



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading doi:10.1016/S2221-1691(12)60054-9 © 2012 by the Asian Pacific Journal of Tropical Biomedicine. All rights reserved.

Tomato lycopene attenuates myocardial infarction induced by isoproterenol: Electrocardiographic, biochemical and anti-apoptotic study

Aman Upaganlawar*, Vaibhav Patel, Balaraman R.

¹Pharmacy Department, Faculty of Technology and Engineering, The Maharaja Sayajirao University of Baroda, Vadodara 390 002, Gujarat, India

ARTICLE INFO

Article history:

Received 5 September 2011

Received in revised form 3 October 2011

Accepted 28 October 2011

Available online 28 May 2012

Keywords:

Myocardial infarction

Lycopene

Isoproterenol

Apoptosis

Antioxidants

Necrosis

ABSTRACT

Objective: To assess the protective effects of lycopene on electrocardiographic, hemodynamic, biochemical and apoptotic changes in isoproterenol induced myocardial infarction. **Methods:** Myocardial infarction was induced in rats by subcutaneous injection of isoproterenol (200 mg/kg) for two consecutive days at an interval of 24 h. Rats were treated with lycopene (10 mg/kg/day, *p.o.*) for a period of 30 days and isoproterenol (ISO) was injected on the 29th and 30th day. At the end of experiment *i.e.* on the 31st day electrocardiographic, hemodynamic, biochemical and apoptotic changes were monitored from control and experimental groups. **Results:** ISO injected rats showed a significant alteration in electrocardiograph pattern and hemodynamic changes (*i.e.* systolic, diastolic and mean arterial pressure). It also showed significant increase in C-reactive protein, myeloperoxidase, nitrite levels and Caspase-3 protease activity. In addition, it also exhibited alteration in the levels of electrolytes (Na^+ , K^+ and Ca^{2+}), vitamin E, uric acid and serum protein. Gel electrophoresis of ISO injected rats showed increase in DNA fragmentation. Triphenyl tetrazolium chloride staining of the heart section shows increase area of infarction in ISO injected rats. Pre-co-treatment with lycopene significantly prevented the ISO induced alteration in ECG, haemodynamic, biochemical and apoptotic changes. **Conclusions:** The present result shows that treatment of lycopene in ISO injected rats significantly attenuates induced myocardial infarction.

1. Introduction

Cardiovascular disease (CVD), remain the principal cause of death in both developed and developing countries which includes high blood pressure, coronary heart disease, congestive heart failure, stroke. It is predicted that CVD will be the most important cause of mortality in India by 2020[1]. CVD is primarily caused by chronic deficiencies of vitamins and other essential nutrients with important properties like coenzymes, cellular energy carriers and free radical scavengers. Many dietary antioxidants and some non-nutrient based antioxidants from plants are increasingly being recognized as possible health promoters

in plummeting the risk of CVD[2].

Myocardial infarction (MI) occurs when the blood supply to a part of the heart is interrupted, causing death of heart tissue. High dose of isoproterenol (ISO), a β – adrenergic agonist, has been reported to induce MI in experimental animals which is confirmed by hyperglycemia, hyperlipidemia, increase in cardiac marker enzymes, hemodynamic changes, biochemical alterations and generation of oxidative stress[3–5].

Lycopene (LYP) has been shown to be one of the most efficient singlet oxygen quencher and peroxy radical scavengers among all the carotenoids and is more effective than α –tocopherol[6]. A number of studies have shown that LYP can protect native low density lipoprotein from oxidation and can suppress cholesterol synthesis. Lycopene effectively protects adrenaline induced MII[7], coronary artery disease[8] and it possesses antihyperlipidemic and antioxidant properties[9,10]. We have previously reported the effect of lycopene and its combination with vitamin E on cardiac marker enzymes, endogenous antioxidant level, membrane bound enzymes and histopathological alteration

*Corresponding author: Dr. Aman Upaganlawar, Pharmacy Department, Faculty of Technology and Engineering, The M. S. University of Baroda, Kalabhavan, Baroda-390001, Gujarat, India.

Tel: +91-0265- 2434187

Fax: +91-0265-2418927

E-mail: amanrx@yahoo.com

Foundation Project: Supported by All India Council for Technical Education (AICTE) (Grant No. 1-10/RID/NDF-PG/ (28/2007-08).

in ISO induced MI^[11]. So, from the above observation the present study was extended to evaluate the effect of lycopene on electrocardiograph (ECG) pattern, hemodynamic and biochemical changes along with anti-apoptotic activity in ISO –induced MI in rats.

2. Materials and methods

2.1. Drugs and chemicals

(±)-ISO hydrochloride and vitamin E (DL- α -tocopherol acetate) were purchased from Sigma Aldrich Co. St. Louis, MO, USA. Lycopene powder (LYP) was obtained as gift sample from Genesis Laboratory Ltd., Mumbai. The entire chemical used in this study was of analytical grade.

2.2. Experimental animals

Male adult albino rats (Wistar strain) weighing between 200 and 250 g were used in the present study. The animals were housed in an air conditioned room and were kept in standard laboratory conditions under normal light and dark cycle (12 h light/ dark) and maintained at an ambient temperature (25 ± 2) °C. The animals were fed with standard pellet diet (Amrut feeds, Pranav Agro Industries Ltd.) and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee of Pharmacy Department, the Maharaja Sayajirao University, Vadodara, Gujarat, in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, New Delhi.

