Results
Isoproterenol induced myocardial infarction is the leading cause of death and experimental studies were conducted on animals to evaluate and explore the cardioprotective effect of mangiferin on isoproterenol induced acute MI through biochemical, histological, electrophysiological studies and the results obtained are summarized as follows.

**Dosage fixation for the drug (Figure 1 & 2)**

The effective dosage was fixed by assessing the activities of serum cardiac marker enzymes such as LDH and CPK which are depicted in Figures 1 and 2. Of the different dosages of mangiferin (2.5 mg, 5 mg, 7.5 mg, 10 mg and 20 mg / 100 g body weight) for different time periods (7, 14, 21, 28 and 35 days), pretreatment of mangiferin at a dose of 10 mg for 28 days offered significant protection (p<0.001) against isoproterenol induced cardiac damage when compared to Group 2 rats and no significant changes were observed when compared to Group 1 control animals which shows its protective effect.

Based on the preliminary studies, a dosage of 10mg/100 g body weight of mangiferin for 28 days was fixed as optimum dosage and optimum duration to protect the myocardium effectively from the necrotic damage induced by isoproterenol and hence further biochemical analysis were carried out with this dosage.
Figure 1: Activity of Serum LDH in control and experimental group of rats
Values are expressed as mean ± S.D for 6 animals in each group

Statistical calculation: One way ANOVA followed by posthoc test LSD
a - Compared with Group 1  *** p < 0.001; ** p < 0.01, * p < 0.05, NS - Non significant
Figure 2: Activity of serum CPK in control and experimental group of rats
Values are expressed as mean ± S.D. for 6 animals in each group

Statistical calculation: One way ANOVA followed by posthoc test LSD

a - Compared with Group 1

*** p < 0.001; ** p < 0.01, * p < 0.05, NS - Non significant
Determination of mortality rate

The rate of survival of rats given isoproterenol was 60 to 65% when compared to normal control rats. The remaining group of animals showed 100% survival as compared with control rats.

Determination of rat heart weight to body weight ratio

Figure 3 depicts the heart weight to body weight ratio of the control and experimental animals.

Group 2 rats showed a significant change (p<0.001) in their heart weight to body weight ratio compared to Group 1 control rats which predicts the maximum damage to the heart and increase in its weight and decrease in their body weight due to isoproterenol induced damage. Group 3 rats showed a non significant change in heart weight to body weight ratio when compared to control rats.

In Group 4 rats, pretreatment of mangiferin showed a better protection of their heart which is indicated by a significant decrease (p<0.001) in heart weight to body weight ratio when compared to Group 2 isoproterenol induced animals.

Histopathological study

Plate 1.1 to 1.6 expresses the histopathological examination of the heart tissue of the control and experimental animals.
Figure 3: Heart weight to body weight ratio of control and experimental group of rats. Values are expressed as mean ± S.D. for 6 animals in each group.

Groups

Statistical calculation: One way ANOVA followed by posthoc test LSD

- a - Compared with Group 1 *** p < 0.001, ** p < 0.01, * p < 0.05, NS - Non significant
- b - Compared with Group 2

(Bar charts showing heart weight ratios for control, ISPH induced, Mangiferin alone, and Mangiferin + ISPH groups with significant differences indicated.)
Plate 1.1 shows the section of the heart of a control rat showing a normal architecture with normal myocardial fibers and muscle bundles.

Plate 1.2 shows the section of heart of drug control animal showing normal myocardial fibers.

Plate 1.3 and 1.4 depicts the section of heart of isoproterenol induced rat showing different areas of myocardial necrosis and explains the separation of myocardial fibers with inflammatory mononuclear infiltrate. The cardiac muscle bundles have two areas of foci of extensive inflammatory collections, edema and separated muscle fibers.

Plate 1.5 and 1.6 show the section of heart of mangiferin pretreated isoproterenol administered rat showing slightly separated myocardial fibers with small focus of inflammatory (mononuclear) collections.

**ECG study**

Plate 2 presents the electrocardiographic pattern of normal and experimental group of animals.

Group 1 and Group 3 (Plate 2.1 and 2.4) rats showed normal ECG pattern and an elevation of ST segment was observed (Plates 2.2 and 2.3) in Group 2 isoproterenol induced rats. Mangiferin pretreated and isoproterenol induced Group 4 rats exhibited a near normal ECG pattern (Plate 2.5) with a slight elevation in ST segment.
1.1 Heart from a control rat showing normal architecture

1.2 Heart from mangiferin treated rat showing near normal architecture

1.3 & 1.4 Heart from an isoproterenol administered rat showing separation of myocardial fibers with inflammatory (mononuclear) collections

1.5 Heart from mangiferin pretreated rat showing slightly separated myocardial fibers with small focus of inflammatory (mononuclear) collections

1.6 Another area of heart from mangiferin pretreated rat showing normal myocardial fibers
PLATE 2 ECG PATTERN

2.1 Control rat showing normal ECG pattern

2.2 & 2.3 ECG of isoproterenol induced MI rat showing ST segment elevation

2.4 Mangiferin alone treated rat showing ECG pattern similar to control rat

2.5 Mangiferin pretreated and isoproterenol administered rat showing near normal ECG pattern
Marker enzymes

**Table 1** represents the activities of AST, ALT, CK and LDH in serum and heart tissue of control and experimental animals. A significant increase (p<0.001) in the activities of myocardial marker enzymes in serum with a corresponding decrease in myocardial tissue (p<0.001) was found in isoproterenol induced Group 2 rats when compared to Group 1 control rats. Group 3 rats showed non-significant changes both in serum and heart tissue marker enzyme status as compared to Group 1 control animals.

All the above parameters both in serum and heart tissue of Group 4 mangiferin pretreated animals exhibited significant changes (p<0.001) compared to Group 2 ISPIND induced rats.

**Serum CK-MB isoenzyme**

**Figure 4** represents the activity of serum CK-MB isoenzyme pattern of control and experimental group of rats. A marked increase (p<0.001) in this enzyme level was observed in Group 2 isoproterenol induced rats compared to Group 1 control rats. The Group 4 mangiferin pretreated rats showed a significant decrease in this enzyme level (p<0.001) compared to Group 2 isoproterenol administered rats. Group 3 rats showed non significant change compared to Group 1 control animals.

**Electrophoretic separation of serum LDH isoenzyme**

Agarose gel electrophoretic separation of serum LDH isoenzyme pattern of control and experimental animals are depicted in **Plate 3**.
Table 1: Activity of the serum and heart tissue marker enzymes in control and experimental group of rats

