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Original article

Protective Effect of Diosgenin and Exercise Training on Biochemical and ECG Alteration in Isoproterenol- Induced Myocardial Infarction in Rats

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Abstract

Objective(s)
Several studies have reported improved response of exercised hearts to myocardial infarction (MI). This study was aimed to evaluate the preventive role of treadmill exercise and diosgenin on cardiac marker enzymes, thiobarbituric acid reactive substances (TBARS), total antioxidant status (TAS), lipids, and electrocardiographic (ECG) patterns in isoproterenol (ISO)-induced myocardial infarction (MI) in male Wistar rats.

Materials and Methods
One hundred Wistar rats were divided into ten groups: Control rats (C), saline (S), L-cremephor (LC), exercise (E), diosgenin dissolved in L-cremephor (15 mg/kg/day) (D), exercise+ diosgenin (E+D), ISO injected (150 mg/kg) (ISO), exercise + ISO (E+ISO), diosgenin + ISO (D+ISO) and exercise+ diosgenin+ ISO (E+D+ISO). At the end of the experiment all animals anesthetized and blood samples were collected for biochemical estimation and also the ECG patterns were recorded.

Results
Exercise and diosgenin pretreatment significantly decreased the lactate dehydrogenase (LDH) and TBARS level in ISO injected animals. Exercise and diosgenin pretreatment significantly decreased serum total cholesterol and increased high density lipoprotein (HDL-C). ISO-treated rats showed pathological Q waves along with elevated ST segments. The altered electrocardiograms (ECG) of ISO-treated rats were also restored to near normal by diosgenin and exercise, but exercise and diosgenin had synergistic effects.

Conclusion
The present investigation demonstrates that combination of diosgenin and exercise exhibited significant protection against ISO induced electrocardiographical and biochemical changes. The cadioprotective mechanism(s) appear to be through changing lipid metabolism.

Keywords: Diosgenin, Isoproterenol, Myocardial Infarction, Treadmill

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Introduction

Myocardial infarction (MI) is one of the main causes of mortality and morbidity in the developed world and most of developing countries from cardiovascular diseases. An increased risk of coronary heart disease (CHD) is associated with high levels of serum total cholesterol, triglyceride and decreased levels of high-density lipoprotein (HDL) (1, 2).

Isoproterenol (ISO) is a synthetic ß-adrenergic agonist that causes severe stress in the myocardium, resulting in infarct like necrosis of the heart muscle (3). ISO-induced myocardial infarction serves as a well standardized model to study the beneficial effects of many drugs and cardiac function (4). Some of the mechanisms proposed to explain the mechanisms of ISO-induced damage to cardiac myocytes include hypoxia due to myocardial hyperactivity and coronary hypotension (5), calcium overload (6), and excessive production of free radicals resulting from oxidative metabolism of catecholamines (7). Since ISO promotes lipolysis in the myocardium, it increases the concentration of myocardial lipids (8).

Diosgenin is a plant-derived sapogenin, isolated from wild yams, structurally similar to progesterone and estrogen, and is the precursor for the industrial large-scale synthesis of different hormones including progesterone (9). Diosgenin (as a phytosterogen) is known to possess anti–hyperlipidaemic, anti-inflammatory and antioxidant properties (10).

Several studies have reported improved response of exercised hearts to injurious insults such as ischemia-reperfusion or acute MI (11, 12). It is reported that treadmill exercise induces protection against isoproterenol induced myocardial infarction (13). The findings of recent studies support that exercise has beneficial effect on hypercholesteromic condition with improving vascular function (14), reducing adhesion molecules/iNOS expression (15), reversing of impaired aerobic capacity and changes in blood lipids and enzymes (16). In addition it is concluded that exercise improved antioxidant enzyme activity, and further protected the body against oxidative stress (lipid peroxidation and DNA damage) (17). It has also been suggested that, combination of exercise with phytosterogens had beneficial effect on cardiovascular risk factors and lipid profiles (18).

There are few studies that investigate preventive mechanisms of diosgenin or exercise (9, 10, 13) and there is no study that used the combination of diosgenin and exercise on the isoproterenol-induced myocardial infarction. This study therefore aimed to determine the effect of diosgenin and exercise on biochemical alterations, total antioxidant status (TAS), thiobarbituric acid reactive substances (TBARS), and ECG pattern in isoproterenol-induced myocardial infarction in rats.

Materials and Methods

Animals

One hundred male Wistar rats (3 months old and initial body mass of 170±27 g) were obtained from laboratory animal house of Tabriz University of Medical Sciences. They were kept in standard laboratory conditions under natural light and dark cycle. The rats were fed normal diet and water. All the experimental procedures were conducted according to protocols approved by the Animal Care Committee of the Tabriz University of Medical Sciences.

