Results and Discussion
Healthy human life is always cardinal for human being starting from his birth to the end of life. The numbers of diseases, minor to major, play a key role in disturbing the healthy human life. Along with the modernization as well as sophistication in the life, human health directly or indirectly faces challenge from several diseases resulting sometime in survival and sometimes in surrender to diseases. CVD remain the principal cause of death in both developed and developing countries. It may present as a typical heart attack, as sudden death, or it may be detected at an advanced stage and be described as a silent infarct. The contributing factors for the growing burden of CVDs are increase in prevalence of cardiovascular risk factors, especially hypertension, dyslipidemia, diabetes, overweight or obesity, physical inactivity and the use of tobacco.

The synthetic drugs that constitute the current pharmacological armamentarium are themselves effective in managing the condition but not without setbacks. These hunches have accelerated the need for natural products, which may be used as dietary supplement to prevent the development of an acute myocardial infarction. Natural products, which include crude herbals, extracts, herbomineral formulations, polyherbal formulations, etc., have been used for the treatment of ischemic heart diseases in traditional systems of medicine. Besides, these natural products may also augment the conventional treatment and offer better management of the condition with fewer side effects.

**Qualitative fingerprint analysis of TpFE by HPLC and MS**

The HPLC chromatogram of standard GA (Fig. 1) resolved a peak at retention time 6.31333 min, Area- 37277, Height- 3614.13. The qualitative fingerprint
chromatogram of TpFE is showed in Fig. 2. A sharp peak resolved and eluted at retention time 6.31333 min, Area- 3884.14, Height- 643.23.

The spectrum in Fig. 3 shows that in the negative ion mode, under the employed screening conditions, GA is identifiable at m/z 169.01695. The spectrum of TpFE depicted in Fig. 4 indicates the presence ion at m/z 169.01450, corresponds to GA. The comparative standard-extract analysis demonstrates the presence of GA in the investigated extract.

Fig. 1. HPLC of gallic acid
Fig. 2. HPLC of Terminalia pallida fruits ethanolic extract

Fig. 3. MS of gallic acid
HPLC-MS represents the combination of a high-resolution separation system with a powerful detection/characterization technique, which has been successfully applied to the determination of compounds present in material from a variety of natural product source. A specific application of HPLC-MS natural product mixture analysis is the procedure known as dereplication. This process is rapid, precise and efficient besides has become one of the key processes for maintaining samples from natural source (Strege, 1999). In order to verify the correct identification of the phenolic compound in the ethanolic extract of *T. pallida*, gallic acid standard was used. Standard solution of gallic acid was chromatographed to determine its retention time and MS data for comparison with the chromatogram of the plant extract. Thus, the standardization of ethanolic extract from *T. pallida* could be determined without the need of isolation and purification processes of compounds through classical phytochemistry, which involves methods that are costly and time-consuming.

**Effect of TpFE and MA on body weight of rats:**
Fig. 5 depicts the effect of TpFE on body weight in normal control and ISO-treated rats. A significant (P<0.05) decrease in body weight was observed in ISO-treated rats by 21.7% when compared to control rats. Pretreatment of ISO administered rats with TpFE (300, and 500 mg kg⁻¹) and GA (15 mg kg⁻¹) increased the body weight significantly (P<0.05) by 13.7, 26.5 and 19.8% respectively as compared to ISO-alone treated rats. TpFE in 100 mg kg⁻¹ did not show significant effect on body weight of rats.

Fig. 6 shows the effect of MA on body weight in normal control and ISO-treated rats. Body weight at the end of experimental period in ISO treated group was found to be significantly (P<0.05) decreased by 16.8% as compared to control group. Treatment with MA (15 mg kg⁻¹) and GA (15 mg kg⁻¹) for 7 days significantly (P<0.05) increased the body weight by 18.7 and 10.5% respectively as compared to ISO-treated group. MA (15 mg kg⁻¹) treatment alone did not showed any significant (P<0.05) effect on body weight of rats.

In the present study, there was a significant decrease in the body weight of ISO alone treated rats. Myocardial infarction induced by ISO has been reported to show many metabolic and morphologic aberrations. Several early events, such as ultrastructural changes, histological, biochemical, electrolyte and membrane changes, have been shown to occur within 48 hours after the injection of ISO. Glycogen depletion and fat deposition have been
Histological changes induced by excessive amounts of ISO include degeneration and necrosis of myocardial fibres, accumulation of inflammatory cells, interstitial edema, lipid droplets and endocardial hemorrhage (Lehr, 1972). All these changes may disturb the dietary habits of animal. Loss of body weight could be due to reduced food and water intake.
Effect of TpFE and MA on heart weight of rats:

Data in Fig. 7 represents the effect of TpFE on heart weight in normal control and ISO-administered rats. Rats administered with ISO exhibited significant (p<0.05) increase in heart weight by 44.1% when compared to control rats. Oral pretreatment with TpFE (300, and 500 mg kg⁻¹) and GA (15 mg kg⁻¹) to ISO-induced myocardial infarcted rats decreased the heart weight significant (p<0.05) by 13.7, 26.5 and 19.8% respectively when compared to ISO alone administered rats.

ISO-treated rats showed a significant (p<0.05) increase in heart weight by 37.7% when compared to normal control rats (Fig.8). Pretreatment with MA (15 mg kg⁻¹) and GA (15 mg kg⁻¹) to ISO-treated rats for a period of 7 days decreased significantly (p<0.05) the weight of heart by 26.6 and 22.8% respectively as compared to ISO-alone administered rats.

In the present study, there was a considerable increase in the heart weight of ISO administered rats. Increase in the heart weight might be due to the increased water content, edematous intramuscular space, and extensive necrosis of cardiac muscle fibers followed by invasion of the damaged tissues by inflammatory cells (Nirmala and Puvanakrishnan, 1994). TpFE and MA pretreatment to ISO group significantly reduced the heart weight which may be due to the anti-inflammatory activity. The active constituents of T. pallida fruits like MA (Aladedunye et al, 2008), GA (kroes et al, 1992), OA (Singh et al, 1992) and EA (Stephanie Corbett et al, 2010) have proved for their anti-inflammatory activity.
Effect of TpFE and MA on Cardiac marker enzymes:

The cardio-toxicity in male albino Wistar rats induced by ISO administration in the present study was evaluated primarily, by measuring biochemical
marker enzymes. Figs. 9-11 show the effect of TpFE on the activities of CK-MB, CK, 
LDH, ALT and AST in myocardium of control and ISO-treated rats. ISO 
administration showed significant (p < 0.05) decrease in the activities of CK-MB, CK, 
LDH, ALT and AST by 38.4, 42.2, 43.5, 44.8, 41.8% respectively in the heart. 
Pretreatment with TpFE (100, 300, and 500 mg kg⁻¹) dose-dependently increased the 
activities of these enzymes. TpFE in 500 mg kg⁻¹ significantly (p < 0.05) increased the 
activities of CK-MB, CK, LDH, ALT and AST by 51.3, 67.4, 74.0, 66.6, and 58.6% 
respectively and TpFE in 300 mg kg⁻¹ also significantly (p < 0.05) increased the 
activities of these marker enzymes by 23.4, 29.3, 41.4, 22.3, and 30% respectively. 
TpFE in 100 mg kg⁻¹ did not significantly increase the levels of these enzymes. GA in 
15 mg kg⁻¹ significantly (p < 0.05) increased the activities of these marker enzymes by 
39.6, 52.6, 58.1, 40 and 46.6% respectively as compared to ISO-induced myocardial 
infarcted rats. Oral treatment of TpFE (500 mg kg⁻¹) alone did not show significant 
effect on cardiac marker enzymes.

The levels of cardiac marker enzymes such as CK-MB, CK, LDH, ALT, AST, 
and ALP increased significantly by 103.6, 72.5, 110.0, 90.9, 97.6, and 108.3% 
respectively in serum of ISO administrated rats when compared with the control rats 
(Fig. 12 and 13). Upon pretreatment of rats with TpFE (100, 300, and 500 mg kg⁻¹) 
and GA (15 mg kg⁻¹) the condition was reversed where the levels of these cardiac 
marker enzymes decreased significantly in the serum of ISO-induced myocardial 
infarcted rats when compared with untreated ISO-induced myocardial infarcted rats. 
TpFE in 500 mg kg⁻¹ bw significantly (p < 0.05) decreased the activities of enzymes 
by 43.4, 31.5, 47.9, 39.7, 45.9, and 45% respectively, and also TpFE in 300 mg kg⁻¹ 
significantly (p < 0.05) decreased the activities of enzymes by 26.8, 18.8, 25.2, 24.1, 
27.9, and 33% respectively. TpFE in 100 mg kg⁻¹ significantly (p < 0.05) decrease the 
activities of CK-MB, LDH, ALT, AST and ALP by 7.0, 10.2, 10.9, 7.3 and 11.0% 
when compared to ISO administered rats. GA in 15 mg kg⁻¹ significantly (p < 0.05) 
decreased the activities of enzymes by 30.2, 25.0, 38.9, 30.4, 34.7 and 36.2% 
respectively whereas TpFE in 100 mg kg⁻¹ did not significantly (p < 0.05) decreased
the activities of these marker enzymes. TpFE (500 mg kg\(^{-1}\)) alone did not show any significant (p < 0.05) effect on either of these enzymes.

