabolism, Ca^{2+} homeostasis, cell signaling, and programmed cell death are the main reasons validaing the new therapeutic approaches that focused on mitochondria as one of the target for protect the myocardium.[5]

It is well known that free oxygen radicals play crucial role in the pathogenesis of chronic disorders such as cancer, diabetes, cardiovascular, and neurological diseases.[6] Various experimental and clinical studies reveals that large quantities of reactive oxygen species including superoxide, H_2O_2, and hydroxyl radicals are produced in the failing myocardium.[7] Therefore, therapeutic interventions that employ antioxidants with free radical scavenging activities have the efficacy to be used to combat oxidative stress related to various cardiovascular diseases, including myocardial infarction (MI). Owing to the costly medications and the extensive poverty burden, various traditional and folkloric approaches have been proposed such as the use of some medicinal plants.[8]

Galangin (3,5,7-trihydroxyflavone), a flavonoid dietary ingredient, it exist in honey and root of *Alpinia officinarum Hance* has long been utilized in traditional medicine.[9] Galangin is also present in high concentrations in propolis, which is a resinous material made by bees, used in many countries for the management of many diseases, including airway affections, cutaneo-mucosal and viral infections.[10, 11] It has various pharmacological activities like, antioxidant,[12] anti-obesity,[13] and antiviral[14] properties. Galangin suppresses cell proliferation and promotes apoptosis in several human malignancies, such as leukemia,[15] breast cancer,[16] pancreatic cancer,[17] it also has anti-inflammatory properties.[18]

Thus, the current study is carried out to infer that consumption of Galangin has mitochondrial protective role in isoproterenol induced myocardial infarction in rats. My current work attempted to authenticate the molecular mechanism of its therapeutic effect by studying the Anti-oxidant, lipid fractions, and other biochemical markers in the mitochondrial fractions.

MATERIALS AND METHODS

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 galangin

Galangin powder was suspended in 0.5% Carboxymethyl Cellulose (CMC) and each animal belonging to three different groups received 1.0 ml of galangin suspension at a dose of 20 mg/kg body weight everyday respectively by intragastric intubation.[19]

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.[20]

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 galangin via intragastric intubation at a daily dose of (20 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 IV rats received galangin 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.[21] Thiobarbituric Acid Reactive Substances

(TBARS) were estimated by the method of Fraga et al.[22] Superoxide Dismutase (SOD) Activity was assayed in the mitochondrial heart by the method of Kakkar et al.[23] Catalse were estimated by the method of Beers RF and Seizer.[24] Glutathione Peroxidase (GPx) was estimated by the method of Rotruck et al.[25] Reduced glutathione (GSH) were estimated by the method of Ellman.[26] From the Mitochondrial Fraction, the lipids were extracted by the method Folch et al.[27] Cholesterol in the mitochondrial lipid fraction was estimated by the method of Zilversmit.[28] 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.[29] Phospholipid content in the Mitochondrial Lipid Fraction was estimated by the method of Zlatkis.[30] The activities of Isocitrate Dehydrogenase (ICDH) were estimated by the method of king.[31] Succinate Dehydrogenase (SDH) were estimated by the method of Slater and Bonner.[32] Malate Dehydrogenase (MDH) were estimated by the method of Mehler et al.[33] α-Ketoglutarate Dehydrogenase (α-KGDH), were estimated according to the standard procedure of Reed and Mukherjee.[34]

Transmission electron microscopic study

Small pieces of heart were taken and rinsed in 0.1 M phosphate buffer (pH 7.2). Approximately 1 mm heart pieces were trimmed and immediately fixed into 3% ice-cold glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) and kept at 4 ◦C for 12 h. Then, tissues processing for TEM study were carried out. The grids containing sections were stained with 2% uranyl acetate and 0.2% lead acetate. Then the sections were examined under a transmission electron microscope (20,000×).

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 $P<0.05$.[35]

RESULTS

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

Table 1 describes the effect of galangin 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). Galangin 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 galangin (20 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).

Effect of galangin 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 galangin (20 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).

Effect of galangin 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). Treatment with galangin (20 mg/kg) daily for a period of 15 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).

