Effect of vitamin E alone and in combination with lycopene on biochemical and histopathological alterations in isoproterenol-induced myocardial infarction in rats

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Background: The present study has been designed to evaluate the combined cardioprotective effect of vitamin E and lycopene on biochemical and histopathological alteration in isoproterenol-induced myocardial infarction in rats. Materials and Methods: Adult male albino rats of Wistar strain were treated with isoproterenol (200 mg/kg, s.c.) for 2 days at an interval of 24 h to develop myocardial infarction. Vitamin E (100 mg/kg/day, p.o.) and lycopene (10 mg/kg/day, p.o.) were administered alone and in combination for 30 days. Change in body weight and organ weight were monitored. Levels of serum marker enzymes (AST, ALT, LDH and CK-MB), lipid peroxidation, endogenous antioxidants (GSH, GPX, GST, SOD and CAT), membrane bound enzymes (Na+/K+ ATPases, Mg2+ATPases and Ca2+ATPases) were evaluated. LDH isoenzyme separation was carried out using gel electrophoresis. Histopathology of heart tissue was performed. Results: Induction of rats with isoproterenol resulted in a significant elevation in organ weight, lipid peroxidation, serum marker enzymes (AST, ALT, CK-MB and LDH), and Ca2+ATPases, whereas it caused a significant (P < 0.001) decrease in body weight, activities of endogenous antioxidants (GSH, GPX, GST, SOD and CAT), Na+/K+ and Mg2+ATPases. ISO treated rats showed high intensity band of LDH1-LDH2 isoenzymes. Treatment with the combination of Vitamin E and lycopene for 30 days significantly attenuated these changes as compared to the individual treatment and ISO treated groups. Histopathological observations were also in correlation with the biochemical parameters. Conclusion: These findings indicate the synergistic cardioprotective effects of vitamin E and lycopene during ISO-induced myocardial infarction in rats.

Key words: Isoproterenol, myocardial infarction, oxidative stress, vitamin E, lycopene

INTRODUCTION

Myocardial infarction commonly known as heart attack is a disease that occurs when the blood supply to a part of the heart is interrupted, causing the death of heart tissue. It is a complex phenomenon affecting the mechanical, electrical, structural and biochemical properties of the heart. It is well recognized that there is an increasing generation of reactive oxygen species such as superoxide anion and hydroxyl radicals and other reactive species in ischemic tissue, bringing about oxidative damage of membrane lipids, proteins, carbohydrates, and DNAs. Energy depletion of the cells and necrotic type cell death was also found due to oxidative stress. Hence, therapeutic intervention with antioxidants may be useful in preventing these deleterious changes.

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DOI:10.4103/0976-500X.64532
Isoproterenol, a β-adrenergic agonist, has been found to cause severe stress in the myocardium resulting in infarct like necrosis of the heart muscle. Some of the mechanisms proposed to explain ISO-induced damage to cardiac myocytes include hypoxia due to myocardial hyperactivity, coronary hypotension, calcium overload, hypertrophy, depletion of energy reserve and excessive production of free radicals resulting from oxidative metabolism of catecholamine.

Vitamin E in humans is the major antioxidant in lipid phases. Vitamin E has been shown to slow or inhibit the oxidative modification of LDL that is responsible for the development and progression of atherosclerosis in human and animals. It has been shown to reduce smooth muscle cell proliferation, platelet adherence and aggregation, protein kinase C activation and isoproterenol-induced myocardial infarction in rats. Epidemiological data indicated an inverse association between cardiovascular risk and vitamin E intake from dietary sources and/or supplements. Despite these promising experimental and epidemiological data, most randomized controlled trials have failed to confirm the role of vitamin E supplementation in cardiovascular prevention.

Lycopene is a natural pigment synthesized by plants and microorganisms. It is highly lipophilic and is most commonly located within cell membranes and other lipid components. It is therefore expected that in the lipophilic environment, lycopene will have maximum ROS scavenging effects. In epidemiological studies and supplementation human trials, lycopene was found to reduce cardiovascular risks due to its antioxidant properties. Lycopene, because of its high number of conjugated double bonds, exhibits higher singlet oxygen quenching ability compared to β-carotene or α-tocopherol. Chronic administration of lycopene can protect myocardium against ischemia reperfusion injury.