Fig.9. Effect of TpFE on heart tissue CK-MB
Fig. 10. Effect of TpFE on heart tissue CK, LDH

Fig. 11. Effect of TpFE on heart tissue ALT, AST
Fig. 12. Effect of TpFE on Serum CK, CK-MB, LDH

Fig. 13. Effect of TpFE on Serum AST, ALT, ALP
Figs. 14-17 showed significant ($p<0.05$) decrease in the activities of cardiac marker enzymes such as CK-MB, CK, LDH, ALT, AST, ALP and GGT (46.8, 51.7, 36.3, 36.7, 69.5, 43.9 and 66.1% respectively) in ISO group when compared to those of control group. Pretreatment of MA (15 mg kg$^{-1}$) to ISO administered rats significantly ($p<0.05$) increased the above mentioned enzyme activities by 66, 94.8, 49.1, 49.7, 165.6, 62.8 and 154.5% respectively when compared to ISO alone administered rats. GA (15 mg kg$^{-1}$) also significantly ($p<0.05$) increased the activity of these enzymes (50.7, 61.1, 37.8, 29.1, 134.3, 46.1 and 118.1 respectively). There is no significant change with treatment of MA (15 mg kg$^{-1}$) alone.

Data in Table 1, figs. 18 and 19 depict the effect of MA on the activities of serum marker enzymes like CK-MB, CK, LDH, ALT, AST, ALP and GGT. In ISO administered rats, a significant ($P<0.05$) elevation in the activities of these marker enzymes was observed by 90.8, 117.2, 100.5, 63.2, 102.5, 69.7 and 42.8% respectively, when compared to control. The activities were significantly ($P<0.05$) cutback by 40.2, 41.6, 45.3, 25.7, 39.7, 35.6 and 21.8% in MA pretreated group, and 36.0, 35.3, 41.0, 20.3, 27.3, 26.9 and 18.1% respectively in GA pretreated group for CK-MB, CK, LDH, ALT, AST, ALP and GGT respectively when compared to ISO administered group. Our results are consistent with a previous report of Hansi and Stanely (2009).
Fig. 14. Effect of MA on heart tissue CK, CK-MB

Fig. 15. Effect of MA on heart tissue LDH
Fig. 16. Effect of MA on heart tissue AST, GGT

Fig. 17. Effect of MA on heart tissue ALT
Table 1: Effect of mas linolic acid (MA) on cardiac marker enzymes: creatine kinase (CK), alanine transaminase (ALT), aspartate transaminase (AST) and gamma glutamyl transferase (GUT) in the serum of normal control and isoproterenol (ISO)-induced myocardial infarcted rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>CK (U/L)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>GUT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>512.1 ± 21.9*</td>
<td>25.0 ± 1.1*</td>
<td>164.0 ± 8.8*</td>
<td>7.7 ± 0.4*</td>
</tr>
<tr>
<td>MA (15mg/kg)</td>
<td>510.4 ± 25.8*</td>
<td>24.3 ± 1.9*</td>
<td>163.4 ± 8.1*</td>
<td>7.6 ± 0.3*</td>
</tr>
<tr>
<td>ISO (85mg/kg)</td>
<td>1112.3 ± 11.7b</td>
<td>40.8 ± 2.8b</td>
<td>332.1 ± 7.7b</td>
<td>11.0 ± 0.7b</td>
</tr>
<tr>
<td>MA (15mg/kg) + ISO</td>
<td>648.7 ± 37.0c</td>
<td>30.3 ± 1.6c</td>
<td>241.2 ± 3.1c</td>
<td>8.6 ± 0.6c</td>
</tr>
<tr>
<td>GA (15mg/kg) + ISO</td>
<td>719.3 ± 20.6d</td>
<td>32.5 ± 1.0d</td>
<td>245.3 ± 1.6d</td>
<td>9.0 ± 0.1d</td>
</tr>
</tbody>
</table>

Values are mean ± SD; (p<0.05). Values that do not share a common superscript (a, b, c, and d) differ significantly from each other (p<0.05, Duncan's multiple range test).

Fig. 18. Effect of MA on serum CK-MB, ALP
Fig. 19. Effect of MA on serum LDH

The serum enzymes namely LDH, AST, ALT and ALP also serve as sensitive indices to assess the severity of myocardial infarction (Sheela-Sasikumar and Shyamala-Devi, 2000) along with the golden markers CK-MB and CK. In the present investigation, we observed a decrease in the activities of myocyte injury markers like CK-MB, CK, LDH, ALT, AST, ALP and GGT in hearts of ISO administered rats. When myocardial cells are damaged due to deficient oxygen supply or glucose, the integrity of cell membrane gets disturbed and it might become more porous that results in the leakage of these enzymes (Arya et al., 2006). As a result of necrosis and leakage, the levels of diagnostic indicators of MI will increase in serum. Oral pretreatment of TpFE and MA restored the activities of myocardial marker enzymes. This could be due to protective effect of TpFE and MA on the myocardium, reducing the myocardial damage and thereby restricting the leakage of CK-MB, CK, LDH, ALT, AST, ALP and GGT. Similar observation was reported by Kubavat and Asdaq (2009).
TpFE pretreatment for 30 days decreased the activities of marker enzymes in the serum and increased the activities of marker enzymes in heart tissue of ISO administered rats which could be due to the free radical scavenging property of the extract due to the presence of phytochemicals such as flavonoids, saponins, anthraquinones, alkaloids, phenolic acids, tannins, steroids, anthocyanidins and terpenoids (Kameswara rao et al, 2003, Malaya Gupta et al, 2002). T. pallida fruit consists of active principles like OA, MA, EA and GA (Gunasekar et al., 1993) have been proved for their cardioprotective activity (Senthil et al, 2007, Hansi and Stanely, 2009). MA pretreatment for 7 days significantly normalized the levels of all marker enzymes in serum and heart tissue which may be due to protective antioxidant effect of MA on myocardium (Montilla et al, 2003). MA had reduced the extent of cardiac damage induced by ISO and thereby restricting the leakage of these enzymes from myocardium.

**Effect of TpFE and MA on Lipid peroxidation:**

Figs. 20 and 21 show the effect of TpFE on the levels of myocardial LPO marker, MDA in heart and serum of control and ISO-administered rats. Cardiotoxicity-induced with ISO exhibited a significant (p < 0.05) increase by 157.1 and 90% in the level of MDA in both heart tissue and serum respectively as compared to control rats. Although TpFE in doses 100, 300 and 500 mg kg⁻¹ and GA in dose 15 mg kg⁻¹ counteracted the deleterious effect of ISO by decreasing the MDA levels significantly (p < 0.05) by 19.4, 48.6, 56.9 and 52.7% respectively in heart tissue, whereas by 8.7, 19.2, 42.1 and 28% respectively in serum as compared to ISO group. Treatment of TpFE (500 mg kg⁻¹) alone did not show significant effect on the above parameter.

Figs. 22 and 23 summarize the effect of MA on LPO in both heart and serum. The content of MDA, in heart and serum was significantly (p<0.05) elevated by 200 and 100% respectively in the ISO-administered group when compared to normal control group. Animals treated with MA (15 mg kg⁻¹) and GA (15 mg kg⁻¹), followed by ISO injection showed significant (p<0.05) decrease in MDA levels by 58.3 and
41.6% respectively in heart tissue, whereas by 50 and 25% respectively in serum when compared with ISO-administered rats. The results are in accordance with previous reports (Sameer Goyal et al., 2010).