DISCUSSION

In the present study, treatment with galangin foreign significant mitochondrial protection in isoproterenol induced myocardial infarction in rats. Mitochondria are the major oxygen deep organelle of the myocardial cell, they are close to reduce oxygen univalently and it assists as a locus in the cell where free radical reactions may initiate. Respiratory chain produces a huge 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.[36]

Oxidative stress is an plays an important role for precipitating factors in AMI.[37] It has also been described that after AMI, myocardial antioxidant ability is reduced and the level of oxidative stress is increased along with increased myocardial apoptosis.[38] Reactive oxygen species (ROS) generation performs a central role in isoproterenol-induced acute myocardial infarction.[39] In the present study, ISO treatment resulted in considerable increase in the levels of lipid peroxidation products in the heart tissue. Elevaed lipid peroxidation appears to be the inaugural stage in making the tissue more susceptible to oxidative damage. This may be answerable for the observed membrane damage revealed by elevated lipid peroxidation levels.[40] Galangin protects the myocardium from lipid peroxidation provoked by free radicals and averts the increased levels of mitochondrial lipid peroxidation products thereby restores normal mitochondrial architecture and activity in ISO induced myocardial infarcted rats. The declined lipid peroxide level in the mitochondria may be due to the potential of galangin in scavenging free radicals.

Antioxidant plays a vital role in protecting cells from the free radical damage. 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.[41] CAT is defending the cellular constituents against oxidative damage. Reduced glutathione assists in decomposing glutathione peroxidase (GPx) and GST to H_2O molecule and other organic hydroperoxides to non-toxic products. In the present study, decreased levels of SOD, CAT, GPx, and GST) were noticed in isoproterenol induced myocardial infarction in rats. During myocardial infarction, superoxide radicals produced at the site of damage, fluctuates SOD, resulting in the loss of activity and accumulation of superoxide radicals, that damages myocardium. As a result of oxidative stress there is increased usage of antioxidant enzymes like SOD.[42] A significant reduction in the activity of CAT with a conjoined increase in LPO observed in ISO group due to enormous production of free radicals by ISO.Pre-treatment with galangin significantly increased the activity of all the antioxidant enzymes (SOD, CAT, GPx, and GST) in isoproterenol induced rats. Galangin, a dietary flavonoid, may protect the cells from oxidative damage by decreasing the free radical production and intensify the antioxidant status. Besides the enzymatic antioxidants, non-enzymatic antioxidants also play a role in scavenging oxygen free radicals, thereby securing the cell function.

Lipids play an important role in cardiovascular disease, not only by contributing to the development of atherosclerosis but also by reforming the composition, structure, and stability of the cellular membrane. High levels of circulating cholesterol and its accumulation in the heart tissue have been correlated with cardiovascular damage.[43]

In present study, altered levels of lipids were ascertained in the mitochondrial fraction of the heart. The mitochondrial lipids suggest clear evidence for renovated cardiac function and ultrastructure in MI. Elevated levels of mitochondrial cholesterol are well allied with MI.[44] We detected an increased level of cholesterol in the mitochondria of heart tissue in isoproterenol-induced rats which indicates redistribution of cholesterol in the mitochondria of ischaemic cells. Increased activity of liver HMG-CoA reductase, give rise to excessive production and accumulation of cholesterol, resulting in progression of MI.[45] Increased cholesterol levels in the mitochondrial membrane alter the permeability of ions and the fluidity. Our results are line with previous finding[46] Phospholipids are crucial components for integrity of cellular membrane and subcellular organelles[47, 48] We also noticed significant decrease in the levels of phospholipids in mitochondrial fraction of the heart in ISO treated rats. In myocardial mitochondria, the primary role of phospholipids is to stabilize the conformation of membrane bound enzymes.[49] The significant increase in the levels of FFAs and decrease in phospholipids observed in the mitochondrial fraction of heart is due to the fast degradation of membrane phospholipids by phospholipases. Farber and young[50] have suggested that accelerated degradation of membrane phospholipases and

lysophospholipases have been linked to membrane dysfunction and irreversible ischemic injury. The metabolic products of isoproterenol produced much free radicals and the phospholipid rich mitochondrial membrane is vulnerable to free radical attack. This may be the cause for reduced levels of phospholipids in mitochondrial fractions of the heart of soproterenol-induced rats.

Treatment with galangin significantly increased the level of phospholipids in the isoproterenol-induced rats. galangin has the ability to decrease the free radical formation it may conserve 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 inverses triglyceride accumulation and leads to contractile dysfunction and it may also due to diminish activity of lipoprotein lipase, leads to decreased uptake of TGs from the circulation.[51]

Galangin administration considerably restored these alterations, thereby maintaining the normal fluidity and function of the myocardial membrane. Galangin may channel fatty acids to triacylglycerol synthesis and switch lipids from toxic metabolic pathways. Deposition of free fatty acids is a result of changes in myocardial lipid metabolism. All the changes in the metabolism of the sub cellular fraction may bring about the damage of the membranes of the cardiac myocyte mitochondria, which may be induce the disorders of electrolyte metabolism and contractile properties of the myocardium.

