

Cardioprotective Effect of Vitamin E in Combination with Lycopene on Lipid Profile, Lipid Metabolizing Enzymes and Infarction Size in Myocardial Infarction Induced by Isoproterenol

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Abstract: Background: Consumption of dietary constituents containing polyphenols, flavonoids and vitamins are associated with reduced risk of cardiovascular disorders. In the present myocardial infarction was induced in male albino rats by subcutaneous injection of isoproterenol (ISO) (200 mg kg^{-1}) for 2 consecutive days at an interval of 24 h. **Result:** ISO injected rats showed a significant ($p < 0.001$) increase in total cholesterol, triglycerides and free fatty acids levels in both serum and cardiac tissue. However, a rise in the levels of serum phospholipids was observed in ISO-injected rats. Further, ISO injected rats showed a significant decrease in the level of high-density lipoprotein in serum and PLs levels in the heart. The activities of lecithin: cholesterol acyl transferase and lipoprotein lipase was significantly ($p < 0.01$, $p < 0.001$) decreased and cholesterol ester synthetase was significantly ($p < 0.001$) increased. ISO injected rats also showed significant increase in area of infarction. Administration of Vitamin E (100 mg/kg/day , p.o.) alone and in combination with lycopene (10 mg/kg/day , p.o.) for 30 consecutive days significantly attenuated these alterations and restored the levels of serum and heart lipids, lipid metabolizing enzymes along with area of infarction. **Conclusion:** These findings indicate that addition of lycopene along with vitamin E showed better effects rather than vitamin E.

Key words: Isoproterenol, lycopene, vitamin E, lipid profile, lipid metabolizing enzymes, infarction size

INTRODUCTION

Myocardial Infarction (MI) is a condition in which an imbalance between myocardial oxygen supply and demand occurs (Mohanty *et al.*, 2004). Studies shown that high levels of total cholesterol, triglycerides, low density lipoprotein cholesterol and low levels of high density lipoproteins cholesterol are the risk factors of cardiovascular diseases. Reactive oxygen species may contribute to the events of atherogenesis and leading to the progression of atherogenic lesions by promoting oxidation of low-density lipoproteins (Benelli *et al.*, 2002). ISO a synthetic catecholamine in large dose causes a severe stress in the myocardium resulting in infarct like necrosis of the heart muscle (Upaganlawar *et al.*, 2009). It also increases the levels of serum and myocardial lipids, which in turn leads to coronary heart disease (Prince and Rajadurai, 2005). It is well known to stimulate lipid peroxidation through generation of free radicals, which is a causative factor for irreversible damage to the myocardial membrane and thus favors the deposition of lipids in the heart (Sathish *et al.*, 2003).

Vitamin E has been shown to slow up the oxidative modification of low-density lipoproteins that is responsible for the development and progression of atherosclerosis in human and animals (Verlangieri and Bush, 1992). It has been shown to reduce platelet adherence, aggregation (Steiner, 1991) and ISO induced MI (Upaganlawar *et al.*, 2009). Epidemiological data indicated an inverse association between cardiovascular risk and vitamin E or lycopene consumption from dietary sources and/or supplements (Jha *et al.*, 1995; Rissanen *et al.*, 2003). Lycopene is a natural pigment synthesized by plants. It is highly lipophilic and is most commonly located within cell membranes and other lipid components. Lycopene, because of its high number of conjugated double bonds, has been reported to exhibit higher singlet oxygen quenching ability compared to β -carotene or α -tocopherol (Di Mascio *et al.*, 1989) and to act as a potent antioxidant. It is reported to prevent the oxidative damage of critical biomolecules including lipids, low density lipoproteins, proteins and DNA (Southon, 2001).

