

CHAPTER 7

SUMMARY AND CONCLUSION

Myocardial infarction (MI) is described as an acute condition of myocardial necrosis that occurs as a result of imbalance between coronary blood supply and myocardial demand. Potentially modifiable lifestyle and complex traits such as hypertension, dyslipidemia, obesity and diabetes mellitus are major contributors to the etiology of MI. Although clinical care has been improved, public awareness is raised and health innovations are widely used, MI remains the leading cause of death worldwide. Therefore, a better understanding of the molecular and cellular mechanisms involved in the etiology of MI may pave the way of improved diagnostic and treatment strategies that in concert with adequate preventive approaches could be of significant benefit for people worldwide.

Modern synthetic medicine looks at the diseases from a narrow point of view, which would be beneficial in an emergency setup, but might harm the system in the long run. It is therefore necessary to search for native alternative drugs for the treatment of cardiovascular diseases to replace currently available drugs of doubtful efficacy and safety. However, with the emerging evidence that oxidative processes are involved in cardiac and vascular disease, it seems reasonable to suggest that antioxidant therapeutic strategies may have values. This has led to copious interest on phytonutrients owing to their non-toxic nature and commendable antioxidant properties.

Antioxidants are uniquely different from one another and each may have the specific function in the body. However, they are also synergistic, and will work most effectively when they are used together. In proper combination, they can perform a wide range of metabolic activities, free radical scavenging and preventive actions. Several *in vitro* and *in vivo* studies have reported that combination of vitamins with other antioxidants and a combination of hydrophilic and lipophilic antioxidants produce synergistic effects.

In vitro study shows the synergistic effects of green tea polyphenol and vitamin E in micelles, homogenous solutions and in human low density lipoproteins. Studies also show the synergistic effects of lycopene (LYP) with vitamin E in microsomal membranes and LDL oxidation. But, any *in vivo* study on whether Vitamin E or its combination with green tea (GT), lycopene (LYP), Pomegranate fruit extracts (PGFE) or *Lagenaria siceraria* fruit juice (LSFJ) could offer protection to the myocardium during myocardial oxidative stress induced injury has not been previously evaluated. Hence, an attempt has been made in the present investigation to evaluate the cardioprotective effect of vitamin E alone and its combination with GT, LYP, PGFE or LSFJ in experimentally induced myocardial infarction in rats.

The outcome of the investigation carried out is summarized below

- A pilot study was carried out to optimize the dose of antioxidants. Result of the pilot study showed that treatment of Vit.E (100mg/kg), GT (100mg/kg), LYP (10mg/kg), PGFE (100mg/kg) and LSFJ (400mg/kg) significantly decreased the elevated levels of LDH, CK-MB, LPO and significantly increased the GSH levels in ISO injected rats. Since the above doses exhibited maximum protection at minimum concentration, they were selected alone and in combination with Vit.E for evaluating different parameters in ISO induced myocardial infarction in rats.
- In the present study, ISO injected rats showed a significant decrease in body weight and a significant increase in heart weight and heart/body weight ratio compared to control rats. Increased in heart/body weight ratio indicate cardiac hypertrophy in ISO induced myocardial infarction in rats.
- Treatment of Vit.E alone and its co-administration with GT, LYP or PGFE in ISO injected rats (Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) significantly reduced heart/body weight ratio which might be due to potent

antioxidant activity of the combination which reduced the stimulus for hypertrophy. However, co-administration of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not show significant reduction in heart/body weight ratio.

- The present study shows significant alterations of ECG patterns in ISO injected rats as compared to control rats. The characteristic findings were reduction in R-R intervals, P wave intensity, QRS complex and elevation of ST segment, QT interval and heart rate.
- Co-administration of Vit.E and GT, LYP or PGFE in ISO injected rats (Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) showed a protective effect against altered ECG pattern suggesting that these combinations could effectively prevent membrane potential. However, co-administration of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not show significant reduction in ST segment elevation compared to Vit.E alone treated groups (Vit.E+ISO).
- ISO injected rats shows MI which is evident by significant fall in systolic, diastolic and mean blood pressure. Supplementation of Vit.E alone and in combination with GT, LYP and PGFE in ISO injected rats (Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) significantly attenuated these changes in hemodynamic parameters evidenced from improvement in systolic, diastolic and mean blood pressure. However, co-administration of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not show significant improvement in hemodynamic changes.
- The present study showed significant elevation of serum levels of AST, ALT, ALP, LDH, CK-MB, Uric acid and significant decrease in total protein level in ISO injected rats as compared to control rats.

