Research Paper

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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ABSTRACT

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, Mg^{2+}ATPases and Ca^{2+}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 Ca^{2+}ATPases, whereas it caused a significant ($P < 0.001$) decrease in body weight, activities of endogenous antioxidants (GSH, GP_X, GST, SOD and CAT), Na^+/K^+ and Mg^{2+}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.[1] It is a complex phenomenon affecting the mechanical, electrical, structural and biochemical properties of the heart.[2] 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.[3] Energy depletion of the cells and necrotic type cell death was also found due to oxidative stress.[4] Hence, therapeutic intervention with antioxidants may be useful in preventing these deleterious changes.

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Isoproterenol, a β-adrenergic agonist, has been found to cause severe stress in the myocardium resulting in infarct like necrosis of the heart muscle.[5] 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.[6]

Vitamin E in humans is the major antioxidant in lipid phases.[7] 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.[8] It has been shown to reduce smooth muscle cell proliferation,[9] platelet adherence and aggregation,[10] protein kinase C activation[11] and isoproterenol-induced myocardial infarction in rats.[12] Epidemiological data indicated an inverse association between cardiovascular risk and vitamin E intake from dietary sources and/or supplements.[13] Despite these promising experimental and epidemiological data, most randomized controlled trials have failed to confirm the role of vitamin E supplementation in cardiovascular prevention.[14]

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.[15] Lycopene, because of its high number of conjugated double bonds, exhibits higher singlet oxygen quenching ability compared to β-carotene or α-tocopherol.[16] Chronic administration of lycopene can protect myocardium against ischemia reperfusion injury.[17]

Several studies have shown that antioxidants are uniquely different from one another and work synergistically and more effectively when they are used in combinations.[12,18,19] *In vitro* study showed that lycopene and vitamin E acts synergistically in microsomal membranes[20] and LDL oxidation.[21] 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 an 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 phospokinase-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[17] and challenged with ISO on 29th and 30th day. Group VI: Vitamin E (100 mg/kg, p.o.) and lycopene (10 mg/kg, p.o.) in combination for 30 days 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 phospokinase-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.[22] 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 scarified 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] Ca^{2+} ATPase[30] and Mg^{2+} ATPase.[31] Protein was estimated according to the method of Lowery *et al*.[32]

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

Table 1: Effects of vitamin E alone and in combination with lycopene on body weight, heart weight and heart/body weight ratio in ISO treated rats

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**	0.00429 ± 0.00022***
Vit.E+Lyp	204.1 ± 9.99	231.2 ± 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	222.9 ± 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 Mg^{2+} ATPase whereas a significant ($P < 0.001$) increase in the activity of Ca^{2+} ATPase in ISO administered rats as compared to the control group. Vitamin E did not produce any significant effect on Mg^{2+} ATPase activity. Combination of vitamin E and lycopene showed a significant ($P < 0.001$) increase in activities of Na^+/K^+ ATPase, Mg^{2+} ATPase, and a significant reduction in the activity of Ca^{2+} 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-

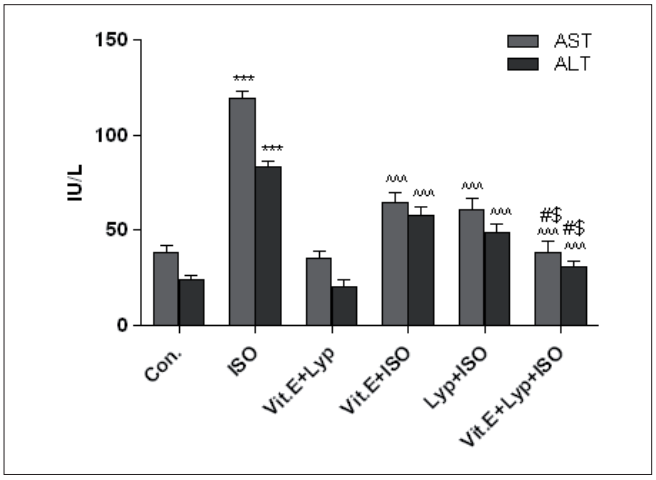

Figure 1: Effects of vitamin E alone and in combination with lycopene on serum AST and ALT levels in normal and ISO treated rats. [Values are expressed as Mean ± SEM (n=6), $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ values compared to control groups, $^{\wedge}P < 0.05$, $^{\wedge\wedge}P < 0.01$, $^{\wedge\wedge\wedge}P < 0.001$ values compared to ISO groups. $^{\#}P < 0.05$ compared to Vit.E+ISO and $^{\$}P < 0.05$ compared to Lyp+ISO group]