Fig.20. Effect of TpFE on heart tissue LPO

Fig.21. Effect of TpFE on serum LPO
Fig. 22. Effect of MA on heart tissue LPO

Fig. 23. Effect of MA on serum LPO
ROS are highly toxic byproducts of aerobic metabolism. They react unfavorably with surrounding macromolecules resulting in severe cell and tissue damage (Halliwell and Chirico, 1993). The process of LPO is one of oxidative conversion of PUFAs to products known as MDA, which is usually measured as TBARS or lipid peroxides, which is the most studied, biologically relevant, free radical reaction (Bakan et al. 2002). LPO has been implicated in the pathogenesis of a number of diseases include atherosclerosis, cancer etc., It is now generally accepted LPO and its product play an important role in liver, kidney, heart and brain toxicity (Lakshmi et al., 2005). LPO in vivo has been identified as one of the basic deteriorative reactions in cellular mechanisms of myocardial ischemia (Handforth, 1962). LPO is an important pathogenic event in myocardial necrosis and accumulation of lipid hydroperoxides reflects damage of the cardiac constituents (Hamberg et al., 1974; Gutteridge, 1982). The increased levels of lipid peroxides in ISO-induced myocardial necrosis might be due to free radical-mediated membrane damage (Zhou et al., 2006; Karthikeyan et al., 2007).

Significant increase is observed in the level of lipid peroxides in serum and heart tissue of ISO treated group which is an indication of oxidative stress. The present results showed that the level of lipid peroxides, measure in term of MDA was significantly increased in serum and heart of ISO-treated group which is an indication of oxidative stress. Pretreatment of TpFE and MA to ISO-administered rats reduced MDA level, clearly showing that the extract and the active compound inhibited the LPO. The MDA levels decreased by TpFE may be due to its phytochemical GA which showed cardioprotective effect in ISO induced myocardial infracted rat by inhibiting LPO (Hansi Priscilla, 2008). MA in this study showed an excellent potential in the inhibition of LPO and this results are supported by Montella et al, 2003. The decreased level of MDA in heart tissue and serum might be also due to the enhanced activities of antioxidant enzymes (SOD, CAT, GPx, GST and GSH). This indicates that the TpFE and MA have the cardioprotective effect by ameliorating tissue oxidative stress.
Effect of TpFE and MA on Lipid Profile:

Fig. 24 depicts the effect of TpFE on myocardial lipids such as TC, TG, LDL-C, VLDL-C, and HDL-C in control and ISO-administered rats. ISO group showed a significant increase (p < 0.05) by 93.0, 57.8, 264.7, 60.2% in the concentrations of TC, TG, LDL-C, and VLDL-C respectively and a significant decrease (p < 0.05) by 50.0% in the concentration of HDL-C in heart when compared to control rats. Pretreatment with TpFE (100, 300, and 500 mg kg⁻¹) dose-dependently decreased the concentrations of TC, TG, LDL-C, and VLDL-C and increased the concentration of HDL-C. TpFE in 500 mg kg⁻¹ and 300 mg kg⁻¹ significantly (p < 0.05) decreased the concentrations of TC, TG, LDL-C, VLDL-C by 49.9, 31.1, 70.9, 29.0% and 19.2, 6.5, 30.6, 5.9% respectively, whereas the concentration of HDL-C significantly (p < 0.05) increased by 100.0 and 44.4% respectively as compared to ISO-alone administered rats. GA (15 mg kg⁻¹) also significantly (p < 0.05) decreased the concentrations of TC, TG, LDL-C, and VLDL-C and increased the concentration of HDL-C. Treatment of TpFE (500 mg kg⁻¹) alone did not show significant changes in lipid profile except HDL-C which is significantly increased as compared to control rats.

Fig. 25 showed the effect of TpFE on serum lipid profile in control and ISO administered groups. Rats administered with ISO showed a significant (p < 0.05) increase in the levels of serum TC, TG, LDL-C and VLDL-C by 61.6, 82.7, 126.2 and 82.7% respectively, except HDL-C which showed a significant (p < 0.05) decrease by 41.6% when compared to control rats. Pretreatment with TpFE (100, 300, and 500 mg kg⁻¹) significantly (p < 0.05) decreased serum TC, TG, LDL-C, VLDL-C levels (TpFE 100 mg kg⁻¹ by 7.9, 8.0, 11.7, and 8.1% respectively, TpFE 300 mg kg⁻¹ by 24.0, 23.8, 34.2 and 23.8% respectively and TpFE 500 mg kg⁻¹ by 35.3, 35.7, 51.9 and 35.8% respectively) and significantly (p < 0.05) increased serum HDL-C levels (by 14.9, 35.4 and 61.4% respectively) when compared to ISO administered rats. GA (15 mg kg⁻¹) also showed a significant (p < 0.05) decrease in all serum lipids (27.5, 28.8, 42.5 and
28.9% respectively) with subsequent increase in the levels of HDL-C (49.6%) than the ISO administered rats.

Fig.26 and table 2 represents the effect of MA on heart and serum lipid profile in normal control and ISO-treated rats. ISO administered rats showed a significant increase (P<0.05) in the concentrations of heart TC, TG, LDL-C, and VLDL-C (88.8, 69.7, 260.0, 75.0% respectively) and a significant increase (P<0.05) in the concentrations of serum TC, TG, LDL-C, and VLDL-C (42.3, 94.9, 137.2 and 95.0% respectively), whereas a significant decrease (p<0.05) in the concentrations of heart and serum HDL-C (40.0 and 44.3% respectively) when compared with those of normal rats. Pretreatment with MA (15 mg kg⁻¹) to ISO-treated rats decreased significantly (P<0.05) the levels of heart TC, TG, LDL-C, and VLDL-C (38.2, 31.5, 59.7 and 28.5% respectively) and serum TC, TG, LDL-C, and VLDL-C (18.6, 42.6, 31.1 and 42.8% respectively), whereas increased the levels of HDL-C in heart (53.3%) and serum (51.7%) of ISO-induced myocardial infarcted rats when compared with untreated ISO-induced myocardial infarcted rats. GA (15 mg kg⁻¹) also significantly (p< 0.05) decreased the levels of heart TC, TG, LDL-C, and VLDL-C (31.3, 19.1, 51.3 and 21.4% respectively) and serum TC, TG, LDL-C, and VLDL-C (16.9, 32.2, 30.0 and 32.3%) whereas significantly (p<0.05) increased the levels of heart HDL-C (46.6%) and serum HDL-C (42.4%). Treatment with MA (15 mg kg⁻¹) alone did not show significant changes in lipid profile when compared to normal control rats.
Fig. 24. Effect of TpFE on heart tissue lipid profile

Fig. 25. Effect of TpFE on serum lipid profile
Table 2. Effect of maslinic acid (MA) on the levels of total cholesterol (TC), triglycerides (TG), very low density lipoprotein cholesterol (VLDL-C), low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) in the serum of normal control and isoproterenol (ISO)-induced myocardial infarcted rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>64.7 ± 5.3*</td>
<td>61.2 ± 1.6*</td>
<td>12.2 ± 0.3*</td>
<td>21.5 ± 5.5*</td>
<td>30.9 ± 3.8*</td>
</tr>
<tr>
<td>MA (15mg/kg)</td>
<td>63.0 ± 1.7*</td>
<td>60.0 ± 2.4*</td>
<td>12.0 ± 0.4*</td>
<td>19.8 ± 1.5*</td>
<td>31.5 ± 2.1*</td>
</tr>
<tr>
<td>ISO (85mg/kg)</td>
<td>92.1 ± 2.2b</td>
<td>119.3 ± 0.7b</td>
<td>23.8 ± 0.1b</td>
<td>51.0 ± 2.5b</td>
<td>17.2 ± 1.0b</td>
</tr>
<tr>
<td>MA (15mg/kg) + ISO</td>
<td>74.9 ± 3.9c</td>
<td>68.4 ± 3.0c</td>
<td>13.6 ± 0.6c</td>
<td>35.1 ± 3.4c</td>
<td>26.1 ± 2.1c</td>
</tr>
<tr>
<td>GA (15mg/kg) + ISO</td>
<td>76.5 ± 1.2c</td>
<td>80.8 ± 2.7d</td>
<td>16.1 ± 0.5d</td>
<td>35.7 ± 1.9c</td>
<td>24.3 ± 1.8c</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. (n=6 rats). Values that do not share a common superscript (a, b, c and d) differ significantly from each other (p<0.05, Duncan's multiple comparison).