- Co-administration of Vit.E and GT, LYP or PGFE in ISO injected rats (Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) significantly prevented the altered marker enzyme levels towards normal. This might be due to the protective effect of Vit.E along with GT, LYP or PGFE 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. Co-administration of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not produce further improvement in cardiac marker enzymes compared to Vit.E alone treated groups (Vit.E+ISO). This prevention in marker enzyme levels could be due to the potent action of these combinations which prevents membrane integrity and/or permeability thereby restricting the leakage of these enzymes from the myocardium.
- In the present study, an increase in the intensity of LDH1 and LDH2 isoenzyme in ISO injected rats was observed. Co-administration of Vit. E with GT, LYP or PGFE in ISO injected rats (Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) significantly reduced the intensity of LDH1 isoenzyme towards normal. The reduction in the intensity of LDH1 isoenzyme by these combinations could be due to potent antioxidant activities. Co-administration of Vit.E and LSFJ (Vit.E+LSFJ+ISO) in ISO injected rats did not show further reduction in the intensity of LDH1 isoenzyme compared to Vit.E alone treated group (Vit.E+ISO).
- A significant increase in lipid peroxidation products and a significant decreased in the activities of GSH, GPX, GST, SOD, CAT and non enzymatic antioxidant such as Vit. E level in the heart of ISO injected rats were observed suggested an enhanced oxidative stress after ISO injection.
- Treatment of Vit. E alone and in combination with GT, LYP or PGFE in ISO injected rats (Vit.E+ISO, Vit.E+GT+ISO, Vit.E+LYP+ISO or

Vit.E+PGFE+ISO) significantly decreased lipid peroxidation and significantly increased activities of oxidative stress enzymes and thereby reduced oxidative stress induced by ISO. Co-administration of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not produce beneficial effects in attenuating lipid peroxidation and oxidative stress compared to Vit.E alone treated group (Vit.E+ISO).

- In the present study ISO injected rats showed a significant decrease in the Na^+/K^+ ATPase and Mg^{2+} ATPase activities and a significant increase in Ca^{2+} ATPase activity. Correspondingly, an increase in sodium and calcium, and a decrease in potassium levels were observed in ISO injected rats.
- Co-administration of Vit.E and GT, LYP or PGFE in ISO injected rats (Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) significantly increased the activities of Na^+/K^+ ATPase and Mg^{2+} ATPase and decreased the activity of Ca^{2+} ATPase compared to ISO injected group. The combination also significantly improved the levels of Na^+ , K^+ and Ca^{2+} ions towards normal. This effects could be due to the ability of these combinations to protect the '-SH' group from oxidative damage through the inhibition of peroxidation of membrane lipids indicated the membrane stabilizing effects of these combination.
- Co-administration of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not show significant protection against altered ATPases activities and electrolyte levels compared to Vit.E alone.
- A significant increase was observed in the levels of serum TC, TG, VLDL, LDL, FFA and PL with significant decrease in HDL level and tissue PL level in ISO injected rats. Treatment of Vit. E alone and its co-administration with GT, LYP or PGFE in ISO injected rats (Vit.E+ISO, Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) shows significant protection against

altered lipid profile. This improvement in serum and tissue lipid profile strongly supports the potent lipid lowering and antioxidant activities of these drugs. Co-administration of Vit.E with LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not show significant protective effects against altered lipid profile compared to Vit.E alone treated group (Vit.E+ISO).

- ISO injected rats showed marked increase in the activities of Cholesterol ester synthase with a decrease in the activity of Lipoprotein lipase and Lecithin: cholesterol acyl transferase. Treatment of Vit. E and its co-administration with GT, LYP or PGFE in ISO injected rats (Vit.E+ISO, Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) showed a significant protection against altered lipid metabolizing enzymes. However, co-administration of LSFJ and Vit.E in ISO injected rats (Vit.E+LSFJ+ISO) did not show further improvement in the activities of lipid metabolizing enzymes compared to Vit.E alone treated group.
- A significant increase in tissue nitrite levels was observed in ISO injected rats compared to control rats indicated the involvement of nitrosative stress in ISO induced MI.
- Supplementation of Vit.E in ISO injected rats (Vit.E+ISO) reduced the nitrite formation but it was found to be statistically non-significant. However, Co-administration of Vit.E and GT or LYP in ISO injected rats (Vit.E+GT+ISO or Vit.E+LYP+ISO) significantly reduced the elevated levels of tissue nitrite suggested the potent antioxidant activity of these combinations. Treatment of Vit.E in combination with PGFE or LSFJ in ISO injected rats (Vit.E+PGFE+ISO or Vit.E+LSFJ+ISO) did not show significant improvement in tissue nitrite level compared to Vit.E alone.
- A significant increase in serum C-reactive protein (CRP) level, and tissue myeloperoxidase (MPO) activity was observed in ISO injected rats compared

to control rats indicated the involvement of inflammatory processes in ISO induced MI. Co-administration of Vit.E and GT, LYP or PGFE in ISO injected rats (Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) significantly decreased the elevated levels of CRP and MPO activity, suggesting potent anti-inflammatory and antioxidant activity of these combinations. However, co-administration of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not produce significant effects on CRP level and MPO activity compared to Vit.E alone.

