RESULTS
AND
DISCUSSION
3. RESULTS AND DISCUSSION

3.1 General observations

No toxic effects of TTFAEt treatment were observed when normal animals were treated for shorter period. In ISO administered rats, the rate of survival was 70-75% when compared to normal control rats during the experimental period of 40 days whereas TTFAEt treated rats appeared to be normal and healthy (as control animals). There was no significant difference in body weight of the treated rats when compared with control rats, either at the beginning or at the end of the study period.

3.2 Serum Lipid Profile:

The levels of total cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol, phospholipids and HDL-cholesterol in serum of normal and experimental rats are shown in Fig 4. Rats administered with -ISO showed a significant (p<0.05) increase in the levels of serum total cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol by 32.91%, 30.23%, 84.37% and 81.16% respectively, but there was a significant decrease in HDL cholesterol (44.41%) levels as compared to normal control rats. Rats pretreated with TTFAEt showed a significant decrease in serum total cholesterol (21.26%), triglycerides (16.19%), LDL- cholesterol (49.98%), VLDL-cholesterol (50.15%) and significant increase in HDL - cholesterol (60.22%) as compared to normal control rats. The marked increase in serum total cholesterol, triglycerides, LDL-cholesterol, VLDL-
Fig: 4 Effect of TTFAEt treatment on Serum Lipid Profile in normal control rats and experimental animals.

Each column is mean ± S.D. for six rats in each group.
cholesterol and a decline in HDL-cholesterol observed in ISO treated rats are in agreement with the earlier reports Shreesh et al., 2008 Su, 2003.

Lipids play an important role in CVD, not only by way of hyperlipidemia and the development of atherosclerosis, but also by modifying the composition, structure and stability of cellular membranes. High levels of LDL cholesterol show a positive correlation with myocardial infarction, whereas high levels of HDL-cholesterol have a negative correlation (Buring, et al., 1992). Increased cholesterol concentration along with triglycerides in serum was considered an important risk factor for atherosclerosis (Hopkins et al., 2005). In particular, many studies revealed LDL-cholesterol to be the most dangerous among the serum lipids, and the oxidation LDL leads to its increased penetration of arterial walls and becomes a major component of atherosclerotic plaque lesions (Aviram, 1993). According to these studies, decreased serum total cholesterol and LDL-cholesterol levels along with increased level of HDL-cholesterol level is important for reducing the risk of cardiovascular disease. High levels of HDL inhibit the uptake of LDL by the arterial wall and facilitate the transport of cholesterol from peripheral tissue to the liver where it is catabolised and excreted from the body (Sheela, 2001). This pathway plays a major role in reducing cholesterol levels and inhibits atherosclerotic plaque formation in the aorta (Young et al., 1956).

In the present study ISO administered rats showed a significant (P<0.05) increase in the levels of serum lipids, phospholipid and decrease in HDL-cholesterol. The abnormal high concentration of serum lipids is mainly due
to increase in the mobilization of free fatty acids from the peripheral depots and myocardium. Catecholamines and other hormones promote lipolysis through cAMP formation. ISO also enhances lipolysis as it is a β-adrenergic agonist. The increase in serum phospholipids indicates the ischemic injury related alterations in lipid composition of myocardial tissue and appears to occur due to destruction of myocardial membrane lipid bi-layer. Paritha and Devi, (1997) also reported an increase in the serum phospholipids in ISO induced rats.