Several studies have shown that antioxidants are uniquely different from one another and work synergistically and more effectively when they are used in combinations. In vitro study showed that lycopene and vitamin E acts synergistically in microsomal membranes and LDL oxidation. The interaction of vitamin E with lycopene during myocardial oxidative stress induced injury has not been previously evaluated. Hence, the present study was designed to evaluate the effect of vitamin E alone and in combination with lycopene on tissue defense system, lipid peroxidation status and histopathological alterations during ISO-induced myocardial infarction in rats.

MATERIALS AND METHODS

Materials
Lycopene powder (10%) was gifted by Genesis Lab Ltd, Mumbai. Vitamin E (DL-α-Tocopherol acetate) and (+)-Isoproterenol hydrochloride were purchased from Sigma Aldrich Co. St. Louis, MO, USA. All other chemicals were of analytical grade.

Experimental animals
Male adult albino rats (Wistar strain) weighing between 200 and 230 g were used in the present study. All experiments were approved by the Institutional Animal Ethics Committee (IAEC) of the Institute. The animals were housed in an air conditioned room and were kept in standard laboratory conditions under natural light and dark cycle (12 h light/dark) maintained at ambient temperature 25 ± 2°C. The animals were fed standard pellet diet (Amrut feeds, Pranav Agro Industries Ltd, Pune, India) and water ad libitum.

Pilot study for dose fixation
Lycopene at the doses of 5, 10 and 15 mg/kg/day and vitamin E at the doses of 25, 50 and 100 mg/kg/day were screened in isoproterenol-induced myocardial infarction in rats. The optimum dose exhibiting maximum cardioprotective effect during 30 days was evaluated by estimating serum lactate dehydrogenase, creatine phosphokinase-MB and the level of tissue lipid peroxidation. Lycopene (10 mg/kg/day, p.o.) and vitamin E (100 mg/kg/day, p.o.) were found to be most effective in functional recovery of biochemical alterations. Hence, these doses were selected for further evaluation (alone as well as in combination) in the present study.

Experimental design and protocol
Animals were randomly allocated into six main groups comprising 10 rats in each group. Six animals were used for biochemical estimations and four animals for histopathological study. Group I: Control: animals received vehicle for 30 days and normal saline on 29th and 30th day. Group II: animals received vehicle for 30 days and intoxicated with ISO (200mg/kg, s.c.) on 29th and 30th day at an interval of 24 h. Group III: Vitamin E (100 mg/kg/day, p.o.) and lycopene (10 mg/kg/day, p.o.) in combination for 30 days. Group IV: Vitamin E (100 mg/kg, p. o.) for 30 days and challenged with ISO on 29th and 30th day. Groups V: lycopene (10 mg/kg, p. o.) for 30 days in olive oil and challenged with ISO on 29th and 30th day. Olive oil was used as vehicle for vitamin E and lycopene. Control and ISO treated groups also received same volume of olive oil thought the treatment period.

Estimation of cardiac marker enzymes
On day 31, blood was collected from retro-orbital plexus under mild ether anesthesia (approx. 2 ml). Blood was centrifuged at 2000 rpm for 20 min to separate serum (Remi centrifuge). Serum lactate dehydrogenase (LDH) and creatine phosphokinase-MB (CK-MB) were determined by using standard kits from Reckon Diagnostic Ltd., India. Serum aspartate transaminase (AST) and serum alanine transaminase
(ALT) were estimated by using the standard kit from Span Diagnostic Pvt Ltd., India. All estimations were carried out using UV spectrophotometer (Shimadzu, India).