2.3. Experimental design and protocol

Animals were divided into various groups. Group I rats received distilled water and olive oil (vehicle for LYP) for 30 days and normal saline (1 mL/kg, *s.c.*) on the 29th and 30th day. Group II rats received distilled water and olive oil for 30 days and ISO (200 mg/kg, *s.c.*) in normal saline on the 29th and 30th day at an interval of 24 h. Group III rats received LYP (10 mg/kg/day, *p.o.*) for 30 days. Group IV rats received LYP for 30 days and ISO on the 29th and 30th day.

2.4. Measurements of ECG changes

After 48 h of the first injection of either ISO or vehicle, ECG was recorded under light ether anesthesia through needle electrodes (Lead II) using Biopac MP30 data acquisition system (Biopac Systems, Santa Barbara, CA). The changes in ST interval, QT interval, RR interval, QRS complex, P wave amplitude and QTc interval along with changes in Heart rate were determined from ECG. QTc interval was calculated from Bazett's equation.

2.5. Non invasive blood pressure determination (indirect method)

Rats were trained for at least one week until the blood pressure was recorded with minimal stress and restraint. Systolic blood pressure (SBP), diastolic blood pressure (DBP)

and mean blood pressure (MBP) were measured at the end of treatment period (Tail cuff) using LE 5002 storage pressure meter.

2.6. Biochemical estimations

The levels of Na⁺ and K⁺ were estimated using commercial kits (Monozyme India Ltd, Secunderabad). Level of Ca²⁺ was measured by the O-cresolphthalein complexone method using a reagent kit (Span Diagnostic Ltd, Gujarat, India).

Tissue nitrite content was estimated colorimetrically with the Griess reagent^[12] in protein free supernatant of heart homogenate. Tissue vitamin E level was estimated as given by Desai^[13] and serum protein levels was determined using reagent kit (Span Diagnostic Ltd, Gujarat, India).

2.6.1. Determination of C-reactive protein (CRP)

Quantitative estimation of CRP in serum was performed as per instructions provided by SPINREACT kit (Latex turbidimetry method) S. A. Ctra. Santa Coloma, Spain.

2.6.2. Myeloperoxidase (MPO) assay

MPO was extracted and the activity was measured using a modified spectroscopic method described by Bradley *et al*^[14] which is as follows. Tissue samples (heart) were homogenized in ice cold 50 mM potassium phosphate buffer (pH=6) containing 0.5% Hexadecyl Trimethyl Ammonium Bromide. The homogenate was freeze thawed three times then centrifuged at $11\,000 \times g$ for 20 min at 4 °C. The supernatant (34 μ L) was mixed with the same phosphate buffer (986 μ L) which containing 0.167 mg/mL ortho-dianisidine dihydrochloride (Sigma) and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm was recorded by spectrophotometer. One unit of MPO activity was defined as that consuming 1 nmole of peroxide per minute at 22 °C. The results were expressed as units/mg of protein.

2.6.3. Caspase – 3 protease level

The levels of caspase – 3 protease was estimated as per the instruction provided by BioVision (Caspase-3/CPP32 Colorimetric Assay Kit, USA). Micro-titer plate was read at 405 nm in a microplate reader (BIO RAD, Model 680XR).

2.7. 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*^[15]. 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 were placed in 10% formalin solution which enhances the colour contrast developed. The % infarction was measured using ImageJ Software system.

2.8. DNA fragmentation analysis by gel electrophoresis

DNA extraction was carried out following the instruction provided by GeNei™, Bangalore–India. From the obtained DNA sample, approximately 8 μ g of DNA was loaded in each lane and run at 70 V on a 0.8% agarose gel and stained with ethidium bromide (0.5 μ g/mL) to separate DNA. Since extensive DNA fragmentation is an important characteristic of apoptosis, visualization of DNA breaks could greatly facilitate the identification of apoptotic cells.

2.9. Statistical analysis

Results of all the above estimations have been indicated in terms of mean \pm SEM. Differences between the groups were statistically determined by analysis of variance (ANOVA) with Bonferroni multiple comparisons test using GraphPad InStat version 5.00, GraphPad Software, California USA. The level of significance was set at $P < 0.05$.

3. Results

3.1. Effect of LYP on ECG pattern and hemodynamic changes

The effects of LYP on ECG pattern in normal and ISO injected rats are shown in Figure 1 and Table 1. Normal control and LYP alone treated groups showed normal pattern of ECG. ISO injected rats showed a significant ($P < 0.001$, $P < 0.01$) increase in ST–segment, QT interval, QTc interval along with a significant ($P < 0.001$, $P < 0.01$) decrease in P wave, QRS complex and RR interval as compared to control rats. Significant elevation of ST segment compared to control rats was indicative of infarcted myocardium (Figure 1). Treatment with LYP for 30 days and intoxicated by ISO significantly ($P < 0.01$, $P < 0.05$) decreased ST– interval, QT interval, QTc interval and significantly ($P < 0.01$, $P < 0.05$) increased P wave, QRS complex and RR interval as compared to ISO treated group (Table 1 and Figure 1).