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Several studies have shown that antioxidants are uniquely different from one another and work synergistically and more effectively when they are used in combinations (Upaganlawar *et al.*, 2009; Punithavathi and Prince, 2009; Upaganlawar and Balaraman, 2010). *In vitro* study showed that lycopene and vitamin E acts synergistically in microsomal membranes and low-density lipoproteins oxidation (Palozza and Krinsky, 1993; Fuhrman *et al.*, 2000). The interaction of vitamin E with lycopene on lipid profile during myocardial infarction has not been previously evaluated. Hence, the present study was designed to evaluate the effect of vitamin E and its combination with lycopene on serum and heart lipid profile, lipid metabolizing enzymes and area of infarction during ISO-induced myocardial infarction in rats.

MATERIALS AND METHODS

Drugs and chemicals: Isoproterenol HCL and Triphenyl Tetrazolium Chloride (TTC) were purchase from Sigma Chemicals (St Louis, MO, USA). Vitamin E was purchase from Hi Media, India. Lycopene powder was gifted by Genesis Lab, Ltd, Mumbai. All other chemicals used were of analytical grade.

Experimental animals: Male Adult albino rats (Wistar strain) weighing between 200 and 250 g were used in the present study. All experiments were approved by the Institutional Animal Ethics Committee (IAEC) of the M.S. University of Baroda, India. The animals were housed in polyacetylin cages (38×23×10 cm) with not more than four rats per cage. They were housed in an air conditioned room and were kept in standard laboratory conditions under natural light and dark cycle (12 h light and 12 h dark) maintained at an ambient temperature 25±2°C. The animals were fed standard pellet diet (Amrut feeds, Pranav Agro Industries Ltd., Sangali, India) and water *ad libitum*.

Experimental design: Animals were randomly allocated into six main groups comprising of ten animals in each group. Four rats from each group were used for TTC staining and six rats for estimation of lipid profile. Group I: Control; Group II: received ISO (200 mg kg⁻¹, s.c.) (Upaganlawar *et al.*, 2009) for 2 consecutive days at an interval of 24 h. Group III: Vit.E (100 mg kg⁻¹, p. o.) and Lycopene (10 mg kg⁻¹, p.o) for 30 days. Groups IV: Vitamin E (100 mg kg⁻¹, p. o.) for 30 days and challenged with ISO on 29th and 30th day. Group V: Lycopene (10 mg kg⁻¹, p.o) for 30 days and challenged with ISO on 29th and 30th day. Groups VI: Vitamin E (100 mg kg⁻¹, p. o.) and Lycopene (10 mg kg⁻¹) in combination for

30 days and challenged with ISO on 29th and 30th day. Vitamin E and lycopene was dissolving in olive oil as vehicle whereas ISO was dissolve in physiological saline. Control and ISO treated groups also received the same quantity of vehicle throughout the experiment period. At the end of experimental period (i.e., on the day 31) blood samples were collected and animals were euthanized. A heart tissue sample of each rat was collected and lipid extraction was carried out for further estimations.

Biochemical estimation: From the sample of heart tissue homogenate lipids were extracted by the method of Folch *et al.* (1957). Blood was collected from the retro-orbital plexus under mild ether anesthesia. Total Cholesterol (TC) and Triglyceride (TG) from heart lipid extracts and serum were estimated using standard diagnostic kits (Reckon diagnostic Ltd. India). The content of free fatty acids (Hron and Menahan, 1981) and phospholipids (Fiske and Subbarow, 1925) from serum and heart lipid extract were estimated. Serum High Density Lipoprotein (HDL) was determined by using standard diagnostic kits (Reckon Diagnostic Ltd., India). Low Density Lipoprotein (LDL) and very Low Density Lipoprotein cholesterol (VLDL) were determined by using Friedwald's Formula (Friedewald *et al.*, 1972). The activities of lipid metabolizing enzymes such as Cholesterol Ester Synthetase (CES) (Kothari *et al.*, 1973), Lecithin: Cholesterol Acyl Transferase (LCAT) (Hitz *et al.*, 1983) and Lipoprotein Lipase (LPL) (Slater and White, 1996) were determined from the heart sample.