**Separation of LDH-isoenzymes**

LDH-isoenzymes were separated by agarose gel electrophoresis.[23] Agarose gel (1%w/v) was prepared and poured immediately on the glass slide. After the gel sets properly, 10 μl of serum samples was loaded into the wells. After the run, the gel was removed and stained by the following method. The staining solution contained 1.0 ml of 1 M lithium lactate, 1.0 ml of 1 M sodium chloride, 1.0 ml of 5 mM magnesium chloride, 2.5 ml of 0.1% w/v nitroblue tetrazolium, 0.25 ml of 0.1%w/v phenazinemethosulphate, 2.5 ml of 0.5 M phosphate buffer (pH 7.5) and 10 mg of NAD in a total volume of 10 ml. The photographs were taken using AlphaEase Fc Imaging system, USA.

**Estimation of myocardial lipid peroxidation and antioxidant enzymes**

The animals were sacrificed using overdose of pentobarbital sodium. Hearts were excised, weighed, and homogenized in chilled tris HCl buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 10,000 rpm at 0°C for 20 min using Remi C-24 high speed cooling centrifuge. The clear supernatant was used for the assay of lipid peroxidation,[23] superoxide dismutase,[24] catalase,[25] reduced glutathione,[26] glutathione peroxidase [27] and glutathione S transferase.[28]

**Estimation of membrane bound ATPases**

The sediment after centrifugation of tissue homogenate was resuspended in ice-cold tris buffer (10 mM, pH 7.4) to get a final concentration of 10%w/v and were used for the estimation of Na+/K+ ATPase,[29] Ca2+ ATPase[30] and Mg2+ ATPase.[31] Protein concentration of 10%w/v and were used for the estimation of LDH-isoenzymes were separated by agarose gel electrophoresis.[32] The animals were sacrificed using overdose of pentobarbital sodium. Hearts were excised, weighed, and homogenized in chilled tris HCl buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 10,000 rpm at 0°C for 20 min using Remi C-24 high speed cooling centrifuge. The clear supernatant was used for the assay of lipid peroxidation,[23] superoxide dismutase,[24] catalase,[25] reduced glutathione,[26] glutathione peroxidase[27] and glutathione S transferase.[28]

**Histopathological study**

After decapitation, the heart was rapidly dissected out and washed immediately with saline and fixed in 10% buffered formalin. Hearts which were stored in 10% formalin were embedded in paraffin sections cut at 5 μm and were stained with hematoxyline and eosin. The stained sections were examined under Olympus (Magnus MLX series) India Pvt Ltd. Photomicroscope and photographed (10X). Four hearts from each group were assessed for light microscopic studies. A minimum of 10 fields per slide were examined and graded for severity of changes using scores on a scale of severe (+ + +), moderate (+ +), mild (+) and absence (A).

**Statistical analysis**

Results are presented as mean ± S.E.M. One-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons using a computer based fitting program (GraphPad Prism 5). Differences were considered to be statistically significant when $P<0.05$. $P>0.05$ was considered as non significant.

**RESULTS**

Effect of vitamin E alone and in combination with lycopene on body weight, heart weight and heart / body weight ratio in ISO treated rats

As shown in Table 1, body weight at the end of experiment period in ISO treated group was found to be significantly ($P<0.01$) decreased, whereas the heart weight and its ratio with body weight was found to be significantly ($P<0.01$) increased as compared to control group. Treatment with vitamin E and lycopene in combination for 30 days significantly ($P<0.01$) increased the body weight and significantly ($P<0.05$, $P<0.01$) decreased the heart weight and heart / body weight ratio as compared to ISO treated group.

**Effect of vitamin E alone and in combination with lycopene on serum cardiac marker enzymes in ISO treated rats**

Figures 1 and 2 show the effect of vitamin E and lycopene combination on serum cardiac marker enzymes. Rats treated with ISO led to a significant ($P<0.001$) increase in the activities of serum marker enzymes such as AST, ALT, LDH and CK-MB as compared to the control groups. Treatment with vitamin E and lycopene in combination for 30 days and challenged with ISO showed a significant ($P<0.001$) reduction in the activities of all serum cardiac marker enzymes