Table 2 shows the effect of LYP on hemodynamic changes *i.e.* on systolic, diastolic and mean blood pressure. LYP alone treated groups showed normal hemodynamic pattern. ISO injected rats showed a significant ($P < 0.01$, $P < 0.001$) decrease in diastolic and mean blood pressure as compared to control group. Treatment with LYP in ISO injected rats showed a significant ($P < 0.05$) increase in mean blood pressure compared to ISO injected rats. LYP didn't show any significant effects on systolic and diastolic blood pressure. ISO injected rats also showed increase in heart rates compared to the control but it was found to be statistically non significant (Table 2).

Table 1

Effect of LYP on ECG changes in normal and ISO induced myocardial infarcted rats (Mean \pm SEM).

Groups (n=6)	ST elevation (mV)	P wave (sec)	QRS complex (sec)	QT interval (sec)	R–R interval (sec)	QTc interval (sec)
Control	0.184 \pm 0.0022	0.028 \pm 0.00037	0.0416 \pm 0.005	0.071 \pm 0.0006	0.170 \pm 0.0007	0.172 \pm 0.022
ISO	0.302 \pm 0.0040 ^{###}	0.024 \pm 0.00030 ^{##}	0.0285 \pm 0.007 ^{###}	0.081 \pm 0.0012 ^{##}	0.158 \pm 0.0017 ^{##}	0.204 \pm 0.029 ^{##}
LYP	0.178 \pm 0.0013	0.029 \pm 0.00054	0.0418 \pm 0.006	0.070 \pm 0.0009	0.170 \pm 0.0005	0.169 \pm 0.041
LYP + ISO	0.232 \pm 0.0025 ^{***}	0.026 \pm 0.00011 [*]	0.0364 \pm 0.004 ^{**}	0.075 \pm 0.0013 [*]	0.161 \pm 0.0016 ^{**}	0.181 \pm 0.032 [*]

^{*} $P < 0.05$, ^{**} $P < 0.01$, ^{***} $P < 0.001$ values compared to ISO groups, [#] $P < 0.05$, ^{##} $P < 0.01$, ^{###} $P < 0.001$ values compared to control groups.

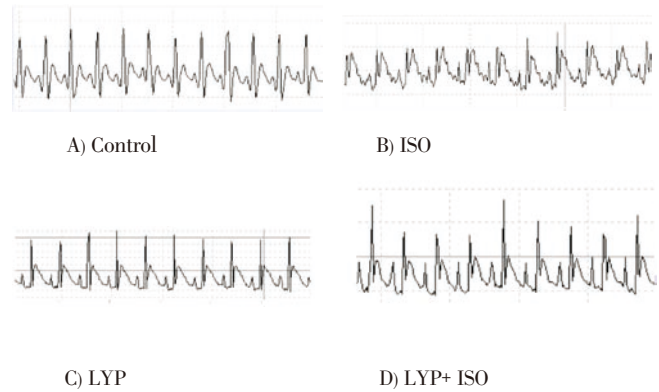


Figure 1. Representative electrocardiographic traces in each group.

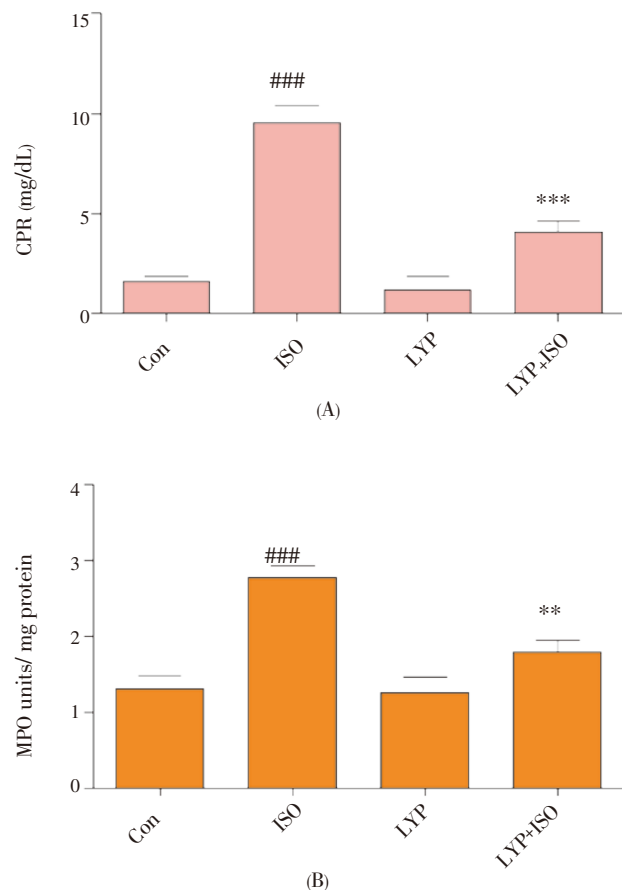


Figure 2. Effect of LYP on serum CRP level (A) and MPO activity (B) in normal and ISO induced myocardial infarcted rats.

Values are expressed as Mean \pm SEM. ^{**} $P < 0.01$, values compared to ISO groups, ^{###} $P < 0.001$ values compared to the control groups.

3.2. Effect of LYP on biochemical changes

Table 2Effect of LYP on hemodynamic changes and heart rate normal and ISO induced myocardial infarcted rats (Mean \pm SEM).