Experimental design

Animals were divided into ten groups and each group consisted of ten rats as following (n= 10):

control (C), saline (S), Isoproterenol (ISO), L-cremephor (LC), diosgenin (D), exercise (E), diosgenin+ISO (D+ISO), exercise+ISO (E+ISO), exercise+diosgenin (E+D), exercise+ diosgenin+ ISO (E+D+ISO)

Experiment 1: Pretreatment by diosgenin

In D and D+ISO groups, animals were orally administered diosgenin (Sigma Chemical. Co., 15 mg/kg/day body weight, dissolved in L-cremephor for a period of 15 days) (19). ISO was injected subcutaneously 24 hr after last

diosgenin administration. L-cremephor was used as vehicle group.

Experiment 2: Exercise

Exercise and exercise+ISO groups were trained as mentioned in treadmill exercise protocol and ISO was injected subcutaneously 24 hr after last exercise bout.

Experiment 3: Exercise and diosgenin

In exercise+diosgenin group, administration of diosgenin started on the first day of 6th week of training. The first dose of ISO was administered 24 hr after last session of exercise and Diosgenin gavages.

Treadmill exercise protocol

Rats were run on a motorized treadmill, at room temperature, for 8 weeks, 5 days/week, 30 min/day at a speed of 20-25 m/min up to a 15% grade (20). The length of the training sessions was progressively increased (from ten minutes duration at fifteen m. min^{-1} with a 15% grade), so that in the end of the second week, the rats could run anticipated workload after a warm-up period of five min at fifteen m. min^{-1}. All of the animals were sacrificed 48 hr after their last exercise bout. In order to avoid possible stress effects, no electric shock was ever applied to animals in either group during exercise training.

Induction of experimental myocardial infarction

Rats received subcutaneous injection of ISO (Sigma Chemical. Co., 150 mg/kg body weight, dissolved in normal saline) at an interval of 24 hr (21).

After 12 hr of the second ISO-injection the rats were anesthetized at the end of experiments by injecting sodium pentobarbital (35 mg/kg, i.p.). Blood samples were drawn from the inferior vena cava before sacrificing and serum was separated by centrifugation for biochemical estimation.

Biochemical estimations

The activities of lactate dehydrogenase (LDH), MB- creatin phosphokinase (CK-MB), cholesterol, triglyceride and high-density lipoprotein cholesterol (HDL-C) of serum were determined using commercial kits (Parsazmoon Co., Karaj, Iran) and auto analyzer (Abbott, Alcyon 300, USA). All analyses were performed in accordance with the manufacturers' recommendations.

Tissue processing, homogenate preparation

At the end of the training programs, 48 hr after the last exercise-training session, and 12 hr after last ISO administration the rats were anaesthetized with pentobarbital sodium (35 mg/kg, i.p.), the heart was quickly removed, washed with ice-cold saline and blotted. Hearts were assigned for oxidative stress analysis, then the atria and great blood vessels were trimmed, the ventricles were weighed, and the apex was cut and quickly frozen in liquid N_2. Duration of the process was less than 2 min. Cardiac homogenates were prepared at 0–4 ºC as previously described (22). In brief, a known weight of ventricle muscle was homogenized in ice cold 0.1 M Potassium phosphate buffer, pH 7.4. The homogenates were centrifuged at 1000 rpm for 1 min at 4°C. The supernatants contained the cytoplasmic protein fraction were collected and protease inhibitor cocktail (104 mM AEBSF, 0.08 mM aprotinin, 2 mM leupeptin, 4 mM bestatin, 1.5 mM pepstatin A, and 1.4 mM E-64) (P8340, Sigma-Aldrich, St Louis, MO) was added to them and stored at -80 ºC until use. The protein concentration of the homogenates was determined using total protein kit (Randox labs. Crumlin, UK) in accordance with kit guideline.

Lipid peroxidation measurement

The amount of TBARS was determined by the TBA (thiobarbituric acid) assay. All reagents used in this assay were obtained from Merck (Darmstadt, Germany). Briefly, 0.50 ml of plasma was added to 3 ml of 1% phosphoric acid, 1 ml of 0.60% TBA, and 0.15 ml of 0.20% butylated hydroxytoluene in 95% methanol. The samples were heated in a boiling water bath for 45 minutes, cooled and 4 ml of 1- butanol was added. The butanol phase was separated by centrifugation at 3000 rpm for 10 minutes and absorbance was measured at 532

nm. The concentration of MDA was calculated and expressed as nmol/mg protein (23).

Analyses of total antioxidant status (TAS)

Serum total antioxidant status (TAS) was determined for a quantitative assessment of *in vivo* antioxidant status using a commercially available kit (Randox) based on the trolox equivalent antioxidant capacity method of following the manufacturers instructions (24). Blood was collected via venipuncture using serum separator tubes, stored at 4 °C and serum separated within 2 hr. Serum samples were then stored at -20 °C waiting further analysis. All samples were then assayed for TAS as a batch. This involved mixing 20 µl calibrator (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid 1.79 mmol/l) or sample with 1 ml of chromogen (metmyoglobin 6.1 µmol/l, ABTS 610 µmol/l) and incubating at 37 °C for 3 min. Initial absorbance was then read at 600 nm in a spectrophotometer (Biorad). After which 200 µl of substrate (hydrogen peroxide 250 µmol/l) was added to calibrator and sample and incubated at 37 °C for 3 min. Final absorbance was then read at 600 nm. The change in absorbance value for samples relative to the change in absorbance of the calibrator was then used to calculate the TAS in all samples.