| Groups     | Body weight (g) | Heart weight (g) | HW/BW |
|------------|-----------------|------------------|-------|
| Initial    | Final           |                  |       |
| Con        | 207.4 ± 3.92    | 232.5 ± 3.39     | 0.660 ± 0.025 | 0.00286 ± 0.00015 |
| ISO        | 202.9 ± 5.55    | 214.2 ± 3.48     | 0.920 ± 0.054 | 0.00049 ± 0.000022 |
| Vit.E+Lyp  | 204.1 ± 9.99    | 213.2 ± 6.77     | 0.664 ± 0.032 | 0.00287 ± 0.00047 |
| Vit.E+ISO  | 211.3 ± 5.11    | 227.7 ± 3.29     | 0.705 ± 0.030 | 0.00309 ± 0.00014 |
| Lyp+ISO    | 210.7 ± 5.43    | 229.9 ± 4.26     | 0.717 ± 0.065 | 0.00321 ± 0.00015 |
| Vit.E+Lyp+ISO | 206.2 ± 7.88 | 228.4 ± 5.65     | 0.701 ± 0.082 | 0.00302 ± 0.00014 |

Values are expressed as Mean ± SEM (n=10), *$P<0.05$, **$P<0.01$, ***$P<0.001$ values compared to control groups, ^$P<0.05$, ^^$P<0.01$, ^^^$P<0.001$ values compared to ISO groups.
as compared to the ISO, vitamin E + ISO and lycopene + ISO treated groups. Combined effect of antioxidants was found to be more effective in maintaining serum marker enzymes as compared to individual antioxidants.

Effect of vitamin E alone and in combination with lycopene on lipid peroxidation and antioxidant enzymes in ISO treated rats

Figure 3 indicates the level of lipid peroxides (LPO) in the hearts of control and experimental group of rats. Maximum induction of LPO was observed in ISO intoxicated rats. The change in LPO was significantly decreased in rats treated with vitamin E and lycopene compared to vitamin E+ISO, lycopene+ISO and ISO intoxicated rats.

Table 2 shows the activities of antioxidant enzymes such as GPX, GST, SOD, CAT and level of GSH in the heart of control and experimental group of rats. Rats injected with ISO showed a significant (*P < 0.001) decrease in the activities of GPX, GST, SOD, CAT and the level of GSH as compared to control groups [Table 2]. Vitamin E in combination with lycopene significantly (P < 0.001) normalized all the parameters and was found to be more effective than vitamin E+ISO, lycopene+ISO and ISO treatment groups.

Effect of vitamin E alone and in combination with lycopene on membrane bound ATPases in ISO treated rats

Figure 4 shows a significant (P < 0.001) decrease in the activities of Na+/K+ ATPase and Mg2+ ATPase whereas a significant (P < 0.001) increase in the activity of Ca2+ ATPase in ISO administered rats as compared to the control group. Vitamin E did not produce any significant effect on Mg2+ ATPase activity. Combination of vitamin E and lycopene showed a significant (P < 0.001) increase in activities of Na+/K+ ATPase, Mg2+ ATPase, and a significant reduction in the activity of Ca2+ ATPase as compared to vitamin E+ISO, lycopene+ISO and ISO treatment groups.

Effect of vitamin E alone and in combination with lycopene on LDH-isoenzymes separation in ISO treated rats

Agarose gel electrophoretic separation of serum LDH-isoenzymes separation in ISO treated rats

| Groups          | GSH (μg of GSH/mg protein) | GPx (μmoles of glutathione oxidized/ min/mg protein) | GST (μmoles of CDNB conjugated/min/mg protein) | SOD(units/mg protein) | CAT (μmoles of H2O2 consumed/min/mg protein) |
|-----------------|---------------------------|---------------------------------------------------|---------------------------------------------|----------------------|---------------------------------------------|
| Control         | 6.920 ± 0.263             | 6.303 ± 0.151                                     | 106.82 ± 5.294                             | 4.816 ± 0.226        | 6.340 ± 0.428                               |
| ISO             | 4.338 ± 0.260**           | 4.032 ± 0.231**                                  | 68.91 ± 4.829**                            | 2.272 ± 0.166**      | 3.687 ± 0.219**                             |
| Vit.E+Lyp       | 7.448 ± 0.491             | 6.801 ± 0.299                                     | 118.33 ± 4.920                             | 4.882 ± 0.463        | 6.763 ± 0.288                               |
| Vit.E+ISO       | 5.785 ± 0.288**           | 5.635 ± 0.336**                                  | 95.55 ± 4.089**                            | 3.566 ± 0.390        | 5.188 ± 0.167**                             |
| Lyp+ISO         | 6.208 ± 0.255**           | 5.502 ± 0.499**                                  | 93.75 ± 5.891**                            | 4.212 ± 0.216**      | 5.042 ± 0.219**                             |
| Vit.E+Lyp+ISO   | 7.600 ± 0.213***          | 6.423 ± 0.382**                                  | 132.20 ± 5.721**                           | 5.122 ± 0.332***     | 6.622 ± 0.214***                            |