Groups (n=6)	SBP (mmHg)	DBP (mmHg)	MBP (mmHg)	Heart rate (BPM)
Control	118.00 \pm 14.52	96.33 \pm 10.87	110.30 \pm 10.32	338.30 \pm 12.88
ISO	101.86 \pm 16.21 ^{ns}	68.98 \pm 14.98 ^{##}	80.33 \pm 16.82 ^{###}	400.09 \pm 14.32 ^{ns}
LYP	114.30 \pm 12.98	99.50 \pm 5.33	109.80 \pm 9.82	340.90 \pm 10.54
LYP+ISO	104.70 \pm 13.54	72.50 \pm 5.55	98.50 \pm 11.10 [*]	359.00 \pm 10.11

* P <0.05, ** P <0.01, *** P <0.001 values compared to ISO groups, # P <0.05, ## P <0.01, ### P <0.001 values compared to control groups; ns – non significant.**Table 3**Effect of LYP on electrolytes, vitamin E, uric acid and protein level in normal and ISO induced myocardial infarcted rats (Mean \pm SEM).

Groups (n=6)	Sodium (Na ₊) (nmol/mg protein)	Potassium (K ⁺) (nmol/mg protein)	Calcium (Ca ²⁺) (nmol/mg protein)	Uric acid (mg/dL)	Protein (mg/dL)	Vitamin E (mmol/mg protein)
Control	6.088 \pm 0.125	8.978 \pm 0.164	10.81 \pm 0.107	4.89 \pm 0.29	7.340 \pm 0.290	2.02 \pm 0.047
ISO	7.728 \pm 0.312 ^{###}	6.162 \pm 0.216 ^{###}	13.83 \pm 0.344 ^{###}	7.78 \pm 0.66 ^{###}	3.531 \pm 0.499 ^{###}	1.09 \pm 0.043 ^{###}
LYP	6.082 \pm 0.122	8.784 \pm 0.121	10.77 \pm 0.083	4.65 \pm 0.42	7.401 \pm 0.388	2.22 \pm 0.031
LYP+ISO	6.542 \pm 0.109 ^{**}	8.332 \pm 0.136 ^{***}	11.32 \pm 0.143 ^{***}	5.55 \pm 0.37 [*]	6.210 \pm 0.267 ^{**}	1.84 \pm 0.035 ^{***}

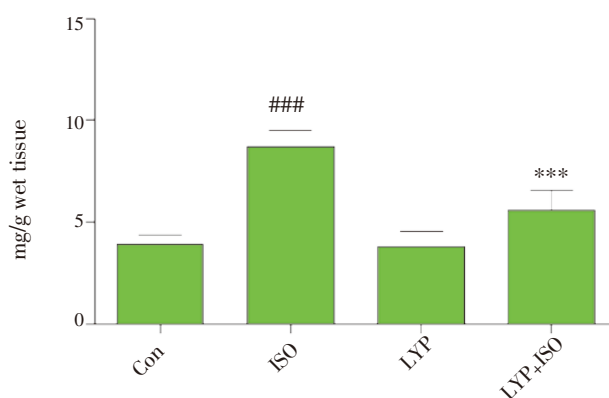
* P <0.05, ** P <0.01, *** P <0.001 values compared to ISO groups, # P <0.05, ## P <0.01, ### P <0.001 values compared to control groups.

Table 3 shows the effect of LYP on certain biochemical parameters in normal and ISO injected rats. ISO injected rats showed a significant (P <0.001) increase in serum uric acid levels and significant (P <0.001) decrease in total protein and tissue vitamin E levels as compared to control rats. Administration of LYP in ISO injected rats showed significant (P <0.001) decrease in uric acid with significant (P <0.001) increase in total protein and vitamin E level as compared to ISO treated group (Table 3). The effect of LYP on electrolytes levels are shown in Table 3. The levels of sodium and calcium were significantly (P <0.001) increased and potassium was significantly (P <0.001) decreased in ISO injected rats compared to the control rats. Administration of LYP for 30 days in ISO injected rats significantly (P <0.001, P <0.05) decreased the levels of sodium, calcium and significantly (P <0.001) increased the potassium levels compared to ISO treated rats (Table 3).

3.3. Effect of LYP on inflammatory markers and tissue nitrite levels

The effects of LYP on inflammatory markers (CRP, myeloperoxidase) and tissue nitrite levels in normal and ISO injected rats are shown in Figure 2 and 3. Rats injected with ISO showed a significant (P <0.001, P <0.01) increase in serum CRP, tissue nitrite level and MPO activity as compared to the control group. Treatment with LYP for 30 days in ISO injected

rats showed a significant (P <0.001, P <0.05) decrease in CRP levels, tissue nitrite level and MPO activity as compared to ISO treated group. Normal and LYP alone treated groups showed normal levels of inflammatory markers and tissue nitrite level.

**Figure 3.** Effect of LYP on tissue nitrite levels in normal and ISO induced myocardial infarcted rats.Values are expressed as Mean \pm SEM. *** P <0.001 values compared to ISO groups, ### P <0.001 values compared to the control groups.