Pretreatment with TTFAEt showed significant decrease in the levels of serum total cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol, phospholipids and an increase in the level of HDL- cholesterol compared to ISO treated rats clearly indicating the protective effect of TTFAEt against ISO induced myocardial stress. The hypolipidemic effect of TTFAEt could be due to the inhibition of lipid absorption by saponins and flavonoids present in the aqueous extract and/or activation of fatty acid synthase, acetyl-coA carboxylase and production of triglyceride precursor (Guo et al., 2007). The hypolipidemic effect of TTFAEt is also due to the saponins which have effects on cholesterol metabolism (Sudheesh et al., 2007). It may be due to enhanced activity of lipid and lipoprotein metabolizing enzymes such as HMG-CoA reductase, lipoprotein lipase and plasma LCAT (Siedal, 1987). This observed hypolipidemic effect of TTFAEt might be due to the action of different active phytochemical constituents like flavonoids and saponins each with single or a diverse range of biological activities (Hughes et al, 2005; Shreesh et al, 2008).
3.3 Serum Cardiac Marker enzymes

The diagnostic serum marker enzymes of myocardial infarction are Creatine Kinase (CK), Lactate dehydrogenase (LDH), Aspartate Transaminase (AST), Alanine Transaminase (ALT). Fig 5 and Fig 6 showed a significant (P<0.05) increase in the activities of serum cardiac markers such as CK, LDH, SGOT, SGPT and XOD respectively in ISO administered rats when compared to normal control rats. Pretreatment with TTFAEt showed significant (P<0.05) decrease in the activities of CK, LDH, SGOT, SGPT and XOD when compared to ISO administered rats which were maintained at near normal levels. These observations are in agreement with earlier findings of Shreesh et al., 2008).

The estimation of CK, LDH, SGOT SGPT and XOD levels are useful parameters for assessing cardiomyocytes damage (Jennings et al., 1990). These enzymes normally exists in cellular compartments and leak out into the serum during myocardial injury due to disintegration of contractile elements and sarcoplasmic reticulum. An increase in the activities of marker enzymes in serum could be due to the leakage of enzymes from myocardium, as a result of ISO induced necrosis (paritha ithyarasi, 1996) and the amount of enzymes appearing in serum is directly proportional to the number of necrotic cells present in the cardiac tissue. Of all the macromolecules that leak from damaged tissue, enzymes because of their tissue specificity and catalytic activity are the best markers of tissue damage. The release of cellular enzymes reflects non-specific alterations in the plasma membrane integrity and permeability as a response to
Fig: 5  Effect of TTFAEt treatment on Serum Cardiac Marker Enzymes in normal control and experimental rats.

Each column is mean ± S.D. for six rats in each group.
Fig: 6  **Effect of TTFAEt on Xanthine Oxidase in normal control and experimental rats.**

Each column is mean ± S.D. for six rats in each group.
β-adrenergic stimulation. During ischemic conditions the adenosine nucleotide pool is degraded to hypoxanthine and xanthine along with conversion of Xanthine dehydrogenase to xanthine oxidase (Roussos, 1967; Bhakuni, et al., 2005).

Xanthine oxidase acts on xanthine and hypoxanthine with the resultant production of free radicals such as superoxide radical (Raghuwanshi et al., 2005). Cardiac pretreated rats in the present study showed significant depletion of serum CK, LDH, SGOT, SGPT and XOD. TTFAEt treatment prevented the leakage of these enzymes and restored their activities as compared to the ISO administered rats. The observed decrease in the activities of serum cardiac marker enzymes in pretreated rats may be due to membrane stabilizing action and free radical scavenging activity of TTFAEt. The above results are supported by the histopathological studies which suggested its cardioprotective effect, these observations are in agreement with (Geetha, et al., 1990).

3.4 Oxidative stress and antioxidant enzymes

A significant (P<0.05) decrease in myocardial GSH level, along with decrease in the activities of glutathione dependent enzymes (GPx, GST and GR), antilipid peroxidative enzymes (SOD and CAT) and increase in lipid peroxidation product MDA in Fig 7 were observed in the heart tissue of ISO administered rats in comparison to normal control rats (Table-1). These observations are in agreement with other reports (Nirmala and Puvanakrishnan, 1996; Sathish et al., 2003). This indicates increased level of tissue antioxidant status and decreased
Fig: 7  Effect of TTFAEt on Lipid peroxidation in normal control and experimental rats.