Values are expressed as Mean ± SEM (n=6), *P < 0.05, **P < 0.01, ***P < 0.001 values compared to control groups, ^P < 0.05, ^^P < 0.01, ^^^P < 0.001 values compared to ISO groups. #P < 0.05 compared to Vit.E+ISO and $P < 0.05 compared to Lyp+ISO group. GSH: reduced glutathione; GPx: glutathione peroxidase; GST: glutathione s-transferase; SOD: superoxide dismutase; CAT: catalase.
isoenzyme patterns of normal and ISO-induced rats are depicted in Figure 5. ISO induction caused an increase in the intensity of LDH-1 and LDH-2 isoenzyme bands compared to normal control rats. Treatment with vitamin E and lycopene in combination significantly decreased the intensity of LDH-1 and LDH-2 isoenzyme bands compared to ISO-intoxicated rats, vit.E+ISO and lycopene+ISO groups [Figure 5].

**Effect of vitamin E alone and in combination with lycopene on histopathological alterations in ISO treated rats**

Figure 6a shows the light micrograph of control heart showing normal architecture without any fraying or infarction. Light micrograph of isoproterenol-intoxicated group shows focal confluent necrosis of muscle fibers with inflammatory cell infiltration, and edema with fragmentation of muscle fibers [Figure 6b]. Treatment with vitamin E +ISO group showed myonecrosis with less edema and inflammatory cells [Figure 6c]. Lycopene+ISO treated rat heart shows mild edema with significant reduction in infarction, showing normal myocardial architecture [Figure 6d]. Combination of vitamin E and lycopene showed less necrosis and edema, whereas absence of inflammatory cells [Figure 6e]. Table 3 shows the change in the degree of histopathological scoring after treatment with the combination of vitamin E and lycopene.
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### Table 3. Effect of vitamin E and lycopene on the degree of histological changes

| Groups       | Necrosis | Oedema   | Inflammatory cells |
|--------------|----------|----------|--------------------|
| Control      | A        | A        | A                  |
| ISO          | +++      | +++      | +++                |
| Vit.E+ISO    | ++       | ++       | +                  |
| LYP+ISO      | +        | +        | +                  |
| Vit.E+LYP+ISO| +        | +        | A                  |

Photomicrographs were used to evaluate the damage in the heart tissues: (A) no change, (+++) severe changes, (++) moderate changes, (+) mild changes.

### DISCUSSION

ISO in large doses induces morphological and functional alterations in the heart leading to myocardial necrosis. It also produces excessive free radicals resulting from oxidative metabolism of catecholamine. There are increasing evidences that cardiotoxicity of ISO occurs because of generation of free radicals and oxidative stress.[1,12,18] In the present study, there was a significant decrease in the body weight, significant increase in heart weight and heart/body weight ratio in isoproterenol treated rats. Increase in the ratio is suggestive of cardiac hypertrophy which may be due to ventricular stiffness, increased water content, edematous intermuscular space and extensive necrosis of cardiac muscle followed by invasion of the damaged tissue by inflammatory cells.[33,34] Treatment with vitamin E and lycopene significantly decreased the heart/body weight ratio, suggesting that the combination could effectively suppress the stimulus for hypertrophy.