3.4. Effect of LYP on macroscopic enzyme assay (TTC) and area of infarction

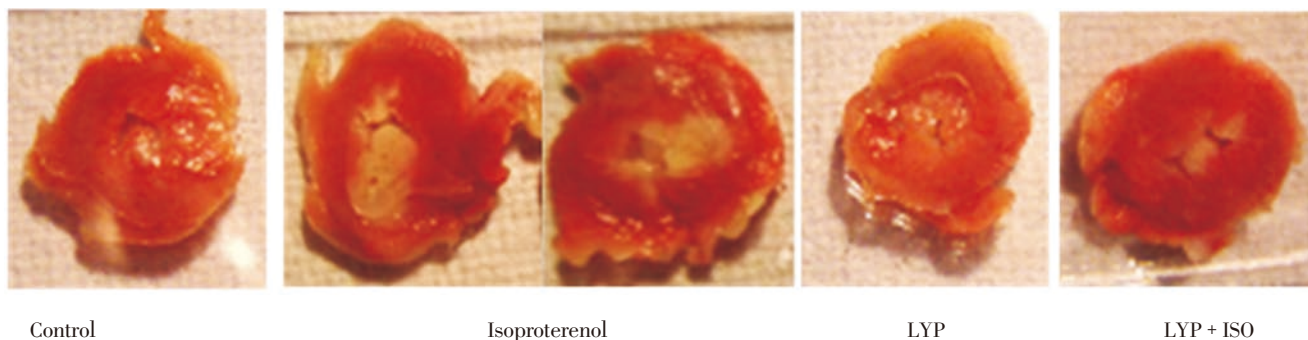
**Figure 4.** Representative myocardial infarction in each group carried out by TTC staining.

Figure 4 shows the heart sections of normal and ISO injected rats stained with TTC. ISO injected rats showed increased infarction area shown by yellowish colour compared to the control rats. The percentage of mean infarct size was also calculated. ISO injected rats showed increased area of infarction compared to control rats. Treatment with LYP for 30 days and intoxicated with ISO showed a significant decrease in infarct size and staining as compared to ISO injected rats (Figure 4, 5).

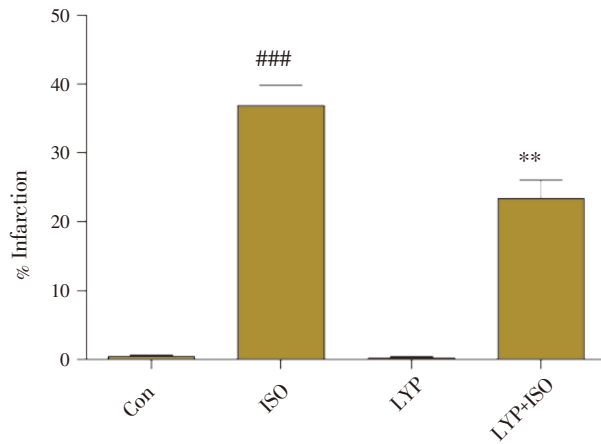


Figure 5. Effect of LYP on area of infarction in normal and ISO induced myocardial infarcted rats. Values are expressed as Mean \pm SEM. ** P <0.01 values compared to ISO groups, ### P <0.001 values compared to the control groups.

3.5. Effect of LYP on caspase-3 protease activity and DNA fragmentation

The effects of LYP on caspase-3 protease activity and severity of DNA damage by gel electrophoresis in normal and ISO injected rats are shown in Figure 6 and Figure 7. ISO injected rats showed a significant (P <0.01) increase in caspase-3 protease activity and severity of DNA damage (Lane B) as compared to control rats. Treatment with LYP for 30 days significantly (P <0.05) reduced caspase-3 activity and showed active prevention in the severity of DNA damage (Lane C and D) compared to ISO injected rats.

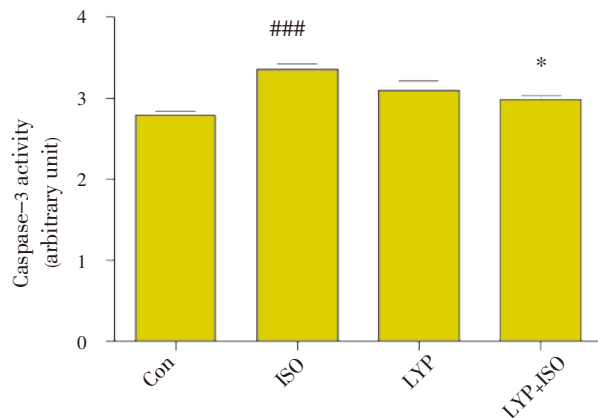


Figure 6. Effect of LYP on caspase-3-protease activity in normal and ISO induced myocardial infarcted rats. Values are expressed as Mean \pm SEM. * P <0.05 values compared to ISO groups, ### P <0.001 values compared to control groups.

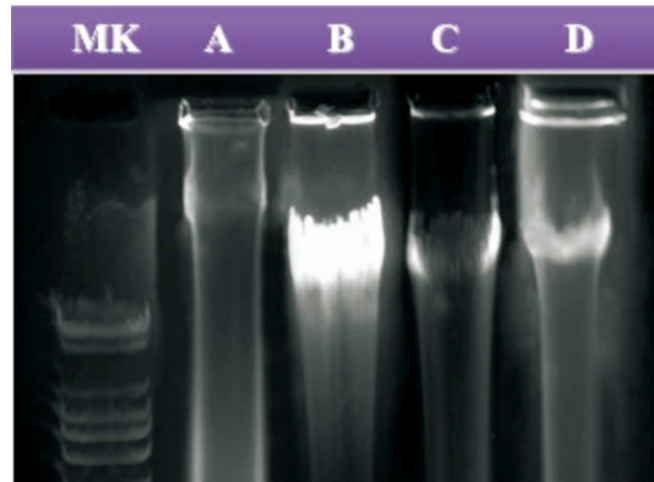


Figure 7. Effect of LYP on DNA damage in normal and ISO induced myocardial infarcted rats.