Electrocardiography

The ECG patterns were recorded by power lab (AD instruments 4/30, Australia). ECG recordings were made in anesthetized (pentobarbital sodium 35 mg/kg, i.p.) animals. The types of alterations (ST segment elevation or depression and Q wave inversion) in experimental animals were considered.

Statistical analysis

Descriptive data (means±SEM) were calculated for each dependent variable. Overall group differences were analyzed using a one-way ANOVA. When appropriate, *post-hoc* analyses were made using a Tukeys HSD test. In all tests, a probability level of 0.05 was used as the decision rule for significance testing.

Results

Serum enzyme levels

Treatment with isoproterenol increased levels of diagnostic marker enzymes (LDH and CK-MB) in comparison with the controls (Table 1).The LDH level appears to be significantly lower in the combined treatment group (E+D+ISO) than in the group injected with isoproterenol only.

LDH level of plasma was increased significantly in exercise rats. Diosgenin administration decreased the LDH level in rats that also exercised. CK-MB level followed the same pattern as LDH level, but because of extreme variation in the concentrations among subjects, it was not significant (Table 1).

Table 1. Levels of MB- creatine phosphokinase (CK-MB) and lactate dehydrogenase (LDH) in plasma of normal and experimental groups of rats.

Group	CK-MB (U/L)	LDH (U/L)
Control (C)	702.87±77.61 [ab]	469.12±72.34 [ad]
Diosgenin alone (D)	571.75±51.86 [ab]	1058.25±94.155 [ad]
Exercise alone (E)	1087.70±171.46 [a]	2645.50±135.07 [ac]
E+D	1299.10±120.56	1686.66±242.59 [acd]
ISO induced (ISO)	1984.75±245.60	3760.71±361.95 [b]
D+ISO	1489.87±167.68	2084.87±111.58 [ac]
E+ISO	1138.75±193.42 [a]	1698.50±109.49 [acd]
E + D +ISO	1690.50±201.86	2618.50±155.03 [ac]

Results are expressed as Mean±SEM, for (n=10) each group.
[a] *P*< 0.05 when compared with ISO, [b] *P*<0.05 when compared with E+D+ISO and D+ISO, [c] *P* < 0.05 when compared with C, [d] *P*< 0.05 when compared with E
[e] *P* <0.05 when compared with ISO, D+ISO, E+ISO, E+D+ISO

extreme variation in the concentrations among subjects, it was not significant (Table 1).

Lipid peroxidation levels
ISO-treated rats showed a significant increase in myocardial TBARS when compared to normal rats. Administration of Diosgenin (10 mg/kg) to ISO-induced rats for a period of 15 days cannot decrease the TBARS level in them. Exercise decreased TBARS levels in ISO group significantly. In addition, combination of diosgenin and exercise decreased TBARS levels significantly in ISO induced rats (Figure 1).

Total antioxidant status (TAS)
Rats induced with ISO, showed a marked decrease in the TAS in heart in comparison with

normal control rats but it was not significant. Pretreatment with diosgenin (10 mg/kg) daily for a period of 15 days, exercise and their combination increased TAS level when compared with ISO alone-induced rats but it was not significant. However, combination of diosgenin and exercise in control rats can significantly increase ($P< 0.05$) TAS level in comparison to ISO- alone (Figure 2).

Serum lipid profile
The administration of isoproterenol significantly increased plasma cholesterol level and decreased HDL-C level in comparison with those observed in the controls. Combination of the exercise and diosgenin maintained the levels of plasma cholesterol and HDL-C as compared to the group injected with isoproterenol only (Table 2).
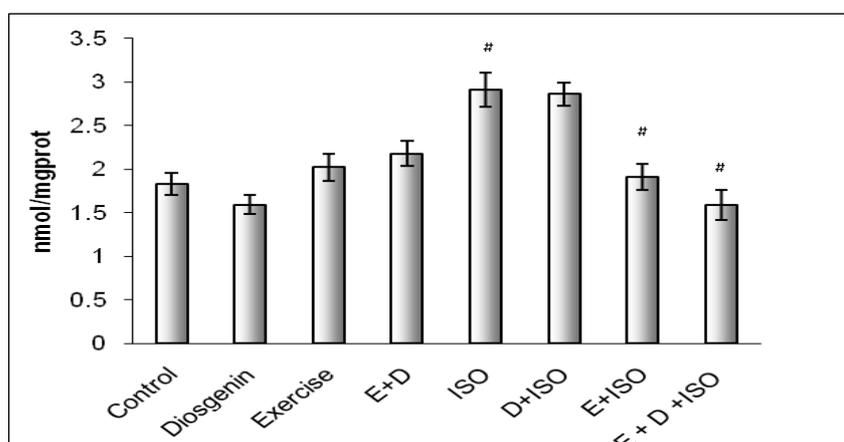