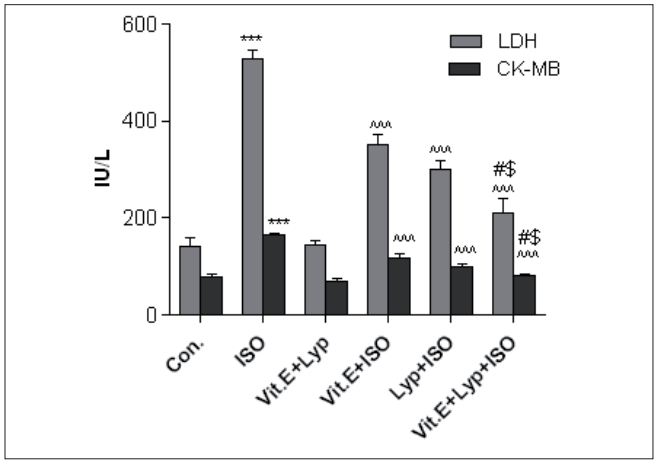

Figure 2: Effects of vitamin E alone and in combination with lycopene on serum LDH and CK-MB levels in normal and ISO treated rats. [Values are expressed as Mean ± SEM (n=6), $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ values compared to control groups, $^{\wedge}P < 0.05$, $^{\wedge\wedge}P < 0.01$, $^{\wedge\wedge\wedge}P < 0.001$ values compared to ISO groups. $^{\#}P < 0.05$ compared to Vit.E+ISO and $^{\$}P < 0.05$ compared to Lyp+ISO group]

Table 2: Effects of vitamin E alone and in combination with lycopene on tissue endogenous antioxidants enzyme activities in normal and 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$^{\wedge\wedge}$	5.635 ± 0.336$^{\wedge\wedge}$	95.55 ± 4.089$^{\wedge\wedge\wedge}$	3.566 ± 0.390$^{\wedge}$	5.188 ± 0.167$^{\wedge\wedge}$
Lyp+ISO	6.208 ± 0.255$^{\wedge\wedge\wedge}$	5.502 ± 0.499$^{\wedge\wedge}$	93.75 ± 5.891$^{\wedge\wedge\wedge}$	4.212 ± 0.216$^{\wedge\wedge\wedge}$	5.042 ± 0.219$^{\wedge\wedge}$
Vit.E+Lyp+ISO	7.600 ± 0.213$^{\wedge\wedge\wedge\#}$	6.423 ± 0.382$^{\wedge\wedge\wedge\#\$}$	132.20 ± 5.721$^{\wedge\wedge\wedge\#\$}$	5.122 ± 0.332$^{***\#}$	6.622 ± 0.214$^{\wedge\wedge\wedge\#\$}$

Values are expressed as Mean ± SEM (n=6), $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ values compared to control groups, $^{\wedge}P < 0.05$, $^{\wedge\wedge}P < 0.01$, $^{\wedge\wedge\wedge}P < 0.001$ 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

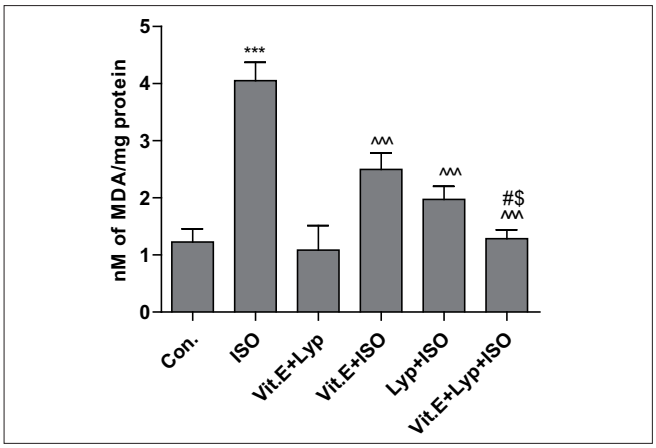

Figure 3: Effects of vitamin E alone and in combination with lycopene on tissue lipid peroxidation in normal and ISO treated rats. Values are expressed as Mean ± SEM (n=6), $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ values compared to control groups, $^{\wedge}P < 0.05$, $^{\wedge\wedge}P < 0.01$, $^{\wedge\wedge\wedge}P < 0.001$ values compared to ISO groups. $^{\#}P < 0.05$ compared to Vit.E+ISO and $^{\$}P < 0.05$ compared to Lyp+ISO group.