Fig. 26. Effect of MA on heart tissue lipid profile
Lipid metabolism plays an important role in myocardial necrosis produced by ischemia (Mathew et al., 1981). The increased concentration of cholesterol could be due to a decrease in HDL-C, since HDL-C is known to be involved in the transport of cholesterol from tissues to the liver for its catabolism (Mathew et al, 1981). Lipoproteins are independent risk factor for CVD. LPO plays an important role in lipoprotein modification, which makes them susceptible to atherosclerosis, which could be the reason for acute MI mediated cardiotoxicity by ISO (Zern et al, 2003). ISO induced myocardial necrosis had been shown to elevate plasma TC, TG, LDL-C, VLDL-C and decrease HDL-C levels (Prince et al., 2008). Strong evidence suggests that hypercholesterolemia induces oxidative stress by causing a reduction in the enzymatic antioxidant defense potential of tissues and generation of oxygen free radicals like superoxide anions. As a result of these metabolic events peroxidation reactions are accelerated leading to cellular injury (Gokkusu et al, 2003). The observed increase in TGs might be due to a decrease in the activity of lipoprotein lipase, resulting in decreased uptake of TGs from the circulation (Sushamakumari et al, 1990). Anandan et al. (2007) have reported that the increase in the myocardial cholesterol content in ISO treated rats is due to increased uptake of LDL-C from the blood by myocardial membranes. These changes in lipid levels might be due to enhanced lipid biosynthesis by cardiac cyclic adenosine monophosphate. Also higher level of LDL-C has a positive correlation, whereas high level of HDL-C has a negative correlation with myocardial necrosis (Buring et al., 1992). ISO causes hyperlipidemia and it increases the LDL-C in the blood, which in turn leads to the build up of harmful deposits in the arteries thus favoring CHD (Rajadurai and Stanely Mainzen Prince, 2006). VLDL contains high concentration of TGs and moderate concentration of cholesterol, PLs and apolipoproteins. The breakdown of VLDL leads to the formation of the cholesterol rich particle, LDL (Draznin et al, 1993). Oxidation of lipoproteins is a characteristic event in the oxidative stress caused by the oxidative damage to PUFAs (Nirmala C and Puvanakrihnan, 1996). In the subendothelial space, LDL is converted to oxidized forms through contact with macrophages and endothelial and smooth muscle cells. Oxidatively modified LDL expresses chemotactic and adhesion
molecules on the surface of endothelial cells. Macrophages in the subendothelial cells intake modified LDL via scavenger receptors, leading to the formation of lipid laden foam cells, the hallmark of early atherosclerotic lesions (Catanzaro and Suen, 1996). HDL is involved in the transport of cholesterol from peripheral tissues to the liver and it acts as a protective factor against CVD (Korytar et al, 2002). It also participates in the regulation of TG catabolism and cholesterol ester synthesis by providing apo C-II for activation and apo-C-III for inhibition of lipoprotein lipase activity (Catanzaro and Suen, 1996). Alterations in lipid metabolism directly reflect the composition of lipoproteins in ISO treated rat.

We observed increased levels of the serum and the heart TC, TG, LDL-C, VLDL-C and decreased levels of HDL-C in the ISO treated rats. HDL-C and LDL-C are significant variables for CHD. Rats pretreated with TpFE and MA showed deceased concentration of heart and serum TC, TG, LDL-C, VLDL-C and increased concentration of HDL-C indicates the beneficial effects of TpFE and MA in reducing hyperlipidemia caused by ISO administration. Increased levels of serum HDL-C observed in rats pretreated with TpFE and MA, facilitates the transport of cholesterol from peripheral tissues to the liver for catabolism and excretion from the body in ISO treated rats. The active compounds GA showed antilipidemic activity in diabetic rats (Punithavathi et al, 2011) and OA showed hypolipidemic activity in alloxan-induced diabetic rats. These compounds may responsbible for the lipid lowering effect of TpFE. MA also significantly lowered the lipid levels which may be due to its anti-hyperlipidemic activity (Jun Lui, 2007).

**Effect of TpFE and MA on serum Electrolytes:**

Data in figs.27 and 28 represent the effect of TpFE on the serum electrolytes such as sodium, potassium and calcium. Serum of ISO-induced myocardial infringated rats showed significant (p < 0.05) increase by 71.6% in the levels of sodium, and significant (p < 0.05) decrease by 44.8 and 50.0% in the levels of potassium and calcium respectively. Pretreatment with TpFE (100, 300, and 500 mg kg$^{-1}$) and GA (15 mg kg$^{-1}$) significantly (p < 0.05) restored the altered levels of sodium by 7.2, 20.5,
33.9 and 25.0% respectively, potassium by 3.7, 14.8, 59.2 and 29.6% respectively, and calcium by 7.5, 42.5, 87.5 and 52.5% respectively in ISO-administered rats when compared to ISO alone administered rats. TpFE in 100 mg kg⁻¹ increased the levels of potassium but not significantly (p < 0.05). There is no significant effect on the levels of electrolytes with the treatment of TpFE in 500 mg kg⁻¹ alone.

Figs.29 and 30 explain the effect of MA on the levels of serum sodium, potassium and calcium in control and experimental rats. ISO administered rats showed a significant (p<0.05) increase in serum levels of sodium (47.1%), and significant (p<0.05) decrease in potassium (38.3%) and calcium (54.7%) when compared to control rats. The rats pretreated with MA (15 mg kg⁻¹) showed a significant (p<0.05) decrease (28.0%) in serum sodium, and a significant increase (51.3 and 97.6%) in potassium and calcium levels when compared to ISO administered rats. GA (15 mg kg⁻¹) pretreatment also showed a significant (p<0.05) decrease (22.1%) in serum sodium, and a significant increase (35.1 and 76.7%) in potassium and calcium levels when compared to ISO administered rats.
Fig. 28: Effect of TpFE on serum calcium, potassium ions

Fig. 29: Effect of MA on serum sodium ions
In the present study an attempt was made to understand the influence of ISO on electrolytes and the effect of TpFE and MA on myocardial infarcted rats. The ions Na$^+$ and K$^+$ play an important role in cardiovascular activity (Nurminen et al., 1998). Na$^+$ together with K$^+$ assists in the maintenance of the body's electrolyte and water balance (Nguyen and Kurtz, 2004). In addition, Na$^+$ and K$^+$ play an important role in the transport of substances across membranes (Marsano and McClain, 1989). Na$^+$ is associated with blood pressure and a reduction in Na$^+$ intake lowers blood pressure in many hypertensive patients. On the other hand, K$^+$, which is present in the intracellular fluid, has been reported to be among the protective electrolytes against hypertension (Nurminen et al., 1998). The Na$^+$-K$^+$ ATPase pump preserves a high intracellular K$^+$ concentration despite an adverse concentration gradient. It is stimulated by catecholamines, insulin, aldosterone and hyperkalemia (Clausen and...
The reduced $K^+$ level and increased $Na^+$ level in serum of ISO administered rats could be due to reduced $Na^+-K^+$ ATPase activity. Kidneys are the major regulators of external $K^+$ homeostasis accounting for approximately 80% of $K^+$ transit from the body. Renal dysfunction can result in gross abnormalities in serum $K^+$ levels (Jay et al., 2000).

Serum $Ca^{2+}$ may be an independent risk factor for MI in middle-aged. It has been reported that the severity of coronary atherosclerosis is closely related to coronary artery calcification which itself may correlate with serum $Ca^{2+}$ and phosphorus concentrations (Rasouli and Mohseni, 2006). The observed decreased level of serum $Ca^{2+}$ in ISO administered rats might be due to pronounced positive inotropic effects mediated by an increased transmembrane $Ca^{2+}$ influx to the myocardial cells (Fuji and Kondo, 1981). The increase in intracellular $Ca^{2+}$ concentration in ISO administration is dependent on extracellular $Ca^{2+}$ concentration (Takuwa et al., 1988). Extracellular $Ca^{2+}$ is required for maintenance of normal structure and functional integrity of myocardial cell membrane and muscle contraction (Crevey et al., 1978). The positive inotropic effects of ISO are mediated by cAMP, when ISO binds to $\beta$-adrenergic receptor on myocyte causes activation of adenylate cyclase which converts ATP to cAMP. In turn, cAMP activates cAMP dependent protein kinase, causes phosphorylation of $Ca^{2+}$ channels of sarcolemma which could lead to enhanced $Ca^{2+}$ influx (Wetzel and Haul, 1988). The elevated $Ca^{2+}$ in cytosol triggers $Ca^{2+}$ induced $Ca^{2+}$ release from sacroplasmic reticulum which further increases $Ca^{2+}$ concentration in the cytosol. This $Ca^{2+}$ causes actin myosin interactions through $Ca^{2+}$ binding to troponin-C and brings about contraction (Huxely and Simmons, 1971; Prashant et al., 2008).

**Effect of MA on serum LCAT:**

Fig. 31 shows the effect of MA on serum LCAT in control and ISO administered rats. Serum LCAT levels decreased significantly ($p<0.05$) by 37% in ISO administered rats when compared with control rats, while MA (15 mg kg$^{-1}$) pretreatment and GA (15 mg kg$^{-1}$) pretreatment increased the levels of LCAT.
LCAT is an enzyme responsible for the conversion of cholesterol to cholesterol esters on the surface of high density lipoproteins (HDLs). The esterification of cholesterol by LCAT leads to the remodeling of the lipoprotein, HDL and results in the formation of large HDL particles that are known to offer protection against CVD. Decreased activity of LCAT inhibits the esterification of cholesterol in ISO treated rats. This leads to high concentration of lipids and lipoproteins in circulation, which are at high risk of atherosclerosis and MI. Increased oxidative stress resulted in the deficiency of LCAT in ISO treated rats. TpFE and MA pretreatment increased the activity of LCAT in ISO induced rats. TpFE and MA increased the activity of LCAT which increases the concentration of good cholesterol (HDL) in ISO treated rats. Thus, the observed increase in LCAT might be due to the blocking of LPO in TpFE and MA pretreated ISO induced rats.