Figure 1. Effect of diosgenin, exercise and their combination on heart tissue thiobarbituric acid-reactive substances (TBARS) in the control and ISO-induced groups. Values are mean±SEM (n= 10). [#]$P< 0.05$ versus ISO alone and D+ISO groups (analysis of variance +Tukey's post hoc test).
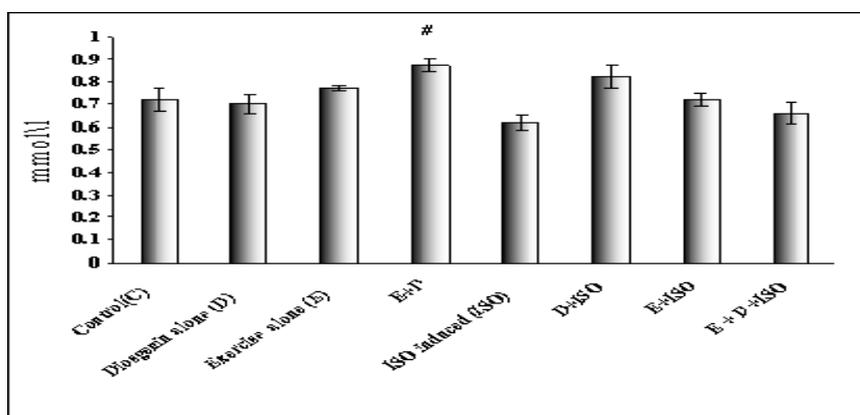

Figure 2. Effect of diosgenin, exercise and their combination on plasma total antioxidant status (TAS) in the control and ISO-induced groups. Values are mean±SEM (n=14). [#]$P < 0.05$ versus ISO alone and exercise+ISO groups (analysis of variance +Tukey's post hoc test).

ECG

The ECG patterns of normal and ISO-treated rats are shown in Figure 3. Rats untreated with ISO (150 mg/kg) did not show any change in ECG patterns. ISO-treated rats showed pathological Q waves along with elevated ST segments. These changes were restored to near normal in diosgenin and exercise and diosgenin+ exercise pretreated rats administered with ISO.

Body weight

Exercise significantly increased body weight in E Group as compared to other groups, except E+ISO group. Diosgenin decreased body weight in D Group significantly in comparison with all groups that exercised (Table 2).

Table 2. Levels of the cholesterol, triglyceride, and high density lipoprotein (HDL-C) in plasma and body weight of normal and experimental groups of rats.

Group	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-C (mg/dl)	Body weight (g)
Control (C)	57.12±4.15 [a]	44.75±4.58	22±1.36	246.37±5.95 [b]
Diosgenin (D)	43.37±3.39[a]	45.00±5.14	21.00±1.25	221.50±8.91[b]
Exercise (E)	47.30±1.57[a]	51.30±3.71	22.20±1.05 [a]	300.50±3.90[c]
E+D	50.40±2.24[a]	54.10±2.80	22.5±1.07 [a]	257.80±5.48 [b c]
ISO induced (ISO)	80.75±5.10	57.75±7.66	17.75±0.70 [a]	257.75±9.30 [b]
D+ISO	74.55±7.35	47.11±3.70	21.00±0.94	249.22±10.15 [b]
E+ISO	62.62±8.74	41.00±8.89	21.55±0.72	267.88±7.52 [c]
E+D +ISO	56.77±4.28 [a]	53.33±3.68	22.66±0.47 [a]	262.22±8.54 [b c]

Results are expressed as Mean±SEM for (n= 10) each group.
[a]$P< 0.05$ when compared with ISO, [b]$P< 0.05$ when compared with E, [c]$P< 0.05$ when compared with D

A. Control   B. Exercise

C. Diosgenin (10mg\Kg)   D. Exercise+Diosgenin

E. ISO (150mg\kg)   F. Diosgenin+ISO

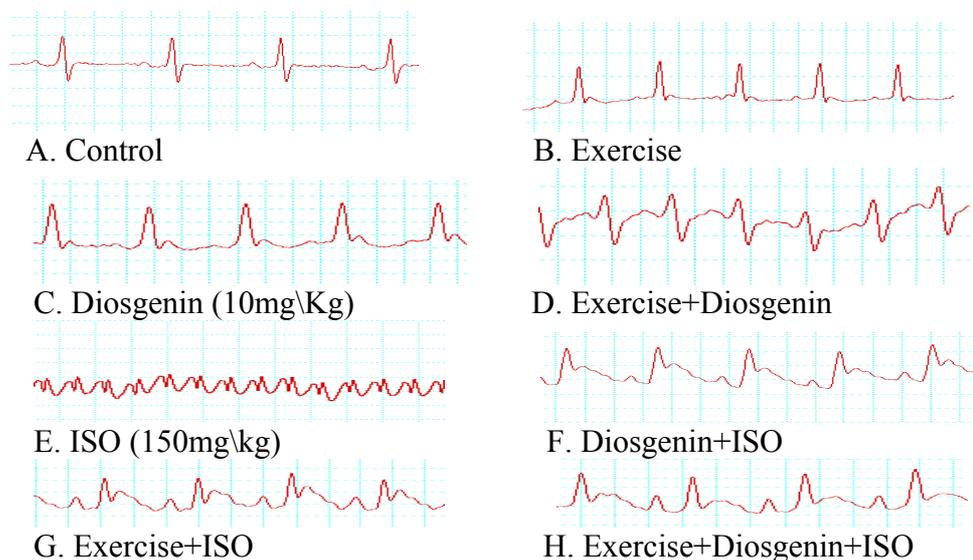G. Exercise+ISO   H. Exercise+Diosgenin+ISO