4. Discussion

MI induced by injection of ISO is a standardized model to study the beneficial effects of numerous drugs and antioxidants. In the present study, we found that lycopene treatment exerts a strong cardioprotection in ISO induced MI in rats.

ECG abnormalities are considered as the main criteria generally used for the diagnosis of MI. In the present study ISO injection shows significant alterations in ECG patterns. The characteristic ECG findings were reduction in R-R intervals, P wave intensity, QRS complex and elevation of ST segment, QT interval and QTc interval. These alterations could be due to the consecutive loss of cell membrane potential in injured myocardium. ST segment elevation was observed in patient with acute myocardial ischemia and in experimental model of ISO in rats[16,17]. ISO undergo auto oxidation and generates free radicals which further produced oxidative stress. Increased oxidative stress causes loss of cell membrane function, leads to ECG changes which include elevation of ST segment which reported by number of clinical and experimental studies[18]. Treatment with LYP in ISO injected rats significantly prevented the altered ECG pattern towards normal suggesting the cell membrane stabilizing potential of LYP which might be due to its potent antioxidant property.

In the present study ISO injected rats showed MI which is evident by significant fall in systolic, diastolic and mean blood pressure and increase in heart rate. These changes in hemodynamic parameters indicated the activation of sympathetic nervous system which is in line with previous reports[19,20]. Supplementation of LYP significantly attenuated these changes in hemodynamic parameters which is evidenced from improvements in systolic, diastolic and mean blood pressure. ISO injected rats did not show increase in heart rate but it was found to be statistically non-significant reports. ISO caused myocardial damage was accompanied by a stress response in pituitary adrenal axis resulting in adrenal hyperactivity which elevates circulating catecholamine. This could account for the elevated heart rate in the present study.

A significant increase in serum uric acid and decrease in total protein and heart vitamin E levels was observed

in ISO injected rats, which is in line with the previous reports^[18,21]. Large cohort studies have shown that uric acid is an important independent risk factor for cardiovascular mortality and in the development of MI. During hypoxic condition tissue are disturbed, the enzyme xanthine dehydrogenase is converted to xanthine oxidase by the oxidation of essential-SH-groups. Xanthine oxidase catalyses the conversion of hypoxanthine to xanthine, uric acid and superoxide^[22]. This could be one of the reasons for the elevated levels of serum uric acid in the present study. A decrease in the level of serum total proteins in the present study could be due to increased free radical and lipid peroxidation production by ISO.

Vitamin E is a major lipid soluble non-enzymatic antioxidant present in cell membranes and lipoproteins that protects against oxidative modification in the body^[23]. The observed decrease in the Vitamin E level, in the present study, might be due to increased utilization for the neutralization of ISO mediated free radicals and lipid peroxidation. Administration of LYP can significantly decrease the level of uric acid and increase the level of serum protein and tissue vitamin E, this might be the antioxidant property of LYP which prevents the SH group of enzyme from oxidation and thereby elevated uric acid level.

In the cell, ATPases are closely associated with the plasma membrane and participate in the energy dependent transport of sodium, potassium, magnesium and calcium translocation. An increase in sodium and calcium along with decrease in potassium were observed in ISO injected rats which might be due to altered ATPases activity in membrane as a result of lipid peroxidation produced by ISO. Increased concentration of sodium might be due to decrease in Na⁺/K⁺ ATPase^[24]. Depletion of ATP by ISO leads to opening of K⁺ channel leading to the decrease in K⁺ ions in the myocardial tissue. Increased levels of intracellular Na⁺ also operate to depress Ca²⁺ effect and augment Ca²⁺ influx. In our previous report we showed the alteration of ATPases during ISO injection. LYP treatment can prevent the altered levels of electrolyte and these effects of LYP could be due to the prevention of 'SH' group of the ATPases from oxidative damage through the inhibition of peroxidation of membrane lipids indicating the membrane stabilizing effects of LYP.

During the acute phase of MI inducible nitric oxide synthase (iNOS) expression and nitric oxide (NO) production increases in the heart^[25]. It has been, reported that β -AR stimulation upregulated iNOS and significant increases production of NO, which create a nitrosative stress and generate the powerful oxidant molecule peroxynitrite (ONOO⁻)^[26]. Administration of LYP in the present study significantly decreased the elevated tissue nitrite levels which might be due to their antioxidant activity which prevents superoxide generation and thereby peroxynitrite formation.

In the present study ISO injected rats showed a significant rise in serum CRP level and decrease in MPO activity indicating the involvement of inflammatory process during ISO toxicity. CRP has been used as a sensitive predictor of acute cardiovascular events compared with other widely used biomarkers^[27]. Several population based observational studies have reported that serum CRP concentrations are inversely associated with dietary intake of fruits, vegetables and tea, which are rich in polyphenolic antioxidants^[28]. Increased MPO activity indicates an acute inflammation and

leukocyte accumulation in heart tissues of the ISO-injected rats^[29]. Treatment of LYP significantly reduced the elevated CRP levels and MPO activity suggesting potent anti-inflammatory activity.