Values are expressed as mean ± SD for six animals in each group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Group 1)</th>
<th>ISPH induced (Group 2)</th>
<th>Mangiferin alone treated (Group 3)</th>
<th>Mangiferin treated + ISPH induced (Group 4)</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum (IU/L)</td>
<td>27.83 ± 1.89</td>
<td>44.70 ± 3.40&lt;sup&gt;***&lt;/sup&gt;</td>
<td>27.05 ± 1.88&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>30.92 ± 1.79&lt;sup&gt;***&lt;/sup&gt;</td>
<td>73.83</td>
</tr>
<tr>
<td>Heart (n moles of pyruvate liberated /min/mg protein)</td>
<td>53.69 ± 3.88</td>
<td>38.03 ± 2.75&lt;sup&gt;***&lt;/sup&gt;</td>
<td>52.78 ± 2.94&lt;sup&gt;a NS&lt;/sup&gt;</td>
<td>48.16 ± 3.20&lt;sup&gt;b***&lt;/sup&gt;</td>
<td>29.68</td>
</tr>
<tr>
<td>ALT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum (IU/L)</td>
<td>25.97 ± 1.79</td>
<td>12.02 ± 0.79&lt;sup&gt;***&lt;/sup&gt;</td>
<td>26.13 ± 1.55&lt;sup&gt;a NS&lt;/sup&gt;</td>
<td>23.41 ± 1.83&lt;sup&gt;b***&lt;/sup&gt;</td>
<td>111.5</td>
</tr>
<tr>
<td>Heart (n moles of pyruvate liberated /min/mg protein)</td>
<td>78.59 ± 5.67</td>
<td>127.60 ± 10.90&lt;sup&gt;***&lt;/sup&gt;</td>
<td>77.57 ± 4.41&lt;sup&gt;a NS&lt;/sup&gt;</td>
<td>88.51 ± 4.35&lt;sup&gt;b***&lt;/sup&gt;</td>
<td>70.15</td>
</tr>
<tr>
<td>LDH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum (IU/L)</td>
<td>139.79 ± 11.21</td>
<td>81.83 ± 5.36&lt;sup&gt;***&lt;/sup&gt;</td>
<td>140.20 ± 9.11&lt;sup&gt;a NS&lt;/sup&gt;</td>
<td>126.21 ± 9.25&lt;sup&gt;b***&lt;/sup&gt;</td>
<td>56.4</td>
</tr>
<tr>
<td>Heart (n moles of pyruvate liberated /min/mg protein)</td>
<td>284.64 ± 23.73</td>
<td>426.78 ± 36.26&lt;sup&gt;***&lt;/sup&gt;</td>
<td>282.83 ± 16.93&lt;sup&gt;a NS&lt;/sup&gt;</td>
<td>316.00 ± 19.43&lt;sup&gt;b***&lt;/sup&gt;</td>
<td>43.47</td>
</tr>
<tr>
<td>CPK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum (IU/L)</td>
<td>17.61 ± 1.23</td>
<td>8.49 ± 0.70&lt;sup&gt;***&lt;/sup&gt;</td>
<td>18.22 ± 1.18&lt;sup&gt;a NS&lt;/sup&gt;</td>
<td>16.12 ± 1.08&lt;sup&gt;b***&lt;/sup&gt;</td>
<td>105.38</td>
</tr>
<tr>
<td>Heart (μ moles of phosphorous liberated /min/mg protein)</td>
<td>284.64 ± 23.73</td>
<td>426.78 ± 36.26&lt;sup&gt;***&lt;/sup&gt;</td>
<td>282.83 ± 16.93&lt;sup&gt;a NS&lt;/sup&gt;</td>
<td>316.00 ± 19.43&lt;sup&gt;b***&lt;/sup&gt;</td>
<td>43.47</td>
</tr>
</tbody>
</table>

Statistical calculation: One way ANOVA followed by posthoc test LSD

a - Compared with Group 1  ** p < 0.001,  * p < 0.05. NS - Non significant
b - Compared with Group 2
Figure 4: Serum CK-MB isoenzyme activity in normal and experimental group of rats
Values are expressed as mean ± SD for 6 animals in each group

STATISTICS
Statistical calculation  One way ANOVA followed by posthoc test LSD
a - Compared with Group 1  *** p < 0.001, ** p < 0.01, * p < 0.05, NS - Non significant
b - Compared with Group 2
<table>
<thead>
<tr>
<th>LDH 1</th>
<th>Group-1</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH 2</td>
<td>Group-2</td>
<td>MI</td>
</tr>
<tr>
<td>LDH 3</td>
<td>Group-3</td>
<td>Drug alone</td>
</tr>
<tr>
<td>LDH 4</td>
<td>Group-4</td>
<td>Drug + MI</td>
</tr>
<tr>
<td>LDH 5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Isoproterenol induction caused an increase in LDH isoenzyme bands, predominantly LDH 1 in Group 2 rats compared to Group 1 control rats. The LDH 1 band location in mangiferin pretreated Group 4 animals were found to be near normal and is almost similar to Group 1 control rats. Group 3 drug control rats also showed normal LDH pattern as compared to Group 1 control rats.

**TTC Test**

Plate 4.1 to 4.4 shows the histochemical approach to detect the myocardial changes in the heart of normal and experimental group of rats through Macroscopic Enzyme Mapping Assay (TTC Test).

A high percentage of mean infarct size with increased staining was observed in Group 2 isoproterenol administered rats when compared to Group 1 control animals. Mangiferin pretreated Group 4 rats showed a moderately low infarct size with reduced staining when compared to Group 2 ISPH induced rats.

**Electrophoretic separation of plasma proteins and determination of A/G ratio**

Plates 5.1 to 5.4 shows the electrophoretic separation of serum total proteins and protein fractions such as albumin and globulins (alpha (1, 2), beta and gamma globulins) of control and experimental rats. The densitometer readings of these separated protein values are provided in Figure 5.
4.1 Group 1 Control rat

4.2 Group 2 ISPH induced MI rat

4.3 Group 3 Mangiferin alone treated rat

4.4 Group 4 Mangiferin pretreated ISPH induced MI rat
PLATE 5 - ELECTROPHORETIC PATTERN OF PLASMA PROTEINS OF RATS

5.1 Group 1 control

5.2 Group 2 ISPH induced MI rat

5.3 Group 3 Mangiferin alone treated rat

5.4 Group 4 Mangiferin pretreated and ISPH induced MI rat
Figure 5: Levels of serum protein and A/G ratio in normal and experimental group of rats. Values are expressed as mean ± S.D. for 6 animals in each group.

Statistical calculation: One way ANOVA followed by posthoc test LSD
a - Compared with Group 1 *** p < 0.001; ** p < 0.01, * p < 0.05, NS - Non significant
b - Compared with Group 2
The serum total protein fractions and albumin: globulin ratios were found to be significantly reduced ($p<0.001$) in Group 2 isoproterenol induced rats when compared to Group 1 control rats. Group 4 mangiferin pretreated rats exhibited a significant increase ($p<0.001$) in these values when compared to isoproterenol induced Group 2 rats. Group 3 drug control (mangiferin alone treated) rats showed non significant changes when compared to Group 1 control animals.

**Serum and heart tissue lipid peroxides**

The levels of lipid peroxide both in serum and heart tissue of control and experimental animals are expressed in Figure 6.

Isoproterenol administered Group 2 rats showed a significant increase in the levels of LPO ($p<0.001$) both in serum and heart tissue when compared to Group 1 control rats. Mangiferin pretreated Group 4 rats showed a significant decrease in LPO level ($p<0.001$) both in serum and heart tissue when compared to isoproterenol induced Group 2 rats. In drug alone treated Group 3 rats, the levels of LPO both in serum and tissue were maintained at near normal levels and showed non significant changes as compared with Group 1 control rats.

**Serum non-enzymic antioxidants**

Table 2 displays the level of serum non-enzymic antioxidants namely uric acid, ceruloplasmin, ascorbate and alpha tocopherol of control and experimental animals.
Figure 6: Levels of serum and heart lipid peroxides in normal and experimental group of rats.

Values are expressed as mean ± S.D. for 6 animals in each group.