Each column is mean ± S.E. for six rats in each group.
Table-1 Effect of TTFAEt treatment on heart tissue antioxidant enzymes in normal control and experimental rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal Control (n=6)</th>
<th>TTFAEt (n=6)</th>
<th>ISO (n=6)</th>
<th>ISO + TTFAEt (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide dismutase</td>
<td>37.390±2.521</td>
<td>38.691±3.119</td>
<td>25.430±2.343*</td>
<td>31.141±1.899*</td>
</tr>
<tr>
<td>(U/min/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catalase</td>
<td>76.50±2.699</td>
<td>71.358±4.291</td>
<td>42.628±3.304*</td>
<td>52.305±2.769*</td>
</tr>
<tr>
<td>(mmoles of H₂O₂ decomposed/min/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione</td>
<td>6.381±0.359</td>
<td>6.276±0.454</td>
<td>3.681±0.645*</td>
<td>5.460±0.462*</td>
</tr>
<tr>
<td>(µg of GSH/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>8.450±0.443</td>
<td>8.656±0.449</td>
<td>3.843±0.609*</td>
<td>7.601±0.1603*</td>
</tr>
<tr>
<td>(µmoles of GSH consumed/min/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione reductase</td>
<td>31.141±4.637</td>
<td>29.558±1.730</td>
<td>11.698±0.438*</td>
<td>26.050±0.942*</td>
</tr>
<tr>
<td>(mmoles of NADPH Oxidized/min/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione -S - transferase</td>
<td>6.003±0.111</td>
<td>5.483±0.353</td>
<td>2.995±0.218*</td>
<td>5.010±0.379*</td>
</tr>
<tr>
<td>(mmoles of GSH-CDNB conjugate formed/min/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value is the mean ± S.E of six animals in each group. Group III were compared with corresponding normal control group. Group IV were compared with group III. According to DMR test* p ≤ 0.05.
lipid peroxidation in ISO induced myocardial infraction condition (Raghuwanshi et al., 2005).

ISO is well known to generate free radicals and stimulate lipid peroxidation which may be a causative factor for irreversible damage to the myocardial membrane. Without the presence of catalytic iron, free radical generation doesn’t occur. Besides Iron is essential for the release of free radicals, and to initiate lipid peroxidation. Iron is essential for maintaining proper cell function and any iron overload may result in deleterious reactions (Minotti, 1993). Without the presence of catalytic iron, free radical generation doesn’t occur. Oxygen free radicals are capable of damaging compounds of all biochemical processes including nucleic acids, proteins, lipoproteins, carbohydrates and connective tissue macromolecules (Bast, 1993).

Initiation of lipid peroxidation by ferrous sulphate takes place either through ferryl perferryl complex or through OH⁻ radical. Ferryl-perferryl complex can also initiate lipid peroxidation on its own in a similar manner as OH⁻ radical, though it is less reactive than OH⁻ (Graf et al., 1984; Braugghler et al., 1986; Govindarajan et al., 2003; Govindarajan et al., 2005). Lipid peroxidation represents oxidative tissue damage caused by hydrogen peroxide, superoxide anion and hydroxyl radicals, which occurs mainly in membranes, where the content of unsaturated fatty acids is relatively high (Das et al., 2002). The oxidation of unsaturated fatty acids in biological membranes may cause impairment of membrane function, decrease in membrane fluidity, inactivation of
membrane receptors and enzymes, increase of non-specific permeability to ions and disruption of myocardial membrane structure (Anandan et al., 2003). This leads to loss of essential fatty acids with the formation of cytosolic aldehyde and peroxide products. Malondialdehyde is a major end product of free radical reaction on membrane fatty acids (Ward, 1995).