Mitochondria are important subcellular organelles involved in the energy production and are prone to oxidative stress. Cardiomyocytes are rich in mitochondria, which are indispensble organelles involved in myocardial injury. Recognition of drug interaction with mitochondria as secondary targets can assist us to understand the underlying mechanisms. Mitochondrial dysfunction plays an salient role in the pathogenesis of many CVDs. In the heart about 45% of the myocardial volume is concerned by mitochondria.[52]

The mitochondrial enzymes (ICDH, SDH, MDH and α- KGDH) catalyse the oxidation of several substrates through the tricarboxylic acid (TCA) cycle, affording reducing equivalents which are channeled through the respiratory chain for the synthesis of adenosine triphosphate (ATP) by oxidative phosphorylation. Inhibition of these enzymes by reactive oxygen species (ROS) may involve the mitochondrial substrate oxidation, resulting in reduced oxidation of substrates, minimized rate of transfer of reducing equivalents to molecular oxygen and reduction of cellular energy.[53] Isocitrate dehydrogenase is mainly exhibited in the heart and skeletal muscle mitochondria. It is NADP dependent and controls the mitochondrial redox balance and the subsequent oxidative damage. SDH is a component of electron chain and is bound to the inner mitochondrial membrane. Malate dehydrogenase , another enzyme present the outer membrane of mitochondria and susceptible to free radical attack.[54] Conversion of a-ketoglutarate to succinyl-CoA and NADH in the heart mitochondria is catalyzed by a-ketoglutarate dehydrogenase. Reduced activities of tricarboxylic acid cycle enzymes revealed aerobic oxidation of pyruvate, leads to declined ATP production in ISO induced rats. The activities of these dehydrogenases were reduced in the myocardial mitochondria of ISO induced rats. It indicates the production of free radicals by ISO. This is in agreement with earlier studies[51] Inhibition of these enzymes activities by ROS may affect the mitochondrial substrate oxidation, resulting in reduced oxidation of substrates, reduced rate of transfer of reducing equivalents to molecular oxygen and depletion of cellular energy.[55] Galangin pre-treated ISO induced myocardial infarcted rat's revealed increased levels of mitochondrial ATP which might be due to the increased activities of TCA cycle enzymes. Thus the improved activities of these enzymes showed the ability of galangin to counter ROS and it may be the reason for reinstating normal function of the mitochondria.

Alteration in the fine structure of Mitochondria is the most prominent TEM finding in cardiac damage induced by ISO. ISO-induced rat's heart showed swelling of Mitochondria, complete loss of Cristae, Vacuolation and change in shape and size. This is in agreement with previous study Senthil Kumaran and Stanely Mainzen Prince.[40] Galangin treatment has revealed that normal architecture of mitochondria is found without swelling. The Mitochondrial size is also greatly decreased. These observations agree closely with the results obtained by other parameters in the study.

In the current study, galangin ingestion was observed to exhibit cardioprotective effects as evidenced by holding the integrity of the lipid peroxidation, mitochondrial membranes restoring the activities of the antioxidants and mitochondrial enzymes to nearby normal

levels of rats induced with myocardial infarction. Moreover, galangin 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 galangin can be correlated directly with its ability to activate the energy status of the anti-oxidant defence system. Thus galangin may be useful as a safe and effective diet containing agent in the management of cardiovascular disease.

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

Groups	Control	galangin (20 mg/kg b.wt)	Isoproterenol (85 mg/kg b.wt)	galangin (20 mg/kg b.wt)+ isoproterenol (85 mg/kg b.wt)
TBARS (nmoles/mg protein)	5.92±1.06^{a}	4.57±0.99^{a}	9.83±1.03^{c}	8.25±1.16^{b}
Superoxide dismutase (SOD) (units/mg protein)	19.2±1.58^{b}	19.01±1.52^{c}	10.97±0.57^{a}	22.05±1.16^{d}
Catalase (CAT) (nmoles of H_2O_2 consumed / min / mg protein)	2.35±0.15^{b}	2.38±0.33^{b}	1.97±0.27^{a}	3.21±0.39^{c}
Glutathione peroxidase (GPx) (nmoles of GSH oxidized/min/ mg protein)	1.47±0.34^{c}	1.49±0.27^{c,d}	0.96± 0.05^{a}	1.31±0.38^{b}
Reduced Glutathione (nmoles GSH reduced /mg protein)	6.98±0.54^{b}	6.72±1.04^{b}	2.31± 0.59^{a}	9.23±0.46^{c}

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 $p<0.05$.