Units
n moles of TBARS / dl in serum
n moles of TBARS / mg of protein in tissue

Statistical calculation: One way ANOVA followed by posthoc test LSD
a - Compared with Group 1  *** p < 0.001, ** p < 0.01, * p < 0.05, NS - Non significant
b - Compared with Group 2
Table 2: Level of serum non enzymic antioxidants in control and experimental group of rats

Values are expressed as mean ± SD for six animals in each group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Group 1)</th>
<th>ISPH induced (Group 2)</th>
<th>Mangiferin alone treated (Group 3)</th>
<th>Mangiferin treated + ISPH Induced (Group 4)</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceruloplasmin (Units/ml)</td>
<td>1.30 ± 0.11</td>
<td>0.44 ± 0.02 a****</td>
<td>1.34 ± 0.12 a***</td>
<td>1.16 ± 0.11 b***</td>
<td>102.84</td>
</tr>
<tr>
<td>Uric Acid (mg/dl)</td>
<td>5.54 ± 0.34</td>
<td>6.56 ± 0.21 a***</td>
<td>5.09 ± 0.49 a***</td>
<td>5.62 ± 0.33 b***</td>
<td>17.57</td>
</tr>
<tr>
<td>Vitamin C (mg dl)</td>
<td>5.90 ± 0.18</td>
<td>3.23 ± 0.16 a***</td>
<td>5.88 ± 0.24 a***</td>
<td>5.59 ± 0.18 b***</td>
<td>263.16</td>
</tr>
<tr>
<td>Vitamin E (mg/dl)</td>
<td>14.92 ± 0.25</td>
<td>9.61 ± 0.94 a***</td>
<td>15.41 ± 0.37 a***</td>
<td>14.01 ± 0.79 b***</td>
<td>96.49</td>
</tr>
</tbody>
</table>

Statistical calculation: One way ANOVA followed by post hoc test LSD
a - Compared with Group 1  *** p < 0.001. ** p < 0.01. * p < 0.05  NS - Non significant
b - Compared with Group 2
The Group 2 isoproterenol induced rats showed a significant increase ($p<0.001$) in serum uric acid and a significant decrease ($p<0.001$) in ceruloplasmin, vitamin C and vitamin E levels compared to Group 1 control animals. Mangiferin pretreated rats Group 4 showed a significant decrease in uric acid ($p<0.001$) level and a significant increase ($p<0.001$) in ceruloplasmin, vitamin C and vitamin E levels compared to Group 2 isoproterenol administrated rats Group 3 drug control rats showed a non significant change in all these parameters when compared to Group 1 control rats.

**Figure 7** represents the level of serum iron and plasma iron binding capacity of control and experimental animals.

In isoproterenol induced Group 2 rats, the plasma iron binding capacity was observed to be decreased significantly ($p<0.001$) with a concomitant increase in free iron concentration ($p<0.001$) when compared to Group 1 control rats. In Group 4 mangiferin pretreated rats, the free iron concentration ($p<0.01$) was found to be decreased with a significant increase in plasma iron binding capacity ($p<0.01$) when compared to Group 2 ISPH induced rats. Group 3 drug alone treated rats showed non significant change in these two parameters when compared to Group 1 control rats.

**Serum, heart tissue glutathione and tissue antioxidant enzymes**

**Table 3** predicts the activity of serum, tissue glutathione and tissue antioxidant enzymes such as glutathione peroxidase, glutathione reductase, glutathione-s-transferase, superoxide dismutase and catalase in control and
Figure 7: Levels of serum iron and plasma iron binding capacity in normal and experimental group of rats

Values are expressed as mean ± S.D. for 6 animals in each group

Units: µg/dl of serum iron
       µg/dl of plasma iron binding capacity

Statistical calculation: One way ANOVA followed by posthoc test LSD

a - Compared with Group 1 *** p < 0.001; ** p < 0.01, * p < 0.05, NS - Non significant
b - Compared with Group 2
Table 3: Level of serum, heart glutathione and activities of heart tissue antioxidant enzymes in control and experimental group of rats

Values are expressed as mean ± SD for six animals in each group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Group 1)</th>
<th>ISPH induced (Group 2)</th>
<th>Mangiferin alone treated (Group 3)</th>
<th>Mangiferin treated + ISPH Induced (Group 4)</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Glutathione (mg/dl)</td>
<td>56.66 ± 3.56</td>
<td>37.04 ± 2.90***</td>
<td>55.85 ± 2.69**</td>
<td>51.80 ± 2.65 b***</td>
<td>56.22</td>
</tr>
<tr>
<td>Heart Glutathione (nmole of GSH/g tissue)</td>
<td>6.70 ± 0.18</td>
<td>4.41 ± 0.18***</td>
<td>6.72 ± 0.21 a**</td>
<td>6.39 ± 0.35 b***</td>
<td>122.46</td>
</tr>
<tr>
<td>Glutathione transferase (nmole of CDNB conjugated 'min' mg protein)</td>
<td>677.56 ± 46.55</td>
<td>461.51 ± 30.49***</td>
<td>702.16 ± 51.31 b**</td>
<td>608.16 ± 41.02 b***</td>
<td>37.86</td>
</tr>
<tr>
<td>Glutathione peroxidase (µg of GSH utilised 'min/mg protein)</td>
<td>3.65 ± 0.26</td>
<td>1.94 ± 0.13***</td>
<td>3.75 ± 0.26 b**</td>
<td>3.33 ± 0.13 b***</td>
<td>93.36</td>
</tr>
<tr>
<td>Glutathione reductase (µg of GSSG utilised 'min/mg protein)</td>
<td>6.42 ± 0.25</td>
<td>4.66 ± 0.23***</td>
<td>6.40 ± 0.19 b**</td>
<td>6.08 ± 0.21 b***</td>
<td>89.39</td>
</tr>
<tr>
<td>SOD (50% of inhibition at the auto oxidation of epinephrine/min/mg)</td>
<td>8.46 ± 0.79</td>
<td>4.05 ± 0.26***</td>
<td>8.38 ± 0.76 b**</td>
<td>7.50 ± 0.56 b***</td>
<td>64.61</td>
</tr>
<tr>
<td>Catalase (nmole of H$_2$O$_2$ decomposed /min/mg protein)</td>
<td>5.65 ± 0.38</td>
<td>3.70 ± 0.29***</td>
<td>5.86 ± 0.31 b**</td>
<td>5.18 ± 0.37 b***</td>
<td>47.69</td>
</tr>
</tbody>
</table>

Statistical calculation One way ANOVA followed by posthoc test LSD

a - Compared with Group 1  *** p < 0.001, ** p < 0.01, * p < 0.05, NS - Non significant

b - Compared with Group 2
experimental group of rats. Activity of serum, tissue glutathione and tissue antioxidant enzymes were found to be decreased (p<0.001) significantly in isoproterenol administered Group 2 rats when compared to Group 1 control animals. Group 4 rats exhibited a marked increase (p<0.001) in these parameters when compared to Group 2 rats. Group 3 drug control (mangiferin alone treated) rats showed non significant changes in all these parameters as compared with Group 1 control rats.

**Lipids, lipoproteins and lipases in serum and heart tissue**

The level of serum and heart tissue cholesterol fractions and serum LDL, VLDL & HDL cholesterol levels of normal and experimental groups of rats are represented in Table 4.

Cholesterol level both in serum and heart tissue showed a significant increase (p<0.001) with concomitant increase in (p<0.001) serum LDL and VLDL cholesterol fractions and a significant decrease in serum HDL cholesterol (p<0.001) level in Group 2 isoproterenol rats when compared to Group 1 control rats.