Macroscopic enzyme mapping (TTC Staining): The Triphenyl Tetrazolium Chloride (TTC) test, used for the macroscopic enzyme mapping of infarcted myocardium was done according to the method of Lie *et al.* (1975). The heart was washed rapidly in cold water to remove excess blood, taking care not to macerate the tissue. The excess epicardial fat was lightly trimmed off. The heart was transversely cut across the left ventricle to obtain slices no more than 0.1 cm in thickness. The heart slices were placed in the covered, darkened glass dish containing pre warmed (1%) TTC solution in phosphate buffer and the dish was incubated between 37-40°C for 30-45 min. the heart slices were turned over once or twice to make certain that it remains immersed and covered by 1 cm of the TTC solution. At the end of incubation period, the heart slices was placed in 10% formalin solution which enhances the colour contrast developed. The % infarction was measured using Image J Software system. The expected reaction of TTC test was as follows: normal myocardium

(succinate dehydrogenase or LDH enzyme active) turned to bright red, ischemic myocardium (succinate dehydrogenase or LDH enzyme deficient) turned to pale grey or grayish yellow or uncolored and fibrous scars turned to white.

Statistical analysis: Results are presented as Mean \pm SEM. One-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons using a computer based fitting program (Prism, Graph Pad). Differences were considered to be statistically significant when $p < 0.05$.

RESULTS

Effect of Vit. E and lycopene on serum lipid profile:

Levels of various lipids in serum of control and ISO injected animals were recorded (Table 1). Rats injected with ISO showed a significant ($p < 0.001$, $p < 0.01$) increase in serum TC, TG, LDL, VLDL, FFA and PL levels with a significant ($p < 0.01$) decrease in HDL level. Treatment of vitamin E or lycopene alone in ISO injected rats (Vit.E+ISO or LYP+ISO) showed a significant ($p < 0.001$, $p < 0.01$, $p < 0.05$) decrease in TC, TG, LDL, VLDL, FFAs, PL and significant ($p < 0.001$, $p < 0.05$) increase in HDL levels as compared to ISO injected rats. However, co-administration of Vit. E and LYP in ISO injected rats (Vit.E+LYP+ISO) significantly ($p < 0.001$, $p < 0.05$) decreased the elevated levels of TC, TG, LDL, VLDL, FFAs, PL and significantly ($p < 0.001$, $p < 0.05$) increased the HDL levels as compared to Vit.E+ISO or LYP+ISO treated groups.

Effect of Vit. E and lycopene on tissue lipid profile:

Table 2 showed the effect of Vit. E and lycopene on heart lipid profile in ISO injected rats. Rats injected with ISO

showed a significant increase ($p < 0.01$, $p < 0.001$) in the levels of heart TC, TG and FFAs with a significant decrease ($p < 0.01$) in PL level compared to control group. Co-administration of Vit.E and LYP in ISO injected rats (Vit. E+LYP+ISO) significantly ($p < 0.01$, $p < 0.05$) decreased TC, TG, FFA level and significantly ($p < 0.05$) increased PL level compared to rats treated with Vit. E+ISO or LYP+ISO treated groups.

Effect of Vit.E and lycopene on the activities of lipid metabolizing enzymes:

The activities of myocardial LCAT, LPL and CES in control and ISO injected rats were observed (Fig. 1a, b). A significant ($p < 0.01$, $p < 0.001$) decreased in the activities of LCAT, LPL and a significant ($p < 0.001$) increased in the activity of CES was observed in ISO injected rats. Co-administration of Vit.E and LYP in ISO injected rats (Vit.E+LYP+ISO) significantly ($p < 0.001$, $p < 0.05$) increased the activities of LCAT, LPL and significantly ($p < 0.001$, $p < 0.05$) decreased the CES activity as compared to Vit.E+ISO or LYP+ISO treated groups. Treatment with Vit.E alone in ISO injected rats did not show significant improvement in LCAT activity.