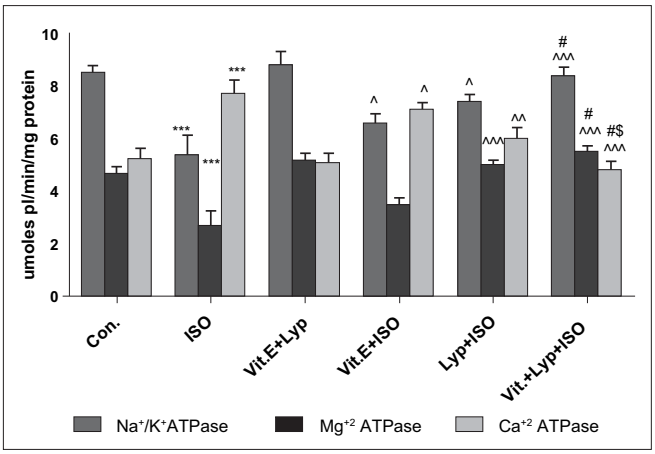

Figure 4: Effects of vitamin E alone and in combination with lycopene on membrane bound ATPases in normal and ISO treated rats**.** Values are expressed as Mean ± SEM (n=6), $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to control groups, $^{\wedge}P<0.05$, $^{\wedge\wedge}P<0.01$, $^{\wedge\wedge\wedge}P<0.001$ values compared to ISO groups. $^{\#}P<0.05$ compared to Vit.E+ISO and $^{\$}P<0.05$ compared to Lyp+ISO group

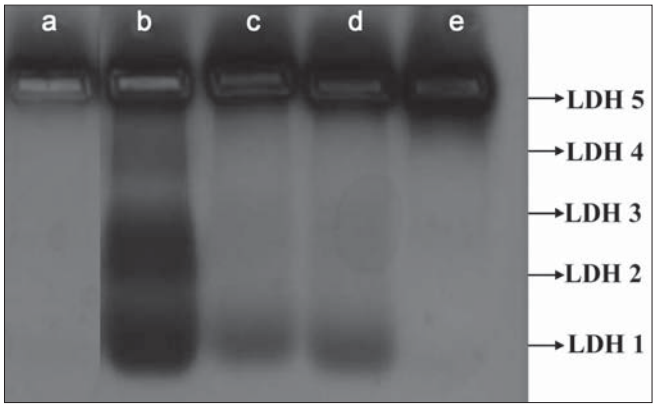

Figure 5: Effects of vitamin E alone and in combination with lycopene on LDH isoenzymes pattern in normal and ISO treated rats
A:Control rats. B:ISO control. C:Vit.E+ISO. D:Lyp+ISO. E: Vit. E+Lyp+ISO

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].

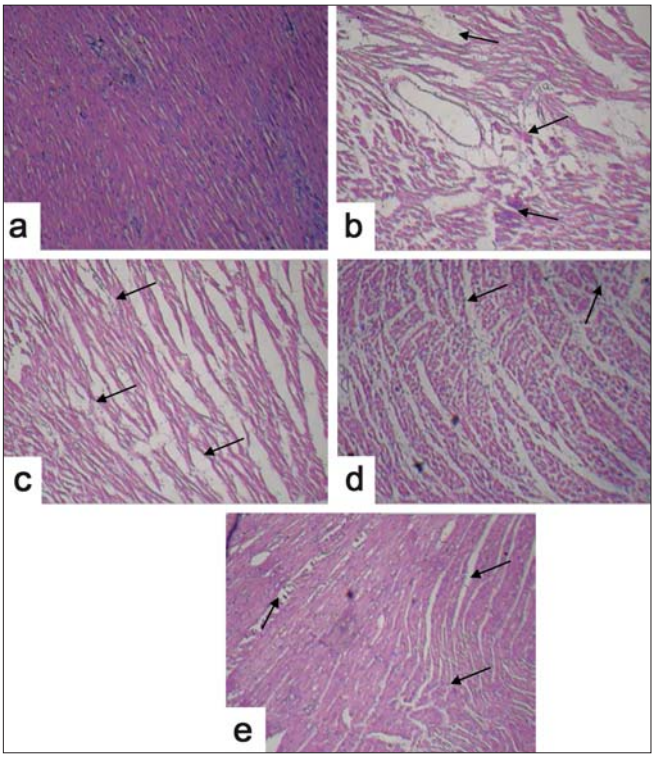

Figure 6: Effects of vitamin E alone and in combination with lycopene on histopathological alteration in normal and ISO – Induced myocardial infracted rats (H&E 10X). A:Control rats. B:ISO control. C:Vitamin E+ISO. D:Lycopene+ISO. E: Vitamin E+Lycopene+ISO

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.

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.[30] 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 Mg^{2+} 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 Mg^{2+} 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 Ca^{2+} ATPase; it may be due to activation of adenylate cyclase activity. Calcium overload in the myocardial cells during ischemia activates the Ca^{2+} dependant ATPase of the membrane depleting high energy phosphate stores, thereby indirectly inhibiting Na^+ and K^+ transport and inactivating Na^+/K^+ ATPase.[2] Treatment with vitamin E and lycopene in combination increased the activities of Na^+/K^+ ATPase and Mg^{2+} ATPase and decreased the activity of Ca^{2+} 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.[11] 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.[39] 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-tocopheroxyl radical by lycopene.[40] 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.

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