Area of infarction indicates loss of membrane integrity which might be due to significant leakage of lactate dehydrogenase enzymes. Further increase in nitrosative stress and reactive oxygen species production led to an enlarged infarct size in the ISO injected MI^[30]. The present study showed a significant increase in the percentage of infarction in ISO injected rats. Present study also shows significant increased nitric oxide production thereby oxidative stress after ISO injection which might be the reasons for increased area of infarction in the present study. Treatment with LYP in ISO injected rats significantly decreased infarction size which might be due to their potent antioxidant activity which prevents leakage of lactate dehydrogenase enzymes and elevated nitrosative stress.

The present study showed a significant increase in caspase-3 activity and DNA breakdown in ISO injected rats. Increased caspase-3 protease activity and DNA damage indicates cardiac apoptosis and necrosis in the present study. Study have reported that β -AR stimulation by catecholamine induces cardiac apoptosis or/and necrosis^[31]. It has been already reported that oxidative stress provoke DNA damage (DNA fragmentation and apoptosis) and treatment with antioxidants inhibit DNA fragmentation and apoptosis^[32]. Oxidation of catecholamine forms quinonoid compounds giving rise to the production of superoxide anions and subsequently hydrogen peroxide, which in the presence of iron forms highly reactive hydroxyl radicals and causes protein, lipid and DNA damage^[33]. Further, increased expression of iNOS in myocardium of animals and patients with heart failure may be responsible for increased numbers of apoptotic cardiomyocytes^[34]. LYP treatment showed significant reduction in caspase-3 protease activity and DNA damage. This indicates that LYP prevents apoptosis and necrosis in ISO injected MI in rats. This beneficial effect might be due to potent antioxidant activity of LYP which scavenge the highly toxic free radicals and prevents the DNA from damage and thereby inhibiting caspase-3 activity. In the present study, significant decrease in tissue nitrite levels by administration of LYP was observed, which might be one of the reasons for anti-apoptotic effects.

In conclusion, the present study showed that acute ISO injection in experimental animals induces MI which is confirmed by ECG pattern, hemodynamic changes, certain biochemical changes and apoptotic markers. Pre-cotreatment with LYP prevents the ISO induced MI by virtue of its potent antioxidant activity. These findings might be helpful to understand the beneficial effects of LYP against ISO induced MI although further study is needed to confirm its mechanism.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are thankful to All India Council for Technical

Education (AICTE) for providing financial assistance under the scheme of National Doctoral Fellowship (NDF) for the present work. AICTE File No. 1–10/RID/NDF–PG/(28)/2007–08.