- Histopathological findings revealed necrotic changes with intense infiltration of leucocytes with edema and fragmentation of muscle fibers in ISO injected rats. Treatment of Vit.E alone and its co-administration with GT, LYP or PGFE in ISO injected rats (Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) significantly protects the myocardial injury by decreasing inflammatory markers as evidenced by reduction of inflammatory cells and edema. LSFJ in combination with Vit.E in ISO injected rats (Vit.E+LSFJ+ISO) showed mild changes in histopathological alterations.
- ISO injected rats showed increased expression of glycoproteins which may be due to ISO induced lipid peroxidation and diminished antioxidant status. Treatment with Vit. E alone and its combination with GT, LYP, PGFE and LSFJ in ISO injected rats (Vit.E+ISO, Vit.E+GT+ISO, Vit.E+LYP+ISO, Vit.E+PGFE+ISO or Vit.E+LSFJ+ISO) showed near normal architecture of membrane and maintenance of membrane bound glycoconjugates compared to Vit.E alone treated groups. The observed effects of these combinations may be due to their membrane stabilizing effects through the termination of lipid peroxidation chain reaction.
- In the present study ISO injected rats shows increased degradation of collagen fibers which was visualized by Masson's trichrome staining.

Treatment of Vit.E alone and its combination with GT, LYP or PGFE in ISO injected rats (Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) showed significant reduction of collagen degradation as compared to Vit.E treatment groups. LSFJ fails to produce protective effects on collagen degradation.

- Area of infarction is suggestive of loss of membrane integrity which might be due significant leakage of LDH enzymes. Present study shows significant increase in % infarction in ISO injected rats. Treatment with Vit.E alone and in combination with GT, LYP or PGFE in ISO injected rats (Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) significantly decreased infarction size suggesting potent antioxidant activity of the combinations which prevents leakage of LDH enzymes and elevated nitrosative stress. Combination of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) slightly reduced the infarction size but was found to be non-significant compared to Vit.E alone treated group (Vit.E+ISO).
- Extensive amount of DNA damage and increased Caspase-3 activity was observed in ISO injected rats. These results suggest the involvement of apoptosis and necrosis in ISO injected rats. Co-administration of Vit.E and GT or LYP in ISO injected rats (Vit.E+GT+ISO or Vit.E+LYP+ISO) significantly reduced the caspase-3 activity and DNA damage as compared to groups treated with Vit.E alone. This effect suggested the protective effects of these combinations against apoptosis and necrosis induced by ISO.
- Treatment with PGFE in ISO injected rats (PGFE+ISO) shows significant effect on DNA damage but does not produce effects on caspase-3 activity. Co-administration of Vit.E and PGFE in ISO injected rats (Vit.E+PGFE+ISO) shows better effects rather than Vit.E alone in protecting ISO induced necrosis but not apoptosis.

- Co-administration of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not show preventive effect on caspase-3 activity and DNA damage compared to Vit.E alone treated group (Vit.E+ISO). This indicates that addition of LSFJ along with Vit.E did not produce beneficial effect on apoptosis and/or necrosis induced by ISO.
- *In vitro* study shows good antioxidant activities of Vit.E, GT, LYP and PGFE in different models viz. DPPH radical scavenging assay, superoxide radical scavenging assay, nitric oxide radical scavenging assay and iron induced lipid peroxidation. LSFJ did not show antioxidant activity against nitric oxide radical scavenging assay and iron induced lipid peroxidation.
- Addition of these antioxidants like GT, LYP and PGFE along with Vit.E shows beneficial effect rather than Vit.E alone in protecting ISO injected myocardial infarction and associated oxidative stress. This beneficial effect might be due to regeneration of vitamin E or the different degree of antioxidant activity of these drugs.

In conclusion, myocardial necrosis and/or apoptosis resulted in substantial alterations in the biochemical variables and marker enzymes with a concomitant increase in oxidative stress and cell damage in the heart tissue.

Co-administration of Vit.E and GT or LYP proved to be more effective than Vitamin E alone in mitigating the oxidative damage induced by ISO, thus suggesting that the combinations act as a potent cardioprotective agent by reducing the oxidative stress and thereby decreasing hemodynamic changes, inflammatory markers, nitrosative stress, apoptosis and necrosis.

Addition of PGFE along with Vit.E shows beneficial cardioprotective effects rather than Vit.E alone by decreasing oxidative stress, inflammatory markers and necrosis but not apoptosis.

LSFJ treatment exhibited mild antioxidant and lipid lowering activity. Co-administration of Vit.E and LSFJ did not produce beneficial cardioprotective effects compared to Vit.E alone. Although LSFJ is traditionally claimed for its cardio-tonic properties, the present study observed little effect. It is also not possible to deny the traditional claim of LSFJ unless it is studied for longer period and/or at higher doses.

Finally, this study will provide new therapeutic implications in the treatment of heart diseases characterized by apoptotic and necrotic cell death.