Figure 3. Effect of diosgenin and exercise on electrocardiographic patterns in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.
A: Normal control rats showing normal ECG pattern. B: Normal rats treated with diosgenin (10 mg/kg) showing normal ECG pattern. C: Exercised rats showing normal ECG pattern D: Exercised rats treated with diosgenin (10 mg/kg) showing normal ECG pattern. E: ISO alone (150 mg/kg) rats show ST segment elevation along with pathological Q waves. F: Diosgenin (10 mg/kg) + ISO show near normal ECG pattern. F: Exercised rats+ISO shows near normal ECG pattern. H: Exercised rats+ diosgenin (10 mg/kg) + ISO return the ECG back to normal.
Since no change of any studied parameter in comparison to control group was seen, data of group seven (saline) and eight (l-chremophore) were ignored.

Discussion

In this study, subcutaneous injection of ISO (150 mg/kg) once a day for two days increased CK-MB and LDH levels. Exercise decreased the serum CK-MB and LDH levels, in addition either diosgenin pretreatment or combination of diosgenin and exercise lowered LDH level in ISO treated rats.

Number of investigations has indicated that when myocardial cells, containing LDH and CK-MB are damaged or destroyed due to deficient oxygen supply or glucose, the cell membrane becomes permeable or may rapture, which results in the leakage of enzymes (25). The release of cellular enzymes reflects the alterations in plasma membrane integrity or permeability as a response to β-adrenergic stimulation. This might be due to the damage caused to the sarcolemma by the β-agonist that has rendered it leaky (21). In addition in this study ISO treatment showed an increase in the levels of lipid peroxidation products (TBARS) in heart. Increased lipid peroxidation appears to be the initial stage to the tissue making it more susceptible to oxidative damage. This may be responsible for the observed membrane damage as evidenced by the elevated lipid peroxidation in terms of TBARS. It was suggested that ISO produces free radicals via β-adrenoceptor mechanism, affects the cell metabolism to such a degree that cytotoxic free radicals are formed, producing myocardial necrosis. This accounts for the increased levels of LDH and CK-MB in isoproterenol injected rats (26).

We found that combination of diosgenin and exercise decreased the activities of LDH, CK-MB and TBARS that could be attributed to the protective effect of this pretreatment on the myocardium, reducing the cardiac damage thereby restricting the leakage of these enzymes.

Lipid peroxidation has been shown to be lower in heart and liver of exercise-trained animals compared with sedentary animals (27). A reduction in the oxidative DNA damage marker, 8-oxo- 7, 8-dihydro-2-deoxyguanosine (8-oxodG), has been reported in skeletal muscle after exercise training (28), and protein carbonyl content in the heart is significantly lower in trained rats compared with sedentary rats (29). Moderate treadmill exercise for 24 week also reduced protein carbonyls and lipid peroxidation in hearts from 52 week old mice (30).

It was shown that a diet containing diosgenin reduces the cognitive deterioration and brain lipid peroxidation in mice (31). In the other hand diosgenin maybe transformed in human intestine into serum dehydroepiandrosterone (DHEA) which is associated with reduced lipid peroxidation (32, 33). According to our study diosgenin has no effect on TBARS level that. Seems it was because of its poor antioxidant activity. However, diosgenin in combination with exercise had better effect.

In accordance with earlier reported studies, in this study it was seen that LDH level in exercised rat is higher than control rats (34, 35). But Diosgenin decreased the level of LDH in exercise group. There are strong evidences that antioxidants can eliminate LDH level in exercise (36), and Diosgenin as an antioxidant (10) may be responsible for this elimination.

Evaluation of the TAS gives more biological relevant information than that of the individual levels of specific antioxidants of a given body fluid such as plasma. The overall TAS considers the cumulative effect of all antioxidants (known and unknown, measurable and not measurable) present in plasma and it is used for evaluating the effect of several physiological conditions on plasma in human and animals. It has been suggested that estimation of TAS may be a useful parameter for assessment of oxidative stress (37). Plasma TAS was increased significantly by combination of exercise and diosgenin in comparison to ISO induced group. Our results are consistent with previous reports (38, 39) and this increase may protect cells from oxidative stress.