The present study reveals that ISO treatment results in marked elevation in the levels of cardiac serum marker enzymes like AST, ALT, LDH and CK-MB. Results of present study are in line with those reported by Kurian et al., 2005.[35] These cardio specific marker enzymes are released from the heart into the blood during myocardial damage due to deficiency of oxygen supply or glucose, the cell membrane become permeable or may rupture and results in the leakage of enzymes in the serum.[36] Combination of vitamin E and lycopene synergistically normalized the levels of serum biomarker enzymes compared to vitamin E+ISO and lycopene+ISO treatment groups. This protection might be due to the effect of vitamin E in combination with lycopene on the myocardium, which had reduced the extent of myocardial damage induced by ISO and thereby restricting the leakage of these enzymes from the myocardium, suggesting the membrane stabilizing potential of the combination.

LDH is a cytosolic enzyme, which exists in five different isoforms (LDH 1 to LDH 5). In the cardiac tissue, LDH 1 and LDH 2 predominate. Hence, detection of elevated concentration of these enzymes becomes a definitive diagnostic criterion for cardiac toxicity. In the present study, we observed an increase in the intensity of LDH1 and LDH2 isoenzyme in serum of isoproterenol treated rats which may be due to ISO-induced necrosis in rats.[37] Vitamin E and lycopene in combination reduces the intensity of LDH1 and LDH2 isoenzyme. This could be due to reduction in the degree of damage and thereby reduction in leakage of enzymes from myocardium.

Significant increase in the level of lipid peroxidation and a significant decrease in the activities of GSH, GPX, GST, SOD and CAT were observed in ISO intoxicated rats, which suggests the involvement of oxidative stress in ISO toxicity. This result is in line with the earlier reported studies.[38] Elevation of lipid peroxides in ISO treated rats could be attributed to the accumulation of lipids in the heart and the irreversible damage to myocardial membranes. Reduction in the activity of SOD and CAT in the present study may be due to the increased generation of highly cytotoxic free radicals due to auto oxidation of catecholamine. GSH exerts its function by reaction with superoxide radical, peroxy radicals and singlet oxygen followed by the formation of oxidized glutathione and other disulfides.[39] The unavailability of GSH may decrease the activity of GPx and GST in ISO treated rats. Combined treatment of vitamin E and lycopene restored the level of LPO and endogenous antioxidants to that of control group indicating the potential antioxidant effects of this combination. Lycopene was found to be more effective in restoring the LPO and antioxidant enzymes than vitamin E.

Membrane bound ATPases plays an important role in the contraction and relaxation of the cardiac muscle by maintaining the normal ion levels inside the myocyte. Na⁺/K⁺ ATPase and Mg²⁺ ATPase are the ‘-SH’ group containing enzymes and is lipid dependant. Reduction in the activity of these enzymes might be due to enhanced lipid peroxidation by free radicals. Reduced activity of Mg²⁺ ATPase and Na⁺/K⁺ ATPase may be responsible for ionic imbalance caused by ISO which damages the membranous proteins. Result of the present study also shows enhanced activity of Ca²⁺ ATPase; it may be due to activation of adenylate cyclase activity. Calcium overload in the myocardial cells during ischemia activates the Ca²⁺ dependant ATPase of the membrane depleting high energy phosphate stores, thereby indirectly inhibiting Na⁺ and K⁺ transport and inactivating Na⁺/K⁺ ATPase.[42] Treatment with vitamin E and lycopene in combination increased the activities of Na⁺/K⁺ ATPase and Mg²⁺ ATPase and decreased the activity of Ca²⁺ ATPase in ISO group. This could be due to the ability of vitamin E and lycopene to protect the ‘-SH’ group from oxidative damage through the inhibition of peroxidation of membrane lipids.

Histopathological findings of vitamin E and lycopene alone and their combination shows a near normal morphology of cardiac muscle. Combination of vitamin E and lycopene shows better morphological protection than alone antioxidant treated groups by absence of necrosis and inflammatory cells which
is in accordance with biochemical changes. These data further confirmed the cardioprotective action of the combination of vitamin E and lycopene.