References

- [1] Rajdurani M, Stanely PMP. Preventive effect of naringin on cardiac markers, electrocardiographic pattern and lysosomal hydrolases in normal and isoproterenol induced myocardial infarction in wistar rats. *Toxicol* 2007; **230**: 178–188.
- [2] Upaganlawar A, Gandhi H, Balaraman R. Isoproterenol induced myocardial infarction: Protective role of natural products. *J Pharmacol Toxicol* 2011; **6**: 1–17.
- [3] Upaganlawar A, Balaraman R. Effect of vitamin E and green tea on hemodynamic, electrocardiographic and some biochemical alterations in experimentally induced myocardial infarction in rats. *Eur J Int Med* 2010; **2**: 135–141.
- [4] Upaganlawar A, Gandhi C, Balaraman R. Effect of green tea and vitamin E combination in isoproterenol induced myocardial infarction in rats. *Plant Foods Hum Nutr* 2009; **64**: 75–80.
- [5] Patel V, Upaganlawar A, Zalawadia R, Balaraman R. Cardioprotective effect of melatonin against isoproterenol induced myocardial infarction in rats: A biochemical, electrocardiographic and histoarchitecture evaluation. *Eur J Pharmacol* 2010; **644**: 160–168.
- [6] Pan SY, Zhou J, Gibbons L, Morrison H, Wen SW, Ccerg TC. Antioxidants and breast cancer risk—a population based case control study in Canada. *BMC Cancer* 2011; **11**: 372.
- [7] Roksana P, Nargis A. Protective effect of tomato against adrenaline-induced myocardial infarction in rats. *Bangladesh Med Res Counc Bull* 2008; **34**: 104–108.
- [8] Riccioni G, Mancini B, Di Ilio E, Bucciarelli T, D’Orazio N. Protective effect of lycopene in cardiovascular disease. *Eur Rev Med Pharmacol Sci* 2008; **12**: 183–90.
- [9] Hsu YM, Lai CH, Chang CY, Fan CT, Chen CT, Wu CH. Characterizing the lipid-lowering effects and antioxidant mechanisms of tomato paste. *Biosci Biotechnol Biochem* 2008; **72**: 677–85.
- [10] Gitenay D, Lyan B, Rambeau M, Mazur A, Rock E. Comparison of lycopene and tomato effects on biomarkers of oxidative stress in vitamin E deficient rats. *Eur J Nutr* 2007; **46**: 468–475.
- [11] Upaganlawar A, Gandhi H, Balaraman R. Effect of vitamin E alone and in combination with lycopene on biochemical and histopathological alterations in Isoproterenol induced myocardial infarction in rats. *J Pharmacol Pharmacother* 2010; **1**: 24–31.
- [12] Guevara I, Iwanejko J, Dembinska-kiec A, Pankiewicz J, Wanat A, Anna P. Determination of nitrite/nitrate in human biological material by the simple griess reaction. *Clin Chim Acta* 1998; **274**: 177–188.
- [13] Desai ID. Vitamin E analysis methods for animal tissues. *Methods Enzymol* 1984; **66**: 138–147.
- [14] Bradely PP, Prieat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation; estimation of neutrophil content with an enzyme marker. *J Invest Dermatol* 1982; **78**: 206–209.
- [15] Lie JT, Pairolero PC, Holley KE, Titus JL. Macroscopic enzyme mapping verification of large, homogenous, experimental myocardial infarcts of predictable size and location in dog. *J Thor Cardiovasc Surg* 1975; **69**: 599–605.
- [16] Peacock WE, Hollander JE, Smalling RW, Bresler MJ. Reperfusion strategies in the emergency treatment of ST-segment elevation myocardial infarction. *Am J Emerg Med* 2007; **25**: 353–366.
- [17] Punithavathi VR, Stanely Mainzen Prince P. The cardioprotective effects of a combination of quercetin and α -tocopherol on isoproterenol-induced myocardial infarcted rats. *J Biochem Mol Toxicol* 2011; **25**: 28–40.
- [18] Prince SMP, Rajkumar S, Dhanasekar K. Protective effects of vanillic acid on electrocardiogram, lipid peroxidation, antioxidants, proinflammatory markers and histopathology in isoproterenol induced cardiotoxic rats. *Eur J Pharmacol* 2011; **668**: 233–240.
- [19] Goyal SN, Arora S, Sharma AK, Joshi S, Ray R, Bhatia J. Preventive effect of crocin of *Crocus sativus* on hemodynamic, biochemical, histopathological and ultrastructural alterations in isoproterenol-induced cardiotoxicity in rats. *Phytomed* 2010; **17**: 227–232.
- [20] Thippeswamy BS, Thakker SP, Tubachi S, Kalyani GA, Netra MK, Patil U. Cardioprotective effect of *Cucumis trigonus* Roxb on isoproterenol induced myocardial infarction in rat. *Am J Pharmacol Toxicol* 2009; **4**: 29–37.
- [21] Devika PT, Prince PSM. (–) Epigallocatechingallate (EGCG) prevents isoproterenol induced cardiac toxicity by stabilizing cardiac marker enzymes and membrane bound ATPases. *J Pharm Pharmacogn* 2008; **60**: 125–133.
- [22] Nivethetha M, Jayasri J, Brindha P. Effects of *Muntingia calabura* L. on isoproterenol-induced myocardial infarction. *Singapore Med J* 2009; **50**: 300–302.
- [23] Prabhu S, Jainu M, Sabiths KE, Devi CSS. Role of mangiferin on biochemical alterations and antioxidant status in isoproterenol-induced myocardial infarction in rats. *J Ethnopharmacol* 2006; **107**: 126–133.
- [24] Al-Numair KS, Chandramohan G, Alsaif MA. Pretreatment with morin, a flavonoid, ameliorates adenosine triphosphates and glycoproteins in isoproterenol induced myocardial infarction in rats. *J Nat Med* 2011; **48**: 1083–1090.
- [25] Pinto VD, Cutini GJ, Sartorio CL, Paigel AS, Vassallo DV, Stefanon I. Enhanced beta adrenergic response in rat papillary muscle by inhibition of inducible nitric oxide synthase after myocardial infarction. *Acta Physiol* 2007; **190**: 111–117.
- [26] Dianyuan Li, Qu Y, Tao L, Liu HR, Hu AH, Gao F. Inhibition of iNOS protects the aging heart against β -adrenergic receptor stimulation-induced cardiac dysfunction and myocardial ischemic injury. *J Surg Res* 2006; **131**: 64–72.
- [27] Verma S, Devaraj S, Jialal I. C-reactive protein promotes atherothrombosis. *Circulation* 2006; **113**: 2135–2150.
- [28] Chun O, Chung S, Song W. Estimated dietary flavonoid intakes and major food sources of U.S. adults. *J Nutr* 2006; **137**: 1244–1252.
- [29] Senthil S, Sridevi M, Pugalendi KV. Cardioprotective effect of oleanolic acid on isoproterenol-induced myocardial ischemia in rats. *Toxicol Pathol* 2007; **35**: 418–423.
- [30] Hu A, Jiao X, Gao E, Koch WK, Sharifi-Azad S, Grunwald Z, Ma XL, Sun JZ. Chronic β -adrenergic receptor stimulation induces cardiac apoptosis and aggravates myocardial ischemia/reperfusion injury by provoking inducible nitric-oxide synthase-mediated nitrate stress. *J Pharmacol Exper Therap* 2006; **318**: 469–475.
- [31] Remondino A, Kwon SH, Communal C, Pimentel DR, Sawyer DB, Singh K, et al. β -adrenergic receptor-stimulated apoptosis in cardiac myocytes is mediated by reactive oxygen species/c-Jun NH₂-terminal kinase-dependent activation of the mitochondrial pathway. *Circ Res* 2003; **92**: 136–138.
- [32] Galang N, Sasaki H, Maulik N. Apoptotic cell death during ischemia/reperfusion and its attenuation by antioxidant therapy. *Toxicol* 2000; **148**: 111–118.
- [33] Dhalla NS, Temsah RM, Netticadan T. Role of oxidative stress in cardiovascular diseases. *J Hypertension* 2000; **31**: 152–161.
- [34] Narula J, Haider N, Virmani V. Apoptosis in myocytes in end-stage heart failure. *N Eng J Med* 1996; **335**: 1182–1189.