In mangiferin pretreated Group 4 rats, the levels of serum LDL, VLDL (p< 0.01) and all serum cholesterol fractions ((p<0.001) showed a significant decrease (p<0.001) with a significant increase (p<0.01) in serum HDL cholesterol level when compared to Group 2 ISPH induced rats. Similarly in heart tissue of mangiferin pretreated Group 4 rats, the levels of total cholesterol, free cholesterol (p<0.01) and ester cholesterol (p<0.05) showed a significant decrease as compared with Group 2 isoproterenol induced rats.
Table 4: Level of serum, heart cholesterol and serum cholesterol lipoprotein fractions in control and experimental group of rats

Values are expressed as mean ± SD for six animals in each group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Group-1)</th>
<th>ISPH induced (Group-2)</th>
<th>Mangiferin alone treated (Group-3)</th>
<th>Mangiferin treated + ISPH Induced (Group-4)</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>90.07 ± 5.64</td>
<td>140.50 ± 6.37***</td>
<td>86.38 ± 4.98 aNS</td>
<td>97.57 ± 6.73 b***</td>
<td>105.17</td>
</tr>
<tr>
<td>Free cholesterol (mg/dl)</td>
<td>37.23 ± 2.63</td>
<td>55.75 ± 3.54 a***</td>
<td>35.25 ± 2.27 aNS</td>
<td>41.58 ± 2.24 b***</td>
<td>69.077</td>
</tr>
<tr>
<td>Ester cholesterol (mg dl)</td>
<td>52.83 ± 3.08</td>
<td>84.75 ± 2.85 a***</td>
<td>51.13 ± 7.23 aS</td>
<td>55.98 ± 4.57 b***</td>
<td>66.31</td>
</tr>
<tr>
<td>Total Cholesterol (mg/g tissue)</td>
<td>5.24 ± 0.12</td>
<td>5.78 ± 0.14 a***</td>
<td>5.18 ± 0.18 aS</td>
<td>5.50 ± 0.19 b**</td>
<td>16.1</td>
</tr>
<tr>
<td>Ester cholesterol (mg/g tissue)</td>
<td>1.72 ± 0.02</td>
<td>1.80 ± 0.02 a***</td>
<td>1.71 ± 0.02 aS</td>
<td>1.76 ± 0.03 b**</td>
<td>14.17</td>
</tr>
<tr>
<td>Free Cholesterol (mg/g tissue)</td>
<td>3.52 ± 0.10</td>
<td>3.97 ± 0.12 a***</td>
<td>3.47 ± 0.17 aS</td>
<td>3.74 ± 0.15 b*</td>
<td>15.35</td>
</tr>
<tr>
<td>LDL_c (mg/dl)</td>
<td>35.77 ± 1.85</td>
<td>42.21 ± 1.78 a***</td>
<td>34.94 ± 1.85 aS</td>
<td>38.22 ± 1.94 b**</td>
<td>18.47</td>
</tr>
<tr>
<td>HDL_c (mg/dl)</td>
<td>19.29 ± 0.98</td>
<td>16.86 ± 0.59 a***</td>
<td>19.68 ± 1.03 aS</td>
<td>18.39 ± 0.62 b**</td>
<td>13.54</td>
</tr>
<tr>
<td>VLDL_c (mg dl)</td>
<td>31.91 ± 1.39</td>
<td>36.58 ± 1.62 a***</td>
<td>31.70 ± 1.40 aS</td>
<td>33.91 ± 1.24 b**</td>
<td>15.14</td>
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</tbody>
</table>

Statistical calculation: One way ANOVA followed by post hoc test LSD

a - Compared with Group 1  *** p < 0.001.  ** p < 0.01,  * p < 0.05.  NS - Non significant
b - Compared with Group 2


Group 3 drug control rats showed a non significant changes for all the above parameters both in serum and heart tissue when compared to Group 1 control rats.

Figure 8 depicts the LDLc/HDLc (risk factor) ratio.

The risk factor ratio (LDLc/ HDLc) was found to be elevated significantly (p<0.001) in Group 2 isoproterenol induced rats when compared to Group 1 control rats and it showed significantly (p<0.001) reduced value in Group 4 mangiferin pretreated rats when compared to isoproterenol administered Group 2 rats. In Group 3 drug control animals, this ratio was observed to be non significant when compared to Group 1 control rats.

Table 5 denotes the level of serum and heart tissue free fatty acid levels, triglycerides, serum VLDL_{1G}, LDL_{1G}, fractions and heart tissue lipase enzyme activity in control and experimental group of rats.

Rats administered with isoproterenol (Group 2) showed a significantly increased value of tracylglycerol in LDL and VLDL fractions with concomitant increase in (p<0.001) serum and heart tissue triglyceride, free fatty acid levels. Similarly, a significant increase (p<0.001) in the activity of tracyl glycerol lipase with significant decrease (p<0.001) in the activity of lipoprotein lipase in heart tissue were observed in Group 2 rats when compared to Group 1 control rats.

In Group 4 mangiferin pretreated rats, the level of VLDL_{TG} and LDL_{TG} (p<0.01) was found to be significantly reduced with a significant
Level of serum LDL/HDL cholesterol ratio (risk factor) in normal experimental group of rats

Values are expressed as mean ± S.D. for 6 animals in each group

Groups

Control
ISPH induced
Mangiferin alone
Mangiferin + ISPH

Statistical calculation: One way ANOVA followed by posthoc test LSD
a - Compared with Group 1  *** p < 0.001  ** p < 0.01  * p < 0.05  NS - Non significant
b - Compared with Group 2
Table 5: Level of serum triglyceride lipoprotein fractions, heart triglycerides, free fatty acids and heart lipase enzyme activity in control and experimental group of rats

Values are expressed as mean ± SD for 6 animals in each group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Group 1)</th>
<th>ISPH induced (Group 2)</th>
<th>Mangiferin alone treated (Group 3)</th>
<th>Mangiferin treated + ISPH Induced (Group 4)</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Triglyceride (mg/dl)</td>
<td>22.42 ± 1.53</td>
<td>31.14 ± 1.77</td>
<td>21.16 ± 1.46 a NS</td>
<td>24.61 ± 1.76 b NS</td>
<td>43.98</td>
</tr>
<tr>
<td>Serum Free Fatty acids (mg/dl)</td>
<td>30.60 ± 2.69</td>
<td>43.37 ± 3.06</td>
<td>31.53 ± 2.55 a NS</td>
<td>36.69 ± 2.94 b NS</td>
<td>25.88</td>
</tr>
<tr>
<td>Serum LDL₁₁₀ (mg/dl)</td>
<td>23.69 ± 1.47</td>
<td>29.25 ± 1.54</td>
<td>23.00 ± 1.67 a NS</td>
<td>26.28 ± 1.90 b NS</td>
<td>17.54</td>
</tr>
<tr>
<td>Serum VLDL₁₁₀ (mg/dl)</td>
<td>30.86 ± 1.11</td>
<td>36.94 ± 1.93</td>
<td>29.87 ± 1.20 a NS</td>
<td>33.15 ± 2.26 b NS</td>
<td>20.45</td>
</tr>
<tr>
<td>Heart Triglyceride (mg/g) tissue</td>
<td>3.25 ± 0.14</td>
<td>4.10 ± 0.19</td>
<td>3.16 ± 0.14 a NS</td>
<td>3.50 ± 0.18 b NS</td>
<td>38.22</td>
</tr>
<tr>
<td>Heart Free Fatty acids (mg/g) tissue</td>
<td>0.49 ± 0.04</td>
<td>0.73 ± 0.05</td>
<td>0.50 ± 0.04 a NS</td>
<td>0.60 ± 0.05 b NS</td>
<td>31.32</td>
</tr>
<tr>
<td>Heart Lipoprotein lipase (mg of glycerol formed/hour/mg protein)</td>
<td>5.60 ± 0.20</td>
<td>4.87 ± 0.15</td>
<td>5.68 ± 0.23 a NS</td>
<td>5.29 ± 0.19 b NS</td>
<td>20.85</td>
</tr>
<tr>
<td>Heart Triglyceride lipase (mg of glycerol formed/hour/mg protein)</td>
<td>2.62 ± 0.18</td>
<td>3.81 ± 0.19</td>
<td>2.59 ± 0.19 a NS</td>
<td>2.94 ± 0.21 b NS</td>
<td>47.04</td>
</tr>
</tbody>
</table>