Reduction noticed in the activities of glutathione-dependent antioxidant enzymes (GPX, GR and GST) and antiperoxidative enzymes (SOD and CAT) in ISO induced myocardial infraction is due to the presence of quinine metabolites of reacts with oxygen to produce superoxide anions and other reactive oxygen species and interfere with SOD, CAT, GSH and glutathione-dependent antioxidant enzymes (GPX, GR and GST) (Shreesh, et al., 2008). The increased generation of reactive oxygen radicals such as superoxide and hydrogen peroxide, which in turn leads to the inactivation of these enzyme activities and causes oxidative stress. Free radical scavenging enzymes such as catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase are the first line cellular defense against oxidative injury decomposing $O_2^-$ and $H_2O_2$ before interacting to form the more reactive hydroxyl radical ($OH$). The equilibrium between these enzymes is an important process for the effective removal of oxygen stress in intracellular organelles.

During normal cellular metabolism, these cellular defenses can adequately cope up with any free radical generated and homeostasis is maintained between radical generation and dissipation. However in certain
clinical conditions there could be an increased generation of reactive oxygen species such that the capacity of scavenging enzymes and antioxidants are surpassed. Lowered activities of these prime antioxidant enzymes may lead to the formation $O_2^-$ and $H_2O_2$, which in turn can form hydroxyl radical (OH$^-$) and bring about a number of reactions harmful to the cellular and subcellular membranes in the heart tissue (Ward, 1995).

Oxidative stress is one of the mechanisms with a central role involved in the pathogenesis of myocardial infarction (Grieve et al., 2004). Oxidative stress can produce major interrelated dearrangements of cellular metabolism including DNA strand breakage, rise in intracellular free calcium, concentration damage to membrane ion transporters and proteins and peroxidation of lipids. The increase in intracellular free calcium can activate proteases, nucleases, protein kinases, thiol proteins and cell surface receptors such as response to oxidative stress will perturb normal cellular metabolism causing dearrangements of cellular components, possibly with decompartmentalization of transition iron with generation of more ROS (Ward, 1995).

Pretreatment with TTFAEt significantly (P<0.05) prevented these adverse changes in heart tissue, and maintained the rats at near normal levels which indicate oxidative stress elicited by ISO which had been nullified due the protective effect of the extract. This is due to the transport of fatty acids into mitochondria for oxidation with the available oxygen to produce energy. TTFAEt showed protection against ISO induced myocardial injury due to the presence of
phytochemicals such as flavonoids, alkaloids and saponins etc., in the aqueous extract of *Tribulus terrestris* fruit (Wang et al., 1997). The protective effect of extract may be due to decreased generation of free radicals, by scavenging the OH radical and other reactive oxygen molecules, by chelation of iron and supplying a competitive substrate for unsaturated fatty acids in the membrane which are responsible for lipid peroxidation. Augmentation of endogenous antioxidants may enhance the myocardial antioxidant reverse and strengthen myocardial defense mechanisms operating in the myocardium (Tripathi et al., 2001).

3.5 Histological observations:

Fig 8 represents the histopathological study of control group revealed the normal architecture of the myocardium, with intact muscle fibres. Heart tissue of ISO administered rats showed hyalinization of muscle fibres, whereas that of TTFAEt pretreated showed no change in the morphology of heart.

In conclusion, the results of present study indicate that the treatment with TTFAEt for 40 days prevents the symptoms of ISO induced myocardial infarction in rats. The overall cardioprotective effect of TTFAEt is probably due to accumulation by its hypolipidemic property and its free radical scavenging ability against ISO induced lipid peroxidation, which is primarily responsible for the irreversible necrosis of the myocardial membrane. Cardioprotective effects of TTFAEt during myocardial ischemia demonstrate its therapeutic potential in the treatment of ischemic heart disease.
Fig 8: HISTOLOGICAL EXAMINATION OF HEART IN CONTROL AND EXPERIMENTAL RATS (HEMATOXYLIN & EOSIN X 100)

A. Normal Control Rats

B. Isoproterenol administered Rats
C. Tribulus terrestris Treated Rats

D. Isoproterenol administered Rats Pretreated With Tribulus terrestris
REFERENCES