There is an urgent need for the clinical development of safe and non-toxic cytoprotective agents for the prevention of cardiovascular diseases. Lipid peroxidation

has been identified as one of the basic deteriorative reactions in cellular mechanisms during myocardial ischemia (7). This study highlights that combination of diosgenin and exercise is one of the promising cytoprotective elements for improving defence mechanisms in the physiological systems against oxidative stress caused by myocardial infarction.

Findings showed that HDL-C level in ISO injected rats decreased significantly and combination of diosgenin and exercise reversed this effect. In addition cholesterol increased in this group and combination of diosgenin and exercise decreased the concentration of total cholesterol.

High levels of HDL cholesterol have a negative correlation with MI (1). Myocardial infarction (MI) is also associated with altered lipid metabolism. The increased concentration of cholesterol could be due to a decrease in HDL-C, since HDL-C is known to be involved in the transport of cholesterol from tissues to the liver for its catabolism (24). Levels of serum total cholesterol were increased while the levels of HDL-C were decreased in ISO treated rats. These changes in lipid levels might be due to enhanced lipid biosynthesis by cardiac cyclic adenosine monophosphate (40). It was frequently reported that an exercise protocol is important for the control of dyslipidemias (41).

Diosgenin has various effects on cholesterol metabolism, one of the most important being the capacity to lower plasma cholesterol concentration in chickens and rabbits fed cholesterol (42). This hypocholesterolaemic effect has been suggested to be dependent on the capacity of diosgenin to inhibit cholesterol absorption, to decrease liver cholesterol concentration, to increase biliary cholesterol secretion and fecal excretion of neutral sterols (43). Lipid profile changes were consistent with these reports. It was reported that diet containing Diosgenin reduces the cognitive deterioration and brain lipid peroxidation in mice (31). Therefore, protective effects of diosgenin that were seen maybe due to this effect. The increase in HDL-C level can be the main combined effect of diosgenin and exercise on serum cholesterol profile.

Increasing of body weight in exercise group was consistent with other reports (12) that may be due to increasing of food intake, muscle mass (44) and its anabolic effect (45). While Diosgenin decreased body weight in exercised rats that may be due to low food consumption (46).

Electrocardiogram is reliable for the early diagnosis of MI. The diagnosis of MI is dependent on documentation that cardiac necrosis has taken place. The main criteria generally used for the definite diagnosis of MI is evolving pattern of ECG-abnormalities (47). Administration of ISO to experimental animals causes ECG-changes suggestive of myocardial ischemia. Significant alterations of ECG patterns were observed in ISO-induced rats when compared with normal rats. The characteristic findings were the appearance of pathological Q waves and ST segment elevation, which are some of the indicative signs of ischemia. This could be due to myocardial necrosis accelerated by ISO. The consecutive loss of cell membrane might be characterized by ST elevation (48). The prominent Q waves and ST-segment elevation in the region of injured myocardium were seen only in conditions of severe ischemia, infarction and in patients with severe heart disease (49). ISO induction also increased the heart rate (50). Also, Prabhu and Devi (2006) have reported similar ECG changes in ISO-induced rats. ECG pattern showed that diosgenin and exercise together were more effective (51). Since combination of exercise and diosgenin enhanced the protective effects on heart, this augmented positive change may be due to elimination of unwanted side effects.

There is an urgent need for the clinical development of safe and non-toxic cytoprotective agents for the prevention of cardiovascular diseases. Lipid peroxidation *in vivo*, has been identified as one of the basic deteriorative reactions in cellular mechanisms during myocardial ischemia.

Conclusion

This study highlights that combination of diosgenin and exercise is one of the promising cytoprotective elements for improving defense mechanisms in the physiological systems against oxidative stress caused by myocardial infarction.