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

Groups	Control	galangin (20 mg/kg b.wt)	Isoproterenol (85 mg/kg b.wt)	galangin (20 mg/kg b.wt)+ isoproterenol (85 mg/kg b.wt)
Triglyceride (nmoles/mg protein)	18.01± 1.54^{a}	16.81±1.36^{a}	31.12±2.25^{c}	24.05±1.22^{b}
Cholesterol (nmoles/mg protein)	33.11± 2.84^{b}	50.12±4.39^{d}	27.91±2.55^{a}	39.12±3.72^{c}
Free fatty acids (nmoles/mg protein)	15.07±1.93^{c}	12.19±1.45^{a}	13.52±1.63^{b}	13.16±1.89^{b}
Phospholipids (nmoles/mg protein)	550.13±17.18^{c}	554.31±31.25^{d}	477.20±20.23^{a}	513.99±22.21^{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 $p<0.05$.

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

Groups	Control	galangin (20 mg/kg b.wt)	Isoproterenol (85 mg/kg b.wt)	galangin (20 mg/kg b.wt) + isoproterenol (85 mg/kg b.wt)
Isocitrate dehydrogenase (ICDH)	749.6±52.15^{c,d}	725.6±51.27^{c}	520.2±36.56^{a}	665.33±34.46^{b}
Succinate dehydrogenase (SDH)	39.15±3.16^{c}	41.72±3.26^{d}	17.97±3.22^{a}	33.05±1.81^{b}
Malate dehydrogenase (MDH)	330.17±10.95^{c}	334.50±11.84^{d}	147.71±9.45^{a}	249.69±9.12^{b}
α-ketoglutarate dehydrogenase (α –KGDH)	78.97±7.05^{c}	93.12±5.74^{d}	59.1± 5.29^{a}	73.22±6.39^{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 $p<0.05$.

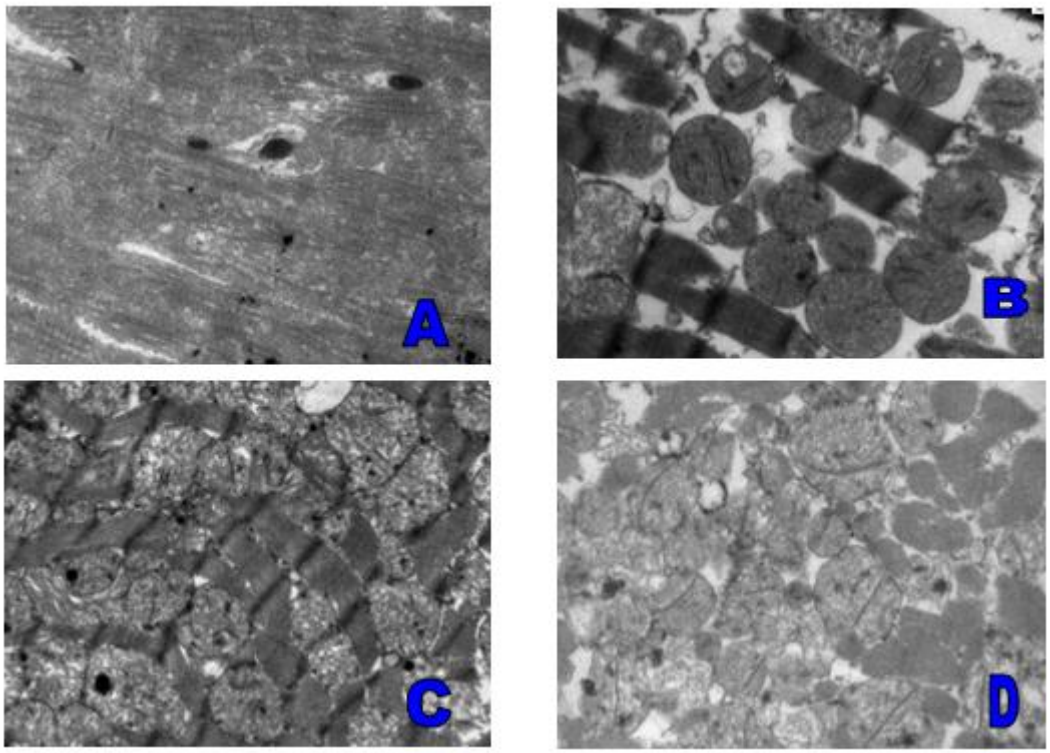

Fig. 1: Transmission electron microscopy images of the heart mitochondria. A) Control rats showing normal architecture of the heart mitochondria with cristae. B) ISO-induced rats showing swelling, disruption of cristae with vacuolation and membrane damage. Galangin treated rats showing mitochondria and myofibrils with Z-bands and no pathological changes (Fig. 2. C). ISO and galangin treated rats showing revealing near normal architecture and mild separation of cristae moderate swelling and greatly decreased size (Fig. 2. D)

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