Statistical calculation: One way ANOVA followed by posthoc test LSD
a - Compared with Group 1  *** p < 0.001, ** p < 0.01, * p < 0.05. NS - Non significant
b - Compared with Group 2
(p<0.001) decrease in serum and heart tissue triglyceride, free fatty acid levels. Similarly, a significant decrease in heart tissue triglyceride lipase (p<0.001) activity with concomitant increase (p<0.01) in lipoprotein lipase activity were observed in Group 4 rat when compared to Group 2 isoproterenol induced rats. Group 3 mangiferin alone treated rats showed a non significant change as compared with Group 1 control rats.

**Figure 9** represents the level of phospholipids in the heart of normal and experimental group of rats.

Group 2 ISPH induced rats showed a significant decrease in tissue phospholipid level when compared to Group 1 control rats whereas a significant increase in tissue phospholipid level was observed in Group 4 mangiferin pretreated rats as compared with Group 2 ISPH rats. A non significant change was observed in Group 3 drug control rats when compared to Group 1 control rats.

**Blood glucose and heart glycogen**

**Figure 10** shows the level of blood glucose and heart tissue glycogen of control and experimental animals. In isoproterenol induced Group 2 rats, the level of blood glucose was found to be significantly increased (p<0.001) while tissue glycogen level was found to be decreased (p<0.001) when compared to Group 1 control animals. Group 4 mangiferin pretreated rats showed a significant decrease (p<0.001) in blood glucose level with a significant increase (p<0.001) in tissue glycogen level as compared
Figure 9: Level of heart phospholipids in normal and experimental group of rats.
Values are expressed as mean ± S.D. for 6 animals in each group.

Statistical calculation: One way ANOVA followed by posthoc test LSD.
a - Compared with Group 1 *** p < 0.001; ** p < 0.01, * p < 0.05, NS - Non significant
b - Compared with Group 2
Figure 10: Level of blood glucose and heart glycogen in normal and experimental group of rats. Values are expressed as mean ± S.D. for 6 animals in each group.

Units: mg/dl of serum glucose
mg/g of tissue glycogen

Statistical calculation: One way ANOVA followed by posthoc test LSD
a - Compared with Group 1  *** p < 0.001, ** p < 0.01, * p < 0.05, NS - Non significant
b - Compared with Group 2
with Group 2 isoproterenol induced rats. In Group 3 mangiferin alone treated rats, these parameters showed a non-significant change (NS) when compared to Group 1 control rats.

**Tissue protein, DNA and RNA**

The levels of total protein, DNA and RNA in heart tissue of the control and experimental animals are tabulated in **Table 6**

Isoproterenol induced Group 2 rats showed a significant increase (p<0.001) in protein, DNA and RNA content in heart tissues when compared to Group 1 control rats. The same parameters showed a significant decrease (p<0.001) in mangiferin pretreated and isoproterenol administered Group 4 rats as compared with isoproterenol induced Group 2 rats. In Group 3 drug alone treated rats, these values were found to be non significant when compared to Group 1 control rats.

**Elemental analysis in serum and heart tissue**

Electrolytes such as sodium and potassium levels both in serum and heart of control and experimental group of rats are presented in **Figure 11**.

Sodium level in serum showed a significant reduction (p<0.001) with a remarkable increase (p<0.001) in serum potassium level, whereas tissue sodium level was significantly increased (p<0.001) with significant decrease in tissue potassium level (p<0.001) in Group 2 isoproterenol induced rats when compared to Group 1 control rats.
Table 6: Level of heart protein and nucleic acids in control and experimental group of rats

Values are expressed as mean ± SD for 6 animals in each group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Group-1)</th>
<th>ISPH induced (Group-2)</th>
<th>Mangiferin alone treated (Group-3)</th>
<th>Mangiferin treated + ISPH Induced (Group-4)</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart protein (mg/g tissue)</td>
<td>152.66 ± 8.64</td>
<td>239.16 ± 17.61&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;***&lt;/sup&gt;</td>
<td>149.50 ± 9.77&lt;sup&gt;aNS&lt;/sup&gt;</td>
<td>171.33 ± 6.56&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;***&lt;/sup&gt;</td>
<td>80.08</td>
</tr>
<tr>
<td>DNA (mg/g tissue)</td>
<td>19.50 ± 0.83</td>
<td>47.98 ± 3.28&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;***&lt;/sup&gt;</td>
<td>18.86 ± 1.10&lt;sup&gt;aNS&lt;/sup&gt;</td>
<td>22.03 ± 1.47&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;***&lt;/sup&gt;</td>
<td>315.62</td>
</tr>
<tr>
<td>RNA (mg/g tissue)</td>
<td>9.50 ± 0.83</td>
<td>23.99 ± 1.62&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;***&lt;/sup&gt;</td>
<td>8.98 ± 0.63&lt;sup&gt;aNS&lt;/sup&gt;</td>
<td>10.82 ± 0.83&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;***&lt;/sup&gt;</td>
<td>275.79</td>
</tr>
</tbody>
</table>

Statistical calculation: One way ANOVA followed by posthoc test LSD

- a - Compared with Group 1<sup>***</sup> p < 0.001, ** p < 0.01, * p < 0.05. NS - Non significant
- b - Compared with Group 2
Figure 11  Level of serum, tissue, sodium and potassium in normal group of rats

Values are expressed as mean ± S.D. for 6 animals in each group

Units µg/g tissue
mg/l serum

Statistical calculation One way ANOVA followed by posthoc test LSD
a - Compared with Group 1  *** p < 0.001, ** p < 0.01, * p < 0.05, NS - Non significant
b - Compared with Group 2
The level of Na⁺ was increased significantly (p<0.001) in serum with significant (p<0.001) decrease in serum K⁺ level, where as heart tissue Na⁺ showed a significant (p<0.05) decrease, with significant increase (p<0.001) in tissue K⁺ levels in Group 4 mangiferin pretreated rats when compared to Group 2 ISPH induced myocardial damaged rats. A non significant change was observed both in serum and heart tissue sodium and potassium levels in Group 3 drug alone treated rats.

**Figure 12** displays serum and tissue magnesium level of control and experimental group of rats. Serum magnesium value was found to be increased significantly (p<0.001) while tissue magnesium value was significantly decreased (p<0.001) in Group 2 isoproterenol induced rats. In Group 4 mangiferin pretreated rats serum Mg²⁺ (p<0.001) showed significantly decreased level and tissue Mg²⁺ (p<0.001) showed a significantly increased level when compared to Group 2 isoproterenol induced rats. Group 3 drug alone treated rats showed a non significant change in serum and tissue Mg²⁺ level when compared to Group 1 control animals.