Acknowledgment

References

1. Grundy SM. Cholesterol and coronary heart disease. A new era. JAMA 2004; 256:2849-2858.
2. Carlson LA, Böttiger LE. Risk factors for ischaemic heart disease in men and women. Results of the 19- year follow-up of the Stockholm Prospective Study. Acta Med Scand 1985; 218; 207-211.
3. Sushamakumari S, Jayadeep A, Kumar JS, Menon VP. Effect of carnitine on malondialdehyde, taurine and glutathione levels in heart of rats subjected to myocardial stress by isoproterenol. Indian J Exp Biol 1989; 27: 134-7.
4. Ithayarasi AP, Devi CS. Effect of alpha-tocopherol on lipid peroxidation in isoproterenol induced myocardial infarction in rats. Indian J Physiol Pharmacol 1997; 41:369-376.
5. Yeager JC, Iams SG.The hemodynamics of isoproterenol-induced cardiac failure in rats. Circ Shock 1981; 8:151–163.
6. Bloom S, Davis DL. Calcium as a mediator of isoproterenol-induced myocardial necrosis. Am J Pathol 1972; 69:459–470.
7. Singal PK, Kapur N, Dhillon KS, Beamis RE, Dhalla NS. Role of free radicals in catecholamine-induced cardiomyopathy. Can J Physiol Pharmacol 1983; 60:1390-1397.
8. Manjula TS, Devi CS. Effect of aspirin on isoproterenol induced changes in lipid metabolism in rats. Indian J Med Res 1993; 98:30-33.
9. Au AL, Kwok CC, Lee AT, Kwan YW, Lee MM, Zhang RZ, *et al.* Activation of iberiotoxin-sensitive, Ca2+-activated K+ channels of porcine isolated left anterior descending coronary artery by diosgenin. Eur J Pharmacol 2004; 502:123-33.
10. Son IS, Kim JH, Sohn HY, Son KH, Kim JS, Kwon CS. Antioxidative and hypolipidemic effects of diosgenin, a steroidal saponin of yam (Dioscorea spp.), on high-cholesterol fed rats. Biosci Biotechnol Biochem 2007; 71:3063-3071.
11. Yamashita N, Baxter GF, Yellon DM.Exercise directly enhances myocardial tolerance to ischaemia-reperfusion injury in the rat through a protein kinase C mediated mechanism. Heart. 2001; 85:331-336.
12. Brown DA, Chicco AJ, Jew KN, Johnson MS, Lynch JM, Watson PA, *et al.* Cardioprotection afforded by chronic exercise is mediated by the sarcolemmal, and not the mitochondrial, isoform of the KATP channel in the rat. J Physiol 2005; 569:913-924.
13. Darrah MI, Engen RL. Beneficial effects of exercise on l-isoproterenol induced myocardial infarction in male rats. Med Sci Sports Exerc 1982; 14:76-80.
14. Jen CJ, Liu YF, Chen HI.Short-term exercise training improves vascular function in hypercholesterolemic rabbit femoral artery. Chin J Physiol 2005; 48:79-85.
15. Yang AL,Chen HI. Chronic exercise reduces adhesion molecules/iNOS expression and partially reverses vascular responsiveness in hypercholesterolemic rabbit aortae. Atherosclerosis 2003; 169:11-7.
16. Weise SD, Grandjean PW, Rohack JJ, Womack JW, Crouse SF. Acute changes in blood lipids and enzymes in postmenopausal women after exercise. J Appl Physiol 2005; 99:609-15.
17. Kim KS, Paik IY, Woo JH, Kang BY. The effect of training type on oxidative DNA damage and antioxidant capacity during three-dimensional space exercise. Med Princ Pract 2010; 19:133-41.
18. Aubertin-Leheudre M, Lord C, Khalil A, Dionne IJ. Effect of 6 months of exercise and isoflavone supplementation on clinical cardiovascular risk factors in obese postmenopausal women: a randomized, double-blind study. Menopause 2007;14(4):624-9
19. Matsuda H, Pongpiriyadacha Y, Morikawa T, Kishi A, Kataoka S, Yoshikawa M. Protective effects of steroid saponins from *Paris polyphylla* var. yunnanensis on ethanol- or indomethacin-induced gastric mucosal lesions in rats: structural requirement for activity and mode of action. Bioorg Med Chem Lett 2003; 13:1101-1106.
20. Hayward R, Ruangthai R, Schneider CM, Hyslop RM, Strange R, Westerlind KC. Training enhances vascular relaxation after chemotherapy-induced vasoconstriction. Med Sci Sports Exerc 2004; 36:428-434.
21. Rajadurai M, Stanely P. Preventive effect of naringin on cardiac markers, electrocardiographic patterns and lysosomal hydrolases in normal and isoproterenol-induced myocardial infarction in Wistar rats. Toxicology 2007; 230:178-188.

22. Rothermel B, Vega RB, Yang J, Wu H, Bassel-Duby R, Williams RS. A protein encoded within the Down syndrome critical region is enriched in striated muscles and inhibits calcineurin signaling. J Biol Chem 2000; 275:8719–8725.

23- Drapr HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990;186:421-31

24. Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and itsapplication to monitoring the antioxidant status in premature neonates. Clin Sci 1993; 84:407-412.

25. Mathew S, Menon PV, Kurup PA. Effect of administration of vitamin A, ascorbic acid and nicotinamide adenine dinucleotide + flavin adenine dinucleotide on severity of myocardial infarction induced by isoproterenol in rats. Indian J Exp Biol 1985; 23:500-504.

26. Sumitra M, Manikandan P, Kumar DA, Arutselvan N, Balakrishna, K, Manohar BM, *et al*. Experimental myocardial necrosis in rats: role of arjunolic acid on platelet aggregation, coagulation and antioxidant status. Mol Cell Biochem 2001; 224:135-42.

27. Venditti P, Di Meo S. Antioxidants, tissue damage, and endurance in trained and untrained young male rats. Arch Biochem Biophys 1996; 31:63–68.