Effect of TpFE and MA on serum NO:

![Graph showing the effect of TpFE and MA on serum NO](image-url)
Fig. 32 illustrates the effect of TpFE on the levels of serum NO in control and ISO administered rats. The levels of serum NO were elevated significantly ($p<0.05$) by 68.0% in ISO administered rats when compared with control rats. The values were restored significantly ($p<0.05$) in TpFE (100, 300, and 500 mg kg$^{-1}$ by 5.0, 16.1, 29.5% respectively) pretreated and GA (15 mg kg$^{-1}$ by 23.7%) pretreated ISO administered rats.

![Graph showing the effect of TpFE on serum nitric oxide](image)

Fig. 32: Effect of TpFE on serum nitric oxide

Fig. 33 depicts the effect of MA on the levels of serum NO in control and experimental rats. The NO levels increased (70.0%) significantly ($p<0.05$) in ISO administered rats when compared to control rats. In ISO administered MA (15 mg kg$^{-1}$) pretreated and GA (15 mg kg$^{-1}$) pretreated rats the NO levels decreased by 33.5 and 24.5% respectively when compared to ISO alone administered rats.

Fig. 33: Effect of MA on serum nitric oxide
Nitric oxide is an important defense against injury from myocardial ischemia/reperfusion. Generation of nitric oxide from L-arginine by nitric oxide synthase requires molecular oxygen as an essential substrate. Under conditions of oxygen deprivation, as encountered during ischemia, oxygen levels are rapidly depleted so that nitric oxide synthase activity will be inhibited. Generation of nitric oxide from the reduction of nitrite under ischemic conditions may represent an important mechanism to maintain nitric oxide production for cell survival and function until aerobic conditions are restored, following reperfusion, so that nitric oxide synthase can resume production of nitric oxide (John et al., 2007).

NO is locally acting vasodilator that has a central role in the regulation of vascular smooth muscle tone. In addition, it inhibits leukocyte and platelet adhesion to the endothelium, leukocyte activation and platelet aggregation, and endothelial permeability [6,24]. It thus optimizes blood flow regulation in the microcirculation. In the heart, endothelium-derived NO modulates myocardial relaxation and diastolic function and the, and reduces oxygen consumption independent of effects on contractile function [26]. NO generated within cardiac myocytes by eNOS and
possibly also nNOS may modulate excitation-contraction coupling via effects on sarcolemmal Ca channels and sarcoplasmic reticular function [27,28]. It can also modulate heart rate and β-adrenergic inotropic responses [27]. NO has vascular and myocardial antiproliferative potential, and can act as a bifunctional regulator of cell apoptosis [27,28].

NO-triggered downstream signal transduction may be cGMP-dependent or -independent [6,24]. The latter usually involves direct reactions of NO with amino, thiol, or diazo groups in proteins, and with haem and Fe or sulphur centres. NO and superoxide molecules leads to the formation of peroxynitrite [29]. Low levels of peroxynitrite can be beneficial via stimulation of guanylyl cyclase, but higher levels generate highly reactive hydroxyl-like species that induce toxic effects [29].

A reduction in endothelial NO production or bioavailability contributes to 'endothelial dysfunction', which is a feature of many cardiovascular pathologies - e.g., hypertension, hypercholesterolaemia, atherosclerosis, diabetes, heart failure [30]. Endothelial dysfunction contributes to disease pathophysiology and, in at least some cases, may even have a primary pathogenetic role. In conditions such as ischaemia-reperfusion, the generation of peroxynitrite from reaction between superoxide and eNOS-derived NO may have damaging effects. The induction of iNOS in cardiovascular tissues has been suggested to be involved in the pathophysiology of several disorders.

Overproduction of NO in the blood vessel wall is associated with vasodilatation, resistance to constrictor stimuli, and endothelial cell damage or dysfunction. In the heart NO inhibits contraction in isolated cardiac myocytes, strips of papillary muscle, or intact perfused hearts. Many of the physiological effects of NO are mediated by interaction with the haem moiety of the enzyme guanylate cyclase and it is possible that toxic effects are due to interaction with haem or iron-sulphur centres of other enzymes. Nitric oxide inhibits mitochondrial respiration, damages DNA, and inhibits replication of cells including vascular smooth muscle cells. At higher concentrations NO reacts rapidly with oxygen and in the presence of 02- (superoxide anion, another product of the inflammatory response)
NO forms ONOO-(peroxynitrite). Depending on the local environment and thiol concentration, peroxynitrite might either revert to NO, cause damage by leading to nitrination, or lead to generation of other toxic radicals including OH- (hydroxyl radical). It remains to be determined why normal physiological production of NO is protective in the cardiovascular system and may prevent atheroma formation whereas excess NO produced after expression of iNOS and under conditions of inflammation, is potentially harmful.

**Effect of TpFE and MA on serum PON:**

Data presented in Fig.34 shows the effect of TpFE on the activity of serum PON in control and experimental rats. Significant (P<0.05) decrease by 39.3% in the levels of serum PON was observed in rats administered with ISO as compared to control rats. TpFE (100, 300, and 500 mg kg⁻¹) pretreatment for a period of 30 days significantly (P<0.05) increased (7.9, 23.4, and 52.5% respectively) the activity of serum PON and also GA (15 mg kg⁻¹) pretreatment significantly (P<0.05) increased the activity by 36.0% when compared to ISO alone administered rats.

![Fig.34: Effect of TpFE on serum PON](image)
Fig. 35 shows the effect of MA on the activity of PON in normal control and ISO-administered rats. Rats treated with ISO, showed a significant (P<0.05) decrease by 37.9% in the activity of serum PON when compared to normal control rats. Pretreatment with MA (15mg kg\(^{-1}\)) and GA (15mg kg\(^{-1}\)) significantly (p<0.05) increased (57.3 and 43.1% respectively) the activity of PON in ISO-induced myocardial infarcted rats. Treatment with MA (15mg kg\(^{-1}\)) alone increased the activity of PON significantly (P<0.05) by 15.8% when compared to normal control rats.

Human and animal studies strongly support the hypothesis that oxidative modification of LDL plays a crucial role in the pathogenesis of atherosclerosis. (Berliner et al 1996 Ref.Atheroscnti) 20 Therefore, mechanisms preventing LDL oxidation appear to be antiatherogenic. HDL-associated PON1 may be, in this respect, a major defense against lipid peroxides from oxidized LDL, and the property of HDL to attenuate the oxidation of LDL seems to be largely attributable to PON1.
Patients who sustained MI did not on average have markedly decreased HDL concentrations, but PON1 activity and concentration were profoundly decreased. Under most circumstances, including in the acute-MI survivors studied, the serum PON activity is therefore likely to depend on the number of PON1 molecules in HDL rather than the serum HDL concentration. The greatly increased ratio of apo AI to PON1 mass found in the MI patients would tend to support this argument.

Serum PON1 prevents oxidative modification of the LDL and is responsible for the antioxidant activity of HDL. Several studies have shown an increase in the products of lipid peroxidation in the plasma of patients with coronary artery disease. Furthermore, LDL isolated from the plasma of patients with diabetes and coronary artery disease has been reported to be more susceptible to oxidation \textit{in vitro} than LDL from normal subjects, and autoantibodies to oxidized LDL are increased in patients with coronary artery disease. The significantly elevated LDL-C to PON1 mass and activity ratios in MI patients in this investigation indicates an increased lipid-burden to protective capacity that may have rendered the patients LDL more susceptible to oxidation \textit{in vivo}.