Serum and tissue calcium levels are represented in **Figure 13**. Calcium level in serum showed significant reduction (p<0.001) whereas tissue calcium level was significantly increased (p<0.001) in Group 2 isoproterenol induced rats when compared to Group 1 control rats. The level of Ca²⁺ was increased significantly (p<0.01) in serum and reduced significantly in tissue (p<0.001) in Group 4 mangiferin pretreated rats when compared to Group 2.
Figure 12: Level of serum and heart magnesium in normal and experimental group of rats

Values are expressed as mean ± S.D. for 6 animals in each group

- **Control**
- **ISPH Induced**
- **Mangiferin alone**
- **Mangiferin + ISPH**

Statistical calculation: One way ANOVA followed by posthoc test LSD

- a: Compared with Group 1  *** p < 0.001, ** p < 0.01, * p < 0.05, NS - Non significant
- b: Compared with Group 2

Units: μg/g tissue, mg/l serum
Figure 13: Level of serum and heart calcium in normal and experimental group of rats
Values are expressed as mean ± S.D. for 6 animals in each group

Statistical calculation: One way ANOVA followed by posthoc test LSD
a - Compared with Group 1  *** p < 0.001, ** p < 0.01, * p < 0.05, NS - Non significant
b - Compared with Group 2
isoproterenol induced myocardial damaged rats. A non significant change was observed both in serum and heart tissue calcium level in Group 3 drug alone treated rats.

Figure 14 predicts serum copper and zinc levels of control and experimental animals.

The level of copper was increased significantly (p<0.001) and zinc level was decreased significantly (p<0.001) in serum of Group 2 isoproterenol induced rats compared to Group 1 control rats. In mangiferin pretreated Group 4 rats, the level of serum copper showed a significant decrease (p<0.001) and a significant increase (p<0.001) was found in serum zinc level compared to Group 2 isoproterenol administered rats and a non significant change (NS) was observed in Group 3 rats both for serum zinc and copper levels when compared to Group 1 control animals.

Mitochondrial Lipid peroxides

The level of mitochondrial LPO in the heart of control and experimental animals are depicted in Figure 15.

The isoproterenol induced Group 2 rats showed a significant increase (p<0.001) in mitochondrial lipid peroxide levels when compared to Group 1 control rats. In mangiferin pretreated Group 4 rats, a significant decrease in mitochondrial LPO (p<0.001) was observed when compared to Group 2 isoproterenol induced rats. The same parameter showed a near
Figure 14: Level of serum copper and zinc in normal and experimental group of rats. Values are expressed as mean ± S.D. for 6 animals in each group.

Statistical calculation: One way ANOVA followed by posthoc test LSD.
- a: Compared with Group 1, *** p < 0.001, ** p < 0.01, * p < 0.05, NS - Non significant
- b: Compared with Group 2
Figure 15: Level of heart mitochondrial lipid peroxides in normal and experimental group of rats
Values are expressed as mean ± S.D. for 6 animals in each group

Statistical calculation: One way ANOVA followed by posthoc test LSD
a - Compared with Group 1  *** p < 0.001, ** p < 0.01, * p < 0.05, NS - Non significant
b - Compared with Group 2
normal level in drug alone treated Group 3 rats and observed a non significant change as compared with Group 1 control animals.

**Mitochondrial glutathione and antioxidant enzymes**

The level of glutathione and the activity of antioxidant enzymes of mitochondria in the heart of control and experimental animals are denoted in **Table 7**.

The Group 2 isoproterenol induced rats showed a significant reduction ($p<0.001$) in glutathione and antioxidant enzyme activities such as GPx, GST, GSH, GR, SOD and Catalase. Mangiferin pretreated Group 4 rats showed a significant increase in mitochondrial GSH, GPx, GST, GRase, SOD ($p<0.001$) and catalase ($p<0.01$) activities when compared to Group 2 isoproterenol induced rats. In Group 3 drug alone treated rats, these parameters showed a non significant change (NS) when compared to Group 1 control animals.

**Mitochondrial protein, membrane bound enzymes and elemental studies**

The activity of mitochondrial membrane bound enzymes such as Na$^+$.K$^+$.-ATPase, Ca$^{2+}$.-ATPase, protease, phospholipase and protein level of control and experimental animals are depicted in **Table 8**.

Protease and phospholipase enzyme activities were observed to be significantly increased ($p<0.001$) with a significant decrease ($p<0.001$) in mitochondrial protein level as well as in the activities of Na$^+$.K$^+$.-ATPase and
Table 7: Activities of mitochondrial antioxidant enzymes and GSH in control and experimental group of rats

Values are expressed as mean ± SD for 6 animals in each group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Group 1)</th>
<th>ISPH induced (Group 2)</th>
<th>Mangiferin alone treated (Group 3)</th>
<th>Mangiferin treated + ISPH Induced (Group 4)</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione (nmol/100mg protein)</td>
<td>4.27 ± 0.018</td>
<td>4.16 ± 0.010 ***</td>
<td>4.28 ± 0.018 NS</td>
<td>4.25 ± 0.018 NS</td>
<td>62.06</td>
</tr>
<tr>
<td>Glutathione per oxidase (nmol of GSH oxidized/min/100 mg protein)</td>
<td>1.97 ± 0.17</td>
<td>1.39 ± 0.11 ***</td>
<td>2.04 ± 0.14 NS</td>
<td>1.78 ± 0.17 NS</td>
<td>22.16</td>
</tr>
<tr>
<td>Glutathione transferase (nmol of CDNB conjugated per min/100 mg protein)</td>
<td>69.23 ± 3.71</td>
<td>52.98 ± 1.53 ***</td>
<td>69.47 ± 6.16 NS</td>
<td>64.43 ± 1.57 NS</td>
<td>25.27</td>
</tr>
<tr>
<td>Glutathione reductase (nmol of NADPH oxidised/min/100mg protein)</td>
<td>3.56 ± 0.27</td>
<td>2.81 ± 0.21 ***</td>
<td>3.76 ± 0.15 NS</td>
<td>3.30 ± 0.18 NS</td>
<td>22.4</td>
</tr>
<tr>
<td>SOD (50% inhibition at the auto oxidation of epinephrine/min/100 mg protein)</td>
<td>12.22 ± 0.68</td>
<td>5.66 ± 0.24 ***</td>
<td>12.35 ± 0.89 NS</td>
<td>11.11 ± 0.79 NS</td>
<td>122.4</td>
</tr>
<tr>
<td>Catalase (n moles of H₂O₂ decomposed min/100mg protein)</td>
<td>1.87 ± 0.096</td>
<td>1.36 ± 0.11 ***</td>
<td>1.91 ± 0.17 NS</td>
<td>1.64 ± 0.12 NS</td>
<td>22.42</td>
</tr>
</tbody>
</table>

Statistical calculation: One way ANOVA followed by posthoc test LSD

a - Compared with Group 1  *** p < 0.001, ** p < 0.01, * p < 0.05. NS - Non significant
b - Compared with Group 2
Table 8: Level of mitochondrial protein and membrane bound enzyme activity in control and experimental group of rats