28. Radák Z, Sasvári M, Nyakas C, Pucsok J, Nakamoto H, Goto S. Exercise preconditioning against hydrogen peroxide-induced oxidative damage in proteins of rat myocardium. Arch Biochem Biophys 2000; 376: 248–251.

29. Radák Z, Kaneko T, Tahara S, Nakamoto H, Ohno H, Sasvári M, *et al*. The effect of exercise training on oxidative damage of lipids, proteins, and DNA in rat skeletal muscle: evidence for beneficial outcomes. Free Radic Biol Med 1999; 27:69–74.

30. Navarro A, Gomez C, López-Cepero JM, Boveris A. Beneficial effects of moderate exercise on mice aging: survival, behavior, oxidative stress, and mitochondrial electron transfer. Am J Physiol Regul Integr Comp Physiol 2004; 286:505–511.

31.Chan YC, Hsu CK,Wang MF,Su TY. A diet containing yam reduces the cognitive deterioration and brain lipid peroxidation in mice with senescence accelerated. Int J Food Sci Technol 2004; 39: 99-107.

32. Araghiniknam M, Chung S, Nelson-White T, Eskelson C, Watson RR. Antioxidant activity of dioscorea and dehydroepiandrosterone (DHEA) in older humans. Life Sci. 1996; 59: 147-57.

33. McAnuf MA, Omoruyi FO, Morrison EY, Asemota HN. Changes in some liver enzymes in streptozotocin-induced diabetic rats fed sapogenin extract from bitter yam (*Dioscorea polygonoides*) or commercial diosgenin. West Indian Med J 2005; 4:97-101.

34. Siu PM, Donley DA, Bryner RW, Always SE. Myogenin and oxidative enzyme gene expression levels are elevated in rat soleus muscles after endurance training. J Appl Physiol 2004; 97:277-285

35. Taylor RP, Ciccolo JT, Starnes JW. Effect of exercise training on the ability of the rat heart to tolerate hydrogen peroxide. Cardiovasc Res 2003; 58:575-581.

36. Su QS, Tian Y, Zhang JG, Zhang H. Effects of allicin supplementation on plasma markers of exercise-induced muscle damage, IL-6 and antioxidant capacity. Eur J Appl Physiol 2008; 103:275-283.

37. Alcerczyk A, Bartosz G. Thiols are main determinants of total antioxidant capacity of cellular homogenates. Free Radic Res 2003; 37:537-541.

38. Iborra RT, Ribeiro IC, Neves MQ, Charf AM, Lottenberg SA, Negrão CE, *et al*. Aerobic exercise training improves the role of high-density lipoprotein antioxidant and reduces plasma lipid peroxidation in type 2 diabetes mellitus. Scand J Med Sci Sports 2008; 18:742-750.

39. González D, Marquina R, Rondón N, Rodriguez-Malaver AJ, Reyes R. Effects of aerobic exercise on uric acid, total antioxidant activity, oxidative stress, and nitric oxide in human saliva. Res Sports Med 2008; 16:128-137.

40. Paritha IA, Devi CS. Effect of alpha-tocopherol on isoproterenol-induced changes in lipid and lipoprotein profile in rats. Indian J Pharmacol 1997; 29:399.

41. Leroith D. Dyslipidemia and glucose dysregulation in overweight and obese patients. Clin Cornerstone 2007; 8:38-52.

42. Laguna J, Gomez A, Pena A, Guzman-Garcia J. Effect of diosgenin on cholesterol metabolism. J Atheroscler Res 1962; 2:459-470.

43. Ulloa N, Nervi F. Mechanism and kinetic characteristics of the uncoupling by plant steroids of biliary cholesterol from bile salt output. Biochim Biophys Acta 1985; 837:181-189.

44. Choe MA, An GJ, Lee YK, Im JH, Choi-Kwon S, Heitkemper M. Effect of early low-intensity exercise on rat hind-limb muscles following acute ischemic stroke. Biol Res Nurs 2006; 7:163-174.

45. Majchrzak KM, Pupim LB, Flakoll PJ, Ikizler TA. Resistance exercise augments the acute anabolic effects of intradialytic oral nutritional supplementation. Nephrol Dial Transplant 2008; 23:1362-1369.

46. Uchida K, Takase H, Nomura Y, Takeda K, Takeuchi N, Ishikawa Y. Changes in biliary and fecal bile acids in mice after treatments with diosgenin and beta-sitosterol. J Lipid Res 1984; 25:236-45.

47. Timmis AD. Early diagnosis of acute myocardial infarction. British Medical Journal 1990 27;301(6758):941-2.

48. Holland RP, Brooks H.TQ-ST segment mapping: critical review and analysis of current concepts. Am J Cardiol 1977; 40:110–129.

49. Nariander NN, Dhawan S. Isoproterenol induced myocardial necrosis and biogenic amine levels in rats. Indian J Exp Biol 1979; 17:292–294.

50. Kela AK, Reddy LP, Thombre DP. ECG findings in normal rats and after administration of isoproterenol. Indian J Physiol Pharmacol 1980; 24:84–90.