Experimental studies using transgenic PON1 knockout mice revealed an antiatherosclerotic characteristic of the PON1 enzyme (Mackness et al., 1998b; 2002). In recent studies, reduced serum PON1 activity has been reported to be associated with increased risk of AHD (Mackness et al., 1998b; 2002; James et al., 2000; Jarvik et al., 2000). Significantly lower PON1 activity has been reported after myocardial infarction when compared with age- and gender-matched controls (Ayub et al., 1999). Decreased serum PON1 activity has been reported with other states associated with increased atherosclerosis, including diabetes, hypercholesterolemia and renal diseases (Mackness et al., 1991; Hasselwander et al., 1998; Ikeda et al., 1998; Ak et al., 2002). Therefore, PON1 activity may reflect the antioxidant and anti-atherogenic capacity (Mackness et al., 1998b; 2002).
Effect of TpFE and MA on Antioxidants:

Figs. 36-40 indicate the effect of TpFE on the activity of antioxidant enzymes in heart of control and experimental rats. A significant (P<0.05) decrease is observed in the level of GSH (53.7%) and activities of GSH dependent enzymes, GPX and GST by 42.7 and 56.7% respectively in the heart tissue. Further a significant (P<0.05) decrease is observed in the activities of antiperoxidative enzymes, SOD, CAT by 52.7, 57.8% respectively in the heart tissue of ISO administered rats as compared to controls. TpFE in 100 mg kg\(^{-1}\) significantly (P<0.05) increased (12.6%) the levels of GSH and did not significantly (P<0.05) increased the activities of antioxidant enzymes when compared to ISO administered rats. Pretreatment with TpFE in 300 mg kg\(^{-1}\) showed a significant (p < 0.05) increase by 67.2, 46.1, 42.8, 68.5, and 118.7%, and TpFE in 500 mg kg\(^{-1}\) showed a further significant (p < 0.05) increase by 101.9, 64.4, 54.7, 108.5, and 131.2%, and also GA in 15 mg kg\(^{-1}\) showed a significant (p < 0.05) increase by 78.0, 57.1, 45.4, 88.5, and 125.0% in the levels of GSH and the activities of antioxidant enzymes such as GPX, GST, SOD and CAT respectively when compared to ISO administered group.

![Graph showing effect of TpFE on heart tissue GSH](image)

**Fig. 36: Effect of TpFE on heart tissue GSH**
Fig. 37: Effect of TpFE on heart tissue GPx

Fig. 38: Effect of TpFE on heart tissue GST
The data presented in Figs.41-44 represent the effect of MA on the activities of antioxidant enzymes in the heart tissues of rats. In the present study, the content of GSH and the activities of GPx, GST, SOD, and CAT were lowered (43.3, 44.9, 61.6, 44.2 and 55.5% respectively) significantly (P<0.05) in ISO administered group when compared to control group. MA (15 mg kg\(^{-1}\)) pretreatment significantly (P<0.05) increased (68.8, 61.4, 112.1, 64.7 and 104.1% respectively) the content of GSH and the activities of these antioxidant enzymes and the data of GA (15 mg kg\(^{-1}\)) (55.4, 45.4, 93.9, 44.1 and 70.8% respectively) is reversed when compared to ISO alone administered rats.
Fig. 42: Effect of MA on heart tissue GPx

Fig. 43: Effect of MA on heart tissue GST

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Fig. 44: Effect of MA on heart tissue GSH

GSH is one of the most abundant non-enzymatic antioxidant bio-molecules present in tissues. It is a highly sensitive indicator of cell functionality and viability. It is an important antioxidant in protecting the myocardium against free radical mediated injury and reduction in cellular GSH content could impair recovery after short period of ischemia. Its functions are removal of reactive oxygen species, such as H2O2, superoxide anions, and alkoxy radicals; maintenance of membrane protein thiols; and provision of a substrate for GPx and GST. Glutathione participates directly in the destruction of hydrogen peroxide and also promotes the formation of reduced form of ascorbate which has high antioxidant activity (Mrtensson and Meister, 1991). GSH depletion is linked to a number of disease states including cancer, neurodegenarative and cardiovascular diseases. In the present study, the reduction noticed in the level of GSH in heart of ISO-induced MI was either due to increased degradation or decreased synthesis of glutathione. Decreased GSH levels in ISO treated rats may be also due to its increased utilization to augment the activities of GPx and GST. Depletion of GSH results in enhanced LPO and excessive LPO. Pretreatment with TpFE and MA prevented the ISO induced LPO and maintained the level of GSH near normal level in heart. This is due to antioxidant activity of TpFE and MA. The increase of GSH may, in turn, activate glutathione-dependent enzymes.
such as GPx and GST. GPx is involved in the reduction of hydrogen peroxide to water by using glutathione as hydrogen donor (Sies, 1993). Reduced activity of this enzyme in the heart of ISO-treated rats has been observed in the current study. Pretreatment with TpFE and MA significantly increased GPx activity and consequently improved the antioxidant status in this system.

Free-radical scavenging enzymes such as SOD and CAT are known to be the first line cellular defense against oxidative stress, disposing of O2 and H2O2 prior to the interaction to form the more harmful hydroxyl (OH-) radical. Both SOD and CAT are key antioxidant enzymes that protect against tissue damage (Halliwell and Gutteridge, 1990). These enzymes are critical for defense mechanisms against the harmful effects of ROS and free radicals in biological systems. The SOD converts superoxide radicals (O_2^-) into H2O2 and O2, thus participating with other antioxidant enzymes, in the enzymatic defense against oxygen toxicity. CAT is a key component of the antioxidant defense system and inhibition of this protective mechanism results in enhanced sensitivity to free radical induced cellular damage. The decrease of CAT may result in a lot of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide (Srinivasan et al., 2007). The activities of these enzymes are lowered due to enhanced LPO in ISO treated group. Antioxidants constitute the foremost defense system that limits the toxicity associated with free radicals. Superoxide radicals generated at the site of damage in MI modulates SOD and catalase resulting in the lowered activities of these enzymes and accumulation of superoxide anion, which also damages the myocardium (Saravanan and Prakash, 2004).

**Effect of TpFE and MA on membrane bound ATPases:**

Fig. shows the effect of TpFE on the activities of membrane bound ATPases in control and ISO administered rats. In ISO administered rats the activities of Na^+K^- ATPase, Ca^{2+} ATPase and, Mg^{2+} ATPase significantly (p < 0.05) decreased by 65.6, 68.0 and 75.0% respectively on comparison with control rats. Pretreatment with TpFE (300 mg kg^-1) showed a significant (p < 0.05) increase by 109.0, 81.2, and
166.6% respectively, TpFE (500 mg kg\(^{-1}\)) showed a significant (p< 0.05) increase by 190.9, 212.5, and 283.3% respectively and GA in 15 mg kg\(^{-1}\) showed a significant (p< 0.05) increase by 163.6, 150.0, and 216.6% on the activities of these enzymes in the heart of ISO administered rats. TpFE (500 mg kg\(^{-1}\)) treatment alone did not show significant effect on the membrane bound enzymes.

Fig.45 illustrates the effect of MA on heart membrane bound transport enzymes of control and experimental rats. The activities of Na\(^+\)/K\(^-\) ATPase, Ca\(^{2+}\) ATPase and Mg\(^{2+}\) ATPase decreased (56.2, 48.4 and 52.5% respectively) significantly (p< 0.05) in the heart tissue of ISO administered rats when compared with the control rats. MA (15 mg kg\(^{-1}\)) pretreatment to ISO administered rats showed a significant (p< 0.05) increase (100.0, 73.5 and 85.7%) and GA (15 mg kg\(^{-1}\)) pretreatment also significantly (p< 0.05) increase (71.4, 58.8 and 71.4%) in the activities of Na\(^+\)/K\(^-\) ATPase, Ca\(^{2+}\) ATPase and Mg\(^{2+}\) ATPase respectively in the heart tissue when compared to ISO alone administered rats.

![Fig.45: Effect of TpFE on heart tissue ATPases](image-url)
Membrane bound ion transport enzymes play a significant role in the contraction and relaxation cycles of the cardiac muscle by maintaining normal ion levels within the myocytes. Any alteration in the properties of these enzymes is known to affect the function of heart. Failure of the cell membrane to maintain normal transmembrane ionic distribution through ion pumps is considered to be a major event in pathogenesis of ischemia and arrhythmia (Vajreswari and Narayanareddy, 1992). LPO and membrane fluidity are the main factors to alter the levels of ATPases. ISO treatment result in the decreased activities of membrane bound ATPases (Chernysheva et al., 1980). The loss of ATPase activity may be responsible for causing functional damage and myocardial cell necrosis. LPO play a key role in the inactivation of membrane bound enzymes with peroxidation of membrane lipids. Peroxidation of membrane lipids could inactivate Na⁺/K⁺ ATPase, Ca²⁺ ATPase and, Mg²⁺ ATPase because of the oxidation of ‘SH’ groups present in its active site leading to the conformational changes in the enzymes (Kako et al., 1988). Mg²⁺ ATPase plays a main role in Ca²⁺ influx and cell bursting. Decrease in the activity of Ca²⁺ ATPase can increase intracellular concentration of free Ca²⁺ and alter the signal transduction pathways and cellular fluidity. Any alterations in the properties of these enzymes
affect the function of the heart. TpFE and MA pretreatment increased the activities of Na⁺/K⁺ ATPase, Ca²⁺ ATPase and, Mg²⁺ ATPase in ISO-treated rats. This might be due to membrane-stabilizing property of TpFE and MA.