Both vitamin E and lycopene are lipid soluble antioxidants. Vitamin E is a chain breaking antioxidant in human plasma and is a low density lipoprotein. It could effectively trap the lipid peroxyl radical to inhibit the free radical initiated lipid peroxidation. Lycopene is a well-known singlet oxygen scavenger and other excited species. During singlet oxygen quenching, energy is transferred from singlet oxygen to lycopene molecule, converting it to the energy rich triplet state.

Further it was reported that the synergistic effects of lycopene and vitamin E combination may be due to the regeneration of vitamin E from its α-tocopherol radical by lycopene. This may possibly be the reason for the better effect of vitamin E and lycopene in attenuating cardiac dysfunction through lowering of cardiac marker enzymes and LPO. In conclusion, the results of the present study indicate that the combined treatment with vitamin E and lycopene synergistically prevents the ISO-induced myocardial infarction than an individual antioxidant treatment in rats.

ACKNOWLEDGMENT

Authors are thankful to All India Council for Technical Education (AICTE) for providing financial assistance in the form of National Doctoral Fellowship (AICTE File No. 1-10/RID/NDF-PG/(30)/2007-08) for this work. Authors are also thankful to Dr. Vikas Toke, Jyot pathology, Baroda for carrying out the histopathological study.

REFERENCES

1. Rajadurai M, Stanely Mainzen Prince P. Preventive effect of naringin on cardiac markers, electrocardiographic patterns and lysosomal hydrodases in normal and isoproterenol-induced myocardial infarction in wistar rats. Toxicology 2007;230:178-88.
2. Petrich ER, Schanne OF, Zamuno AP. Electrophysiological responses to ischemia and reperfusion. In: Karmazyn M, editor. Myocardial ischemia: Mechanism, reperfusion, protection. Basel: Birkhauser Verlag; 1996. p. 115–33.
3. Dikshit M, Van Oosten MH, de Graff S, Srimal RC. Free radical scavenger mechanisms in experimentally induced ischemia in the rabbit heart and protective effect of verapamil. Arch Int Pharmacodyn Ther 1992;318:55-65.
4. Radons J, Heller B, Bürkle A, Hartmann B, Rodriguez ML, Kröncke KD, et al. Nitric oxide toxicity in islet cells involves poly (ADP-ribose) polymerase activation and concomitant NAD+ depletion. Biochem Biophys Res Commun 1994;200:279-70.
5. Weidt BC. Myocardial infarction in young vs. old male rats: pathophysiological changes. Am Heart J 1978;96:70-80.
6. Mohanty I, Arya DS, Dinda A, Talwar KK, Joshi S, Gupta SK. Mechanisms of cardioprotective effects of Withania somnifera in experimentally induced myocardial infarction. Basic Clin Pharmacol Toxicol 2004;94:184-90.
7. Traber MG, Sies H. Vitamin E in humans: Demand and delivery. Annu Rev Nutr 1996;16:321-47.
8. Verlagier AJ, Bush MJ. Effects of δ-(±)-α-tocopherol supplementation on experimentally induced primate atherosclerosis. J Am Coll Nutr 1992;11:131-8.
9. Meydani M. Vitamin E. Lancet 1995;345:170-5.
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provide cardioprotection in the experimental model of myocardial ischemia reperfusion injury. Life Sci 2006;79:38-4.

37. Priscilla DH, Prince PS. Cardioprotective effect of gallic acid on cardiac troponin-t, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in Wistar rats. Chem Biol Interact 2009;179:118-24.

38. Rajadurai M, Stanely Mainzen Prince P. Preventive effect of naringin on lipid peroxide and antioxidant in isoproterenol-induced cardiotoxicity in Wistar rats: biochemical and histopathological evidences. Toxicology 2006;228:259-68.

39. Wertz K, Siler U, Goralczyk R. Lycopene: modes of action to promote prostate health. Arch Biochem Biophys 2004;430:127-34.

40. Palozza P, Krinsky NI. β-carotene and α-tocopherol are synergistic antioxidants. Arch Biochem Biophys 1992;297:184-97.

Source of Support: Financial assistance in the form of National Doctoral Fellowship (AICTE File No. 1-10/RID/NDP-PG/(30)/2007-08) for this work, Conflict of Interest: None declared