Effect of Vit. E and lycopene on area of infarction: The percentage of mean infarct size with increased staining was observed in ISO injected rats when compared to control group. Treatment with Vit. E+ISO and LYP+ISO showed a significant decrease in infarct size and staining as compared to ISO injected rats. Further co-administration of Vit.E and LYP in ISO injected rats (Vit.E+LYP+ISO) showed reduction in infarct size and staining as compared to Vit.E+ISO or LYP+ISO treated groups (Fig. 2).

Table 1: Effect of Vit.E and LYP on serum lipid profile in normal and ISO injected rats

Groups	TC (mg dL ⁻¹)	TG (mg dL ⁻¹)	HDL (mg dL ⁻¹)	LDL (mg dL ⁻¹)	VLDL (mg dL ⁻¹)
Con.	92.50 \pm 4.23	50.56 \pm 2.21	35.58 \pm 2.99	43.81 \pm 4.86	10.11 \pm 0.44
ISO	139.82 \pm 5.64 ^{###}	78.79 \pm 5.61 ^{###}	22.10 \pm 1.92 ^{###}	102.00 \pm 5.16 ^{###}	15.75 \pm 1.12 ^{###}
Vit.E+LYP	89.99 \pm 6.87	48.00 \pm 5.89	39.54 \pm 3.88	34.11 \pm 0.55	9.62 \pm 0.84
Vit.E+ISO	116.70 \pm 5.49*	63.45 \pm 2.63*	35.06 \pm 4.27*	68.97 \pm 3.02 ^{***}	12.79 \pm 0.58*
LYP+ISO	106.52 \pm 4.04**	57.73 \pm 2.30 ^{***}	38.18 \pm 1.98**	57.22 \pm 1.69 ^{***}	11.14 \pm 0.46 ^{***}
Vit.E+LYP+ISO	87.86 \pm 5.98 ^{***ab}	51.85 \pm 3.03 ^{***a}	41.87 \pm 3.44 ^{***a}	37.62 \pm 2.93 ^{***ab}	10.37 \pm 0.60 ^{***a}

Values are expressed as Mean \pm 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 injected groups. ^a $p < 0.05$ compared to Vit. E+ISO and ^b $p < 0.05$ compared to LYP+ISO

Table 2: Effect of Vit. E and LYP on tissue lipid profile in normal and ISO injected rats

Groups	TC (mg g ⁻¹ wt. tissue)	TG (mg g ⁻¹ wt. tissue)	FFA (mg g ⁻¹ wt. tissue)	PL (mg g ⁻¹ wt. tissue)
Con.	8.230 \pm 0.637	6.147 \pm 0.637	0.871 \pm 0.077	25.620 \pm 1.988
ISO	13.690 \pm 1.340 ^{##}	11.260 \pm 0.840 ^{###}	1.450 \pm 0.117 ^{###}	16.620 \pm 1.902 ^{##}
Vit.E+LYP	7.832 \pm 0.435	6.176 \pm 0.422	0.868 \pm 0.222	24.879 \pm 1.883
Vit.E+ISO	10.080 \pm 0.483*	7.337 \pm 0.823**	1.102 \pm 0.077	23.640 \pm 1.982*
LYP+ISO	9.342 \pm 0.732**	7.158 \pm 0.710**	0.881 \pm 0.122**	24.576 \pm 3.111**
Vit.E+LYP+ISO	8.315 \pm 0.572 ^{***a}	5.411 \pm 0.529 ^{***ab}	0.877 \pm 0.194 ^{***a}	26.872 \pm 2.996

Values are expressed as Mean \pm 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 injected groups. ^a $p < 0.05$ compared to Vit.E+ISO and ^b $p < 0.05$ compared to LYP+ISO