**Effect of TpFE and MA on HMG CoA-reductase:**

Fig.47 showed the effect of TpFE on the activity of HMG Co-A reductase activity in the liver of rats. HMG Co-A reductase decreased (48.1%) significantly (p<0.05) in ISO administered rats when compared to control group. Pretreatment with TpFE (100, 300, and 500 mg kg⁻¹) to ISO administered group showed significant (p<0.05) increase (17.8, 42.8 and 82.1%) and pretreatment with GA (15 mg kg⁻¹) showed significant (p<0.05) increase (67.8%) in the activity of HMG Co-A reductase when compared with ISO administered group.

Data depicted in Fig.48 reveal the effect of TpFE on the activity of HMG Co-A reductase in the heart of rats. HMG Co-A reductase decreased (53.8%) significantly (p<0.05) in ISO administered rats, as compared to control rats. TpFE (300 and 500 mg kg⁻¹) pretreatment to ISO group showed significant (p<0.05) increase (38.3 and 88.3%) and GA (15 mg kg⁻¹) pretreatment to ISO group showed significant (p<0.05) increase (55.0%) in the activity of HMG Co-A reductase when compared with ISO alone administered group.

![Fig.47: TpFE-Liver-HMG CoA](image)
Fig. 48: Effect of TpFE on heart tissue HMG CoA

Fig. 49 reveals the effect of MA on the activity of HMG Co-A reductase in heart and liver of rats. HMG Co-A reductase in heart and liver decreased (62.5 and 41.4% respectively) significantly (p < 0.05) in ISO administered rats when compared to control rats. MA (15 mg kg⁻¹) pretreatment to ISO group showed significant (p < 0.05) increase (133.3 and 62.5% respectively) and GA (15 mg kg⁻¹) pretreatment to ISO group also showed significant (p < 0.05) increase (100.0 and 45.8% respectively) in the activity of HMG Co-A reductase when compared with ISO alone administered group.
We observed significant increase in the activity of HMG-CoA reductase in liver and heart of ISO treated rats. HMG-CoA reductase is the rate limiting enzyme in the cholesterol biosynthesis. An increase in the activity of HMG-CoA reductase leads to excessive accumulation of cholesterol resulting in the formation of foam cells, a pre-requisite step in the development of atherosclerosis [40]. HMG-CoA reductase inhibitors are known to decrease the secretion of VLDL and LDL levels. Cellular cholesterol homeostasis is an important factor in the prevention of CVD such as MI. The concentration of cholesterol can be regulated by cholesterol biosynthesis, removal of cholesterol from circulation, absorption of dietary cholesterol and excretion via bile and feces. Our findings clearly show that pretreatment with TpFE and MA regulates cholesterol biosynthesis by inhibiting HMG-CoA reductase in ISO treated rats. Thus, the decreased cholesterol levels in extract and compound treatment might be correlated to the decreased activity of HMG CoA reductase in ISO treated rats. The antioxidant property indirectly helps to decrease the levels of lipids, by reducing or inhibiting lipid peroxidation.
Effect of MA on Lipoprotein lipase:

Fig.50 represents the effect of MA on the activity of LPL in liver of control and ISO administered rats. The rats injected with ISO exhibited significant (p< 0.05) decrease (57.5%) in the activity of LPL when compared to control rats. Oral pretreatment with MA (15 mg kg⁻¹) and GA (15 mg kg⁻¹) to ISO-induced myocardial infarcted rats significantly (p< 0.05) increased (102.2 and 77.7% respectively) the activity of LPL when compared to ISO alone administered rats.

![Graph](image)

Fig.50: Effect of MA on liver tissue LPL.

Many papers have been published showing that an increase in LPL is anti-atherogenic and a decrease in LPL is atherogenic. Whether LPL, directly or indirectly, promotes or protects against atherosclerosis remains unclear. However, understanding the role of LPL in atherosclerosis is clinically relevant.

LPL hydrolyzes triglycerides in plasma lipoproteins such as chylomicrons and VLDL and causes a wide variety of alterations in lipoprotein metabolism. This effect includes the stimulation of the hepatic removal of the lipolyzed lipoproteins, and transfer of surface components of triglyceride-rich lipoproteins to HDL. LPL molecules remain associated with chylomicrons after hydrolysis and, therefore, might assist in their hepatic uptake [29]. The net result of all the interactions between
lipoproteins and LPL or cell surface receptors is the retention and accumulation of lipoproteins in the arterial subendothelial matrix and their rapid uptake by cells. There are three potential pathways by which LPL may enhance cellular uptake of lipoproteins. The first is receptor mediated uptake of lipoproteins bound to the cell surface via LPL [33,34]. Secondly, LPL may act directly as a ligand for receptors [31] and, finally, the entire (heparin sulphate proteoglycans) HSPG–LPL–lipoprotein complex may be internalised by a receptor-independent pathway [35, 36]. Benlian et al. (5) have reported that several LPL-deficient patients have developed relatively advanced atherosclerosis. Furthermore, several clinical studies have shown that fibric acid derivatives induce LPL activity, lower plasma triglycerides, and suppress atherosclerosis (10–12).

**Effect of TpFE and MA on Inducible Nitric Oxide Synthase (iNOS):**

Fig.51 showed the effect of TpFE to ISO administered rats on iNOS expression. The iNOS enzyme expression in ISO rats was more when compared to control groups. Further, it is evident that rats pretreated with TpFE (100, 300 and 500 mg kg⁻¹) the expression was negatively modulated with restoration of the protein to normal level.

Fig.52 represents the effect of MA on iNOS expression. A significant increase in the expression of iNOS protein in ISO administered rats was detected by western blot when compared with control rats. Pretreatment with MA (15 mg kg⁻¹) and pretreatment with GA (15 mg kg⁻¹) decreased the expression of iNOS protein in ISO injected group.

We observed increased iNOS expression in ISO injected group. iNOS is physiologically present at very low levels, but its expression can increase in heart failure.27 This can considerably increase NO levels in the heart because iNOS produces large quantities of NO independently of calcium dynamics in the cell. Our data show that upregulation of iNOS is an early event in ISO-induced heart failure. Increased iNOS expression has been observed in a model of ISO-induced heart failure.
Wright et al. concluded that inhibition of constitutive NOS was deleterious, but that selective inhibition of iNOS might be beneficial. They also speculated that iNOS expression in the heart might account for myocardial depression. Subsequent studies by these and other

Set 1

![iNOS blots of control, TpFE, ISO and TpFE(100,300,500mg/kg)+ISO](image)

Set 2

![iNOS blots of control, MA, ISO, MA+ISO, GA+ISO](image)
workers led to the hypothesis that iNOS expression was deleterious not only with respect to hypotension and vascular hyporeactivity in endotoxic shock, but also in relation to myocardial dysfunction (Brady et al, 1992; Balligand et al, 1993; de Belder, 1993).

The human iNOS promoter contains a hypoxia-responsive element. Therefore it is not surprising that iNOS protein has been detected in cardiac myocytes and in infiltrating macrophages of patients several days after myocardial infarction [79,80,105]. In a rabbit heart infarction model, administration of specific NOS inhibitors significantly improved ventricular performance and increased myocardial blood flow in the surviving myocardium [117], suggesting tissue-destructive effects of NO production via iNOS. Moreover, after cardiac transplantation iNOS protein expression in myocytes and VSMC appears to correlate with contractile dysfunction [111]. Data on animal stroke models imply that iNOS is expressed in areas of infarcted or injured brain. In mutant mice deficient in nNOS or iNOS, infarct volume and neurological deficits were significantly smaller than those in normal mice (118). It has been suggested that iNOS is expressed in aneurysmal atherosclerotic human aorta'7 and in the megakaryocytes of patients with atherosclerosis.'6.