Values are expressed as mean ± SD for 6 animals in each group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Group 1)</th>
<th>ISPH induced (Group 2)</th>
<th>Mangiferin alone treated (Group 3)</th>
<th>Mangiferin treated + ISPH Induced (Group 4)</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (mg/g tissue)</td>
<td>36.41 ± 1.70</td>
<td>24.35 ± 1.13**</td>
<td>36.72 ± 2.10**</td>
<td>33.83 ± 1.47**</td>
<td>74.85</td>
</tr>
<tr>
<td>Na⁺K⁺ ATPase (µmole of pi liberated/min/mg protein)</td>
<td>0.21 ± 0.018</td>
<td>0.13 ± 0.009**</td>
<td>0.23 ± 0.018**</td>
<td>0.19 ± 0.019**</td>
<td>32.1</td>
</tr>
<tr>
<td>Ca²⁺ ATPase (µmole of pi/min/mg protein)</td>
<td>0.915 ± 0.059</td>
<td>0.65 ± 0.037**</td>
<td>0.94 ± 0.041**</td>
<td>0.85 ± 0.050**</td>
<td>42.81</td>
</tr>
<tr>
<td>Protease (nmole aminoacid liberated/hr mg protein)</td>
<td>161.96 ± 11.67</td>
<td>200.86 ± 17.86**</td>
<td>156.44 ± 7.64**</td>
<td>181.35 ± 7.48**</td>
<td>17.18</td>
</tr>
<tr>
<td>Phospholipase (nmole free fatty acid liberated per minute /mg protein)</td>
<td>0.27 ± 0.017</td>
<td>0.67 ± 0.016**</td>
<td>0.26 ± 0.013**</td>
<td>0.29 ± 0.021**</td>
<td>756.6</td>
</tr>
</tbody>
</table>

Statistical calculation: One way ANOVA followed by posthoc test LSD

a - Compared with Group 1  *** p < 0.001,  ** p < 0.01,  * p < 0.05. NS - Non significant
b - Compared with Group 2
Ca\textsuperscript{2+}-ATPases (p<0.001) in isoproterenol administered Group 2 rats as compared with Group 1 control rats. A significant elevation in the activity of Na\textsuperscript{+}-K\textsuperscript{+} - and Ca\textsuperscript{2+}-ATPases (p<0.001) with a concomitant decrease in phospholipase (p<0.001) and protease (p<0.05) activities and significant increase (p<0.01) in mitochondrial protein level were noticed in mangiferin pretreated Group 4 rats when compared to Group 2 ISPH induced rats. Group 3 rats exhibited non significant changes as compared with Group 1 control rats.

Mitochondrial elements such as Na\textsuperscript{+}, K\textsuperscript{+} and Ca\textsuperscript{2+} levels in normal and experimental animals are reported in Figure 16.

The level of sodium and calcium were observed to be significantly increased (p<0.001) while potassium was observed to be decreased significantly (p<0.001) in Group 2 isoproterenol induced rats compared to Group 1 control rats. In Group 4 mangiferin pretreated rats, Na\textsuperscript{+} (p<0.05) and Ca\textsuperscript{2+} (p<0.001) level were significantly decreased and K\textsuperscript{+} value was found to be significantly increased (p<0.001) when compared to Group 2 isoproterenol administered rats. No significant change was observed in Group 3 drug alone treated rats as compared with Group 1 control animals.

**Enzymes of TCA cycle and inner mitochondria**

The activity of mitochondrial tricarboxylic acid cycle enzymes and cytochrome oxidase and NADH dehydrogenase activities of control and experimental animals are represented in Table 9.
Figure 16: Level of heart mitochondrial sodium, potassium and calcium in normal and experimental group of rats
Values are expressed as mean ± S.D. for 6 animals in each group

Mitochondrial sodium

Mitochondrial potassium

Mitochondrial calcium

Statistical calculation: One way ANOVA followed by posthoc test LSD
a - Compared with Group 1  *** p < 0.001, ** p < 0.01, * p < 0.05, NS - Non significant
b - Compared with Group 2
Table 9: Activity of heart mitochondrial TCA cycle enzymes in control and experimental group of rats

Values are expressed as mean ± SD for 6 animals in each group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Group 1)</th>
<th>ISPH induced (Group 2)</th>
<th>Mangiferin alone treated (Group 3)</th>
<th>Mangiferin treated + ISPH Induced (Group 4)</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malate dehydrogenase (nmoles of NADH oxidized/minute/mg protein)</td>
<td>335 45 ± 16 67</td>
<td>250 66 ± 17 45 ***</td>
<td>337 97 ± 20 78 a ***</td>
<td>310 56 ± 23 10 b ***</td>
<td>25 53</td>
</tr>
<tr>
<td>Succinate dehydrogenase (nmoles of succinate oxidised/min/mg protein)</td>
<td>250 16 ± 14 13</td>
<td>175 14 ± 12 71 ***</td>
<td>251 57 ± 16 93 a ^s</td>
<td>231 06 ± 15 83 b ***</td>
<td>34 22</td>
</tr>
<tr>
<td>α-keto glutarate dehydrogenase (nmoles of ferrocyanide formed/hour/mg protein)</td>
<td>62 83 ± 2 22</td>
<td>53 13 ± 3 74 ****</td>
<td>63 50 ± 2 51 a ^s</td>
<td>59 51 ± 4 20 b ***</td>
<td>12 53</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase (nmoles of alpha ketoglutarate formed/hr/mg protein)</td>
<td>714 73 ± 37 62</td>
<td>582 98 ± 41 95 ^***</td>
<td>721 08 ± 39 05 ^s</td>
<td>649 30 ± 46 42 b ^*</td>
<td>14 67</td>
</tr>
<tr>
<td>NADH dehydrogenase (nmoles of NADH oxidized/minute/mg protein)</td>
<td>138 61 ± 9 73</td>
<td>90 01 ± 6 28 ^***</td>
<td>121 28 ± 8 48 a ^**</td>
<td>128 94 ± 9 00 b ^***</td>
<td>36 94</td>
</tr>
<tr>
<td>Cytochrome ‘C’ oxidase (nmoles/min/mg protein)</td>
<td>0 308 ± 0 021</td>
<td>0 24 ± 0 019 ^***</td>
<td>0 31 ± 0 024 a ^s</td>
<td>0 28 ± 0 014 b **</td>
<td>16 46</td>
</tr>
</tbody>
</table>

Statistical calculation: One way ANOVA followed by posthoc test LSD

a - Compared with Group 1  *** p < 0.001. ** p < 0.01. * p < 0.05. NS - Non significant
b - Compared with Group 2
The citric acid cycle enzymes such as malate-dehydrogenase, succinate dehydrogenase, alpha keto glutarate dehydrogenase and isocitrate dehydrogenase showed a highly significant decrease (p<0.001) in their activities in Group 2 isoproterenol induced rats and also a significant decrease (p<0.001) was exhibited by inner mitochondrial enzymes such as cytochrome oxidase and NADH dehydrogenase in Group 2 isoproterenol induced rats. Mangiferin pretreated Group 4 rats showed significant increase in these enzyme activities such as MDH, SDH, NADH DH (p<0.001), AKGDH, Cytochrome oxidase (p<0.01), ICDH (p<0.05) when compared to isoproterenol administered Group 2 rats. The enzyme levels showed non significant changes in Group 3 rats as compared with Group 1 control animals except NADH dehydrogenase.

Plasma Lactate

Figure 17 depicts the level of plasma lactate content of control and experimental animals.