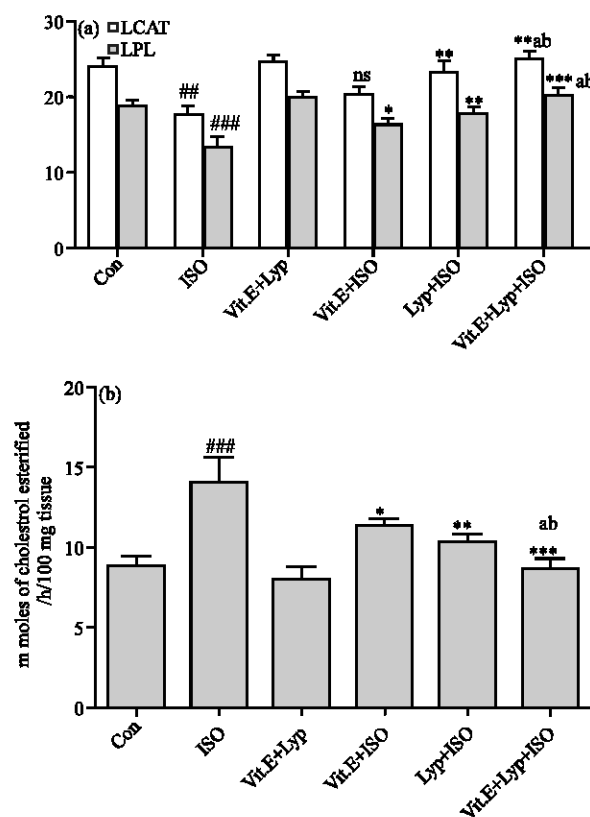


Fig. 1(a-b): Effect of Vit. E and LYP on (a) LCAT and LPL (b) CES levels in normal and ISO injected rats Units: LCAT (μ moles of cholesterol esterified/h/100mg tissue) LPL (μ moles of free fatty acids liberated/100 mg tissue). Values are expressed as Mean \pm SEM (n = 6). #p<0.05, ##p<0.01, ###p<0.001 values compared to control groups, *p<0.05, **p<0.01, ***p<0.001 values compared to ISO injected groups. ^ap<0.05 compared to Vit.E+ISO and ^bp<0.05 compared to LYP+ISO

DISCUSSION

Altered lipid metabolism is considered to accelerate the development of atherosclerosis, which is a major risk factor in myocardial infarction. A high level of circulating cholesterol and its accumulation in heart tissue is well associated with cardiovascular damage. In the present study, ISO injected rats showed increase in the levels of serum and heart tissue lipids with a decreased in heart tissue phospholipids and serum HDL cholesterol level. The observed pattern of altered lipid profile in the serum and heart are in line with the previous report (Karthikeyan *et al.*, 2007). ISO is reported to produce free radicals that may cause cellular cholesterol accumulation

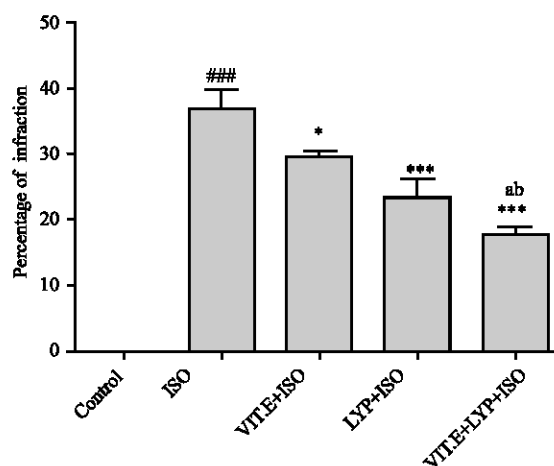


Fig. 2: Effect of Vit. E and LYP on area of infarction in normal and ISO injected rats Values are expressed as Mean \pm SEM (n = 4). ###p<0.001 values compared to control groups, *p<0.05, **p<0.01, ***p<0.001 values compared to ISO injected groups. ^ap<0.05 compared to Vit. E+ISO and ^bp<0.05 compared to LYP+ISO