**Effect of TpFE and MA on Cell lines:**

Fig.53 showed the effect of TpFE on the viability of SK-N-MC cells in control and ISO administered cell lines. The viability of cells significantly decreased in cells treated with ISO when compared with control cells. The viability of ISO incubated cells increased significantly when treated with TpFE and GA.
Fig. 53: Effect of TpFE on neuroblastoma SKN-MC Cell lines

Fig. 54 showed the effect of MA on the viability of SK-N-MC cells in control and ISO administered cell lines. The viability of cells significantly decreased in cells treated with ISO when compared with control cells. The viability of ISO incubated cells increased significantly when treated with MA and GA.
The leading cause of heart attack and stroke is atherosclerosis. The heart and brain are similarly damaged due to the interruption of blood supply. In the present study, the effect of TpFE and MA on cells of the neuroblastoma cell line SKN-MC was investigated. Assessment of cell viability in the present study via MTT assay confirmed that ISO exerts cytotoxic effects on SKN-MC cells. Neuropeptide Y (NPY) receptors in the SK-N-MC human neuroblastoma cell line couple to mobilization of intracellular Ca\(^{2+}\) and inhibition of adenylylcyelase. Treatment of SK-N-MC cells with ISO enhanced the NPY-stimulated Ca\(^{2+}\) mobilization, mainly by increasing the maximal response to NPY. (Ref. Michel et al, 1992). Due to the inhibition of adenylylcyelase and mobilization of Calcium the ISO treated cells viability was decreased. Jang et al. (2002) reported that caffeine induces apoptotic death in neuroblastoma SKN-MC cells by increasing the caspase-3 enzyme activity. TpFE and MA altered the ISO-induced toxicity and exert the protective effect.

**Docking studies:**

After collecting the crystal model, the possible binding sites of HMG coA reductase were searched with CASTP server and were shown in Fig. From the binding site analysis of HMG coA reductase it was identified that, the binding pockets are identical in all chains and the largest binding pocket was taken for further docking studies. The crystal structures of HMG coA reductase are similar and we have therefore taken 1HW9 (chain A) as representative structure for docking studies. The docking of compounds into the active site of HMG coA reductase was performed using the GOLD software and the docking evaluations were made on the basis of GoldScore fitness functions. We preferred Gold fitness score than Chemscore fitness as Gold fitness score is marginally better than Chemscore fitness function.
The selected docked conformations of EA, MA, GA and OA in the HMG CoA binding site are shown in. The docked conformations revealed that all ligands were located in the hydrophobic binding pocket surrounding the binuclear copper active site. In this study, all docked ligands were found to have some interaction between an oxygen atom of the ligands and enzyme. Moreover, these docked conformations also formed an H-bonding interaction with in the active site. In the binding pocket, common H-bonding interactions were formed between all docked ligands and ARG496, SER500, PRO 506, LEU509 and TYR514. The specific H-bonding interaction with TYR514 was only found in the docked conformation of EA. In order to explain the binding of these compounds, the H-bonding interactions with the other surrounding residues in the hydrophobic binding pocket were also investigated. In strong H-bonding interactions between the hydroxyl group (H8) of EA and an oxygen atom of ARG496 and another hydrogen bond between hydroxyl group of enzyme residue TYR514 and oxygen atom (O22) of EA. H-bonding interactions were also formed with SER500 (HG) and oxygen atoms (7, 8) of EA which is important residue coordinated with in the active site. The docked MA, GA and OA are shown in this study. In the case of docked GA, H bonds with ARG496, SER500, LEU509, were formed. Hydrogen bonding interactions between hydroxyl group of GA and oxygen atom of enzyme residue ARG496 was observed. A total of three hydrogen bonds were observed in which two hydrogen bonds are formed between oxygen atom (O7) and enzyme residues SER500 (OG), LEU509 (N).

From the docking of MA into the active site of HMG coA reductase, we observed hydrogen bond between the Hydroxyl group (H44) of MA and oxygen atom of ARG496 and another hydrogen bond between hydroxyl group of LEu509 and oxygen atom (O5) of MA.

From the docking of OA into the active site of HMG coA reductase, we observed that, H7 in OA forms hydrogen bond with oxygen atom of PRO506 and O22 forms another hydrogen bond with Hydrogen atom of LEUS09. The atoms involved in bonding with HMG CoA and their bond lengths along with docking energies were indicated in table.
The docking results agreed well with the observed *in vitro* data, which showed that the HMG CoA inhibitory activity of MA was higher than those of GA and EA. Our investigations show that MA has inhibitory activity on HMG CoA reductase and this can be helpful for further investigations. The docking results data supports the inhibitory activity of Oleanolic acid.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>No. of Hydrogen bonds</th>
<th>Atoms</th>
<th>Bond length</th>
<th>Docking score</th>
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</thead>
<tbody>
<tr>
<td>Ellagic acid</td>
<td>4</td>
<td>TYR514(HH)</td>
<td>22(O)</td>
<td>2.409</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ARG496(O)</td>
<td>8(H)</td>
<td>1.643</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SER500 (HG)</td>
<td>8(O)</td>
<td>2.648</td>
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<td></td>
<td></td>
<td></td>
<td>7(O)</td>
<td>2.595</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>3</td>
<td>ARG496(O)</td>
<td>17(H)</td>
<td>1.644</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SER500(OG)</td>
<td>7(O)</td>
<td>3.509</td>
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<tr>
<td></td>
<td></td>
<td>LEU509(N)</td>
<td>7(O)</td>
<td>2.817</td>
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<tr>
<td>Oleanolic acid</td>
<td>2</td>
<td>LEU509(H)</td>
<td>23(O)</td>
<td>2.492</td>
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<td></td>
<td></td>
<td>PRO506(O)</td>
<td>7(H)</td>
<td>1.509</td>
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<tr>
<td>Maslinic acid</td>
<td>2</td>
<td>ARG496(O)</td>
<td>44(H)</td>
<td>1.272</td>
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<tr>
<td></td>
<td></td>
<td>LEU509(H)</td>
<td>5(O)</td>
<td>2.474</td>
</tr>
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</table>
Fig.55: Ellagic acid docking score 39.07

Fig.56: Gallic acid 29.04
Fig. 57: Maslinic acid  -126.40

Fig. 58: Oleanolic acid  -59.99
**Effect of TpFE and MA on Histopathological studies:**

Fig. 59 shows the effect of TpFE on the extent of histopathological changes in myocardial tissues in normal and ISO-treated rats. Fig. 59A shows the light micrograph of control heart showing normal architecture. Fig. 59C shows the light micrograph of ISO-administered group shows focal confluent necrosis of muscle fibres with inflammatory cell infiltration, edema with fibroblastic proliferation and myophagocytosis along with extravasation of red blood cells. The degree of myocardial damage in TpFE 100 mg kg⁻¹ bw + ISO group was similar to that of ISO group with similar morphological changes (Fig. 59D). In TpFE 300 mg kg⁻¹ bw + ISO group (Fig. 59E), and GA 15 mg kg⁻¹ bw + ISO group (Fig. 59G) there was less edema and myonecrosis with less inflammatory cells. Light micrograph of TpFE 500 mg kg⁻¹ bw + ISO-administered heart tissue shows mild edema but no infarction. The myocardial fibers are normal in architecture (Fig. 59F). TpFE 500 mg kg⁻¹ bw administration alone did not lead to any histopathological changes in the myocardium (Fig. 59B).

The histopathological studies of the control group revealed normal architecture of the myocardium, with intact muscle fibres. Heart tissue of ISO-induced rat showed severe infarcted area with edema, inflammatory cells and separation of cardiac muscle fibres. TpFE pretreated and MA pretreated rats showed near normal architecture of cardiac fibres with less edema and low inflammatory cells.
Fig. 59: Photomicrographs of histopathological examination (H&E, 10x) of the heart of normal, ethanolic extract of TpFE treated and ISO treated experimental animals.

A) Control
B) TpFE (500 mg/kg bw) treated,
C) ISO (85 mg/kg bw, s.c.),
D) TpFE (100 mg/kg bw) pretreated + ISO (85 mg/kg bw, s.c.),
E) TpFE (300 mg/kg bw) pretreated + ISO (85 mg/kg bw, s.c.),
F) TpFE (500 mg/kg bw) pretreated + ISO (85 mg/kg bw, s.c.),
G) GA (15 mg/kg bw) pretreated + ISO (85 mg/kg bw, s.c.).
Fig. 60: Photomicrographs of histopathological examination (H&E, 10x) of the heart of normal, maslinic acid treated and ISO treated experimental animals.

A) Control  
B) MA (15 mg/kg bw) treated,  
C) ISO (85 mg/kg bw, s.c.),  
D) MA (15 mg/kg bw) pretreated + ISO (85 mg/kg bw, s.c.),  
E) GA (15 mg/kg bw) pretreated + ISO (85 mg/kg bw, s.c.),

**Conclusion:**

CVD remains the principal cause of death in both developed and developing countries and 75% of global deaths resulting from IHD occurred in the low and middle-income countries. Oxidative stress, resulting from increased production of free radicals, plays a major role in CVD such as MI and atherosclerosis. MI, commonly known as heart attack, is a disease that occurs when the blood supply to a part of the heart is interrupted, causing damage to the heart tissue.

ISO causes severe stress in the myocardium resulting in infarct-like necrosis of the heart muscle. ISO administration resulted in biochemical changes leading to cardiotoxicity in rats which are similar to those of MI in humans. The involvement of increased oxidative