by increasing cholesterol biosynthesis, decreasing cholesteryl ester hydrolysis and reducing cholesterol efflux (Gesquiere *et al.*, 1999). The decline in the cardiac phospholipid content with a concomitant increase in the serum can be due to ISO mediated peroxidation of unsaturated membrane lipids in biomembranes and tissues causing the leakage of these lipids into circulation (Sathish *et al.*, 2003). Also, the increased peroxidation of membrane phospholipids releases free fatty acids by the action of phospholipase A₂ (Chien *et al.*, 1980). Increased cardiac lipid peroxidation is in concern with altered PL and FFA levels in ISO injected rats observed herein. Treatment with Vitamin E alone and in combination with lycopene showed better protection than alone antioxidant in preventing serum and heart lipid profile towards normal suggested potent lipid lowering and antioxidant activity of this combination.

Present study showed a significant decrease in cardiac LCAT and LPL activities and a significant increase in CES activity in ISO injected rats. HDL is the main substrate for LCAT for cholesterol esterification and incorporation (Onyeneke *et al.*, 2007). A significant increase in the level of HDL in rats treated with Vitamin E alone and in combination with lycopene supports the increase cardiac LCAT activity. LPL in the heart is involved in the uptake of TG rich lipoproteins from circulation. An inverse correlation between TG and LPL activity has been reported (Hodis and Mack, 1995). In the present study hypertriglyceridemia observed in ISO

injected rats might be due to decrease activity of LPL in the myocardium resulting in decrease uptake of TG from the circulation. Accumulation of ester cholesterol occurs when the rate of esterification by cholesterol ester synthetase exceeds the rate of hydrolysis, which in turn results in myocardial membrane damage (Upananlawar and Balaraman, 2010). Vitamin E in combination with Lycopene alters the activities of LCAT, LPL and CES near to the normal by increasing HDL and decreasing TG and cholesterol levels, indicating the potential lipid lowering effects of this combination. In this context Vit. E has been reported to reduce lipid peroxidation formation from unsaturated fatty acids by inhibiting phospholipase A₂ and lipoxygenase activity in ISO injected rats (Yoshihara and Watanabe, 1980). This could be the reason for improving the lipid profile in the present study. Lycopene as a lipid soluble antioxidant proved to prevent lipid peroxidation and thereby phospholipids degradation in the present study. Recently it has been reported that lycopene from tomato paste produced significant improvement in lipid profile and lower atherogenic index in hyperlipidemic rats and high fat diet fed rabbit (Ibrahim *et al.*, 2008). Fuhrman *et al.* (1997) showed that the addition of lycopene to macrophage cell lines decreased cholesterol synthesis and increased LDL receptors, due to its non antioxidant function.

Area of infarction indicates loss of membrane integrity that might be due to significant leakage of LDH enzymes. Further increase in nitrosative stress and ROS production led to an enlarged infarct size in the ISO injected MI (Hu *et al.*, 2006). The present study showed a significant increase in % infarction in ISO injected rats. Present study also shows significant increase in ROS, LDH isoenzyme levels and nitric oxide production after ISO injection which might be the reasons for increased area of infarction in the present study. Treatment with Vit.E alone and in combination with LYP in ISO injected rats (Vit.E+LYP+ISO) significantly decreased infarction size which might be due to their potent antioxidant activity which prevents leakage of LDH enzymes and elevated nitrosative stress.

Addition of lycopene along with Vitamin E shows beneficial effects rather than lycopene alone in preventing altered lipid profile and activities of lipid metabolizing enzymes. These beneficial effects might be due to different in the activities of these antioxidants or might be due to the regeneration of Vitamin E from its α -tocopheroxyl radical by lycopene (Palozza and Krinsky, 1992). Finally concluding, the result of the present study indicates that the combined treatment with vitamin E and lycopene shows beneficial effects in preventing altered lipid profile towards normal rather than Vitamin E alone in ISO-induced myocardial infarction.

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