In Group 2 isoproterenol induced rats, the content of plasma lactate was significantly higher (p<0.001) compared to Group 1 control rats, and the lactate amount was significantly decreased (p<0.001) in Group 4 mangiferin pretreated rats compared to Group 2 isoproterenol induced rats. Group 3 drug control rats showed near normal level (NS) when compared to Group 1 control rats.
Figure 17: Level of plasma lactate in normal and experimental group of rats
Values are expressed as mean ± S.D. for 6 animals in each group

Statistical calculation: One way ANOVA followed by posthoc test LSD
- a: Compared with Group 1  *** p < 0.001, ** p < 0.01, * p < 0.05, NS - Non significant
- b: Compared with Group 2
Mitochondrial ETC and oxidative phosphorylation

The oxidation of succinate in State 3, State 4 and ATP levels are depicted in Table 10. Respiratory control ratio and oxidative phosphorylation ratio (P/O ratio) are depicted in Figure 18. All these parameters showed a significant decrease in their appropriate values \((p<0.001)\) in Group 2 rats as against Group 1 control rats. Group 4 mangiferin pretreated animals exhibited a significant increase in ATP \((p<0.01)\), State 3 \((p<0.001)\), and State 4 \((p<0.01)\) levels while comparing with isoproterenol induced Group 2 rats RCR \((p<0.01)\) and P/O ratio \((p<0.001)\) also showed significant increase in their level in Group 4 mangiferin pretreated rats when compared to isoproterenol induced Group 2 rats. All the above parameters were near normal (NS) in Group 3 drug alone treated rats as compared with Group 1 control animals.

Figures 19, 20 and 21 depict the level of NADH oxidation and the oxidation of cytochromes in heart tissue mitochondria of normal and experimental animals.

In Group 2 isoproterenol induced rats, the oxidation of NADH was found to be increased \((p<0.001)\) significantly when compared to Group 1 control animals. In Group 4 mangiferin pretreated rats, the oxidation of NADH was observed to be lowered significantly \((p<0.001)\) when compared to Group 2 isoproterenol induced rats. Group 3 rats exhibited non significant change while comparing to Group 1 control animals (Figure 19)
Table 10: Level of heart mitochondrial electron transport components in control and experimental group of rats

Values are expressed as mean ± SD for 6 animals in each group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Group 1)</th>
<th>ISPH induced (Group 2)</th>
<th>Mangiferin alone treated (Group 3)</th>
<th>Mangiferin treated + ISPH Induced (Group 4)</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP (nmoles/mg protein)</td>
<td>4.52 ± 0.16</td>
<td>3.81 ± 0.25 a***</td>
<td>4.61 ± 0.13 aNS</td>
<td>4.20 ± 0.32 b**</td>
<td>14.46</td>
</tr>
<tr>
<td>State 3 (+ ADP)</td>
<td>40.53 ± 1.54</td>
<td>30.08 ± 2.20 a****</td>
<td>40.66 ± 1.54 aNS</td>
<td>38.19 ± 2.57 b***</td>
<td>36.73</td>
</tr>
<tr>
<td>State 4 (- ADP)</td>
<td>10.30 ± 0.57</td>
<td>8.36 ± 0.59 a***</td>
<td>10.40 ± 0.65 aNS</td>
<td>9.53 ± 0.63 b**</td>
<td>14.44</td>
</tr>
</tbody>
</table>

Statistical calculation: One way ANOVA followed by posthoc test LSD

a - Compared with Group 1 *** p < 0.001, ** p < 0.01, * p < 0.05, NS - Non significant
b - Compared with Group 2
Figure 18: Level of heart mitochondrial respiratory control ratio and oxidative phosphorylation ratio in normal and experimental group of rats.
Values are expressed as mean ± S.D. for 6 animals in each group.

Statistical calculation: One way ANOVA followed by posthoc test LSD
a - Compared with Group 1  *** p < 0.001; ** p < 0.01; * p < 0.05, NS - Non significant
b - Compared with Group 2
Figure 19: Level of heart mitochondria NADH oxidation in control and experimental group of rats
Values are expressed as mean ± SD for 6 animals in each group

Statistical calculation: One way ANOVA followed by posthoc test LSD
- a - Compared with Group 1 *** p < 0.001; ** p < 0.01, * p < 0.05, NS - Non significant
- b - Compared with Group 2
Figure 20: Level of mitochondrial cytochromes c, c1 oxidation in normal and experimental group of rats
Values are expressed as mean ± S.D. for 6 animals in each group

Statistical calculation: One way ANOVA followed by posthoc test LSD
a Compared with Group 1 *** p < 0.001 ** p < 0.01 * p < 0.05 NS Non significant
b Compared with Group 2
Figure 21: Level of cytochrome b and a oxidation in normal and experimental group of rats

Values are expressed as mean ± S.D. for 6 animals in each group

Statistical calculation: One way ANOVA followed by posthoc test LSD

- Compared with Group 1: *** p < 0.001; ** p < 0.01, * p < 0.05, NS - Non significant
- Compared with Group 2
The oxidation properties of all cytochromes (c, c₁, b and aa₃) were significantly reduced (p<0.001) in Group 2 isoproterenol induced rats compared to Group 1 control rats whereas the Group 4 mangiferin pretreated rats showed significantly increased value in their oxidation (p<0.001) when compared to Group 2 necrotic animals. In Group 3 drug control rats the oxidative nature of cytochromes was found to be near normal and showed non significant changes (NS) to Group 1 control animals (Figures 20 and 21).

**Electron Microscopy Studies**

**Plate 6.1 to 6.8** shows the structure of heart mitochondria of the control and experimental animals under transmission electron microscopy.

Plate 6.1 depicts the heart mitochondria of Group 1 control animals showing normal architecture.

Mitochondria of isoproterenol administered rats (Group 2) showed swelling and membrane disruption (Plates 6.2 and 6.3). The mitochondrial cristae were also disrupted and fragmented.

The mitochondria of Group 3 mangiferin (drug alone) treated rats (Plate 6.4) showed a near normal structure similar to Group 1 control rat heart mitochondria.

The heart mitochondria from mangiferin pretreated rats (Group 4) (Plate 6.5) showed partial swelling and very minute disturbance in cristae with minimum fragmentation.
PLATE 6 - ELECTRON MICROGRAPHS OF HEART MITOCHONDRIA OF RATS

6.1 Control rat mitochondria showing regular arrangement of cristae with intact membranes

6.2 & 6.3 ISPH administered rat heart mitochondria showing explosive swelling and membrane disruption

6.4 Mangiferin alone treated rat heart showing a normal architecture of mitochondria

6.5 Mangiferin pretreated and ISPH induced rat heart showing minimal swelling without disruption of mitochondrial cristae
Mitochondrial Lipids

The level of heart mitochondrial lipid profile of control and experimental animals are listed in Table 11.

The amount of cholesterol, triglyceride and free fatty acids are found to be increased significantly in Group 2 isoproterenol induced rats (p<0.001) with a significant decrease (p<0.001) in phospholipid levels when compared to Group 1 control animals. Group 4 mangiferin pretreated rats showed a significantly decreased level of cholesterol (p<0.01), triglyceride (p<0.05) and free fatty acids (p<0.001) with a significant increase (p<0.01) in phospholipid levels when compared to isoproterenol induced Group 2 rats. Group 3 mangiferin alone treated rats showed a non significant change as compared with Group 1 control rats.

Lysosomal protein and lipid peroxides

The levels of lysosomal protein, lipid peroxides and lysosomal hydrolase enzyme activities of serum and lysosome in control and experimental rats are tabulated in Table 12.

Lysosomal protein level was found to be decreased significantly (p<0.001) with a concomitant increase in lysosomal lipid peroxidation (p<0.001) in Group 2 isoproterenol induced rats as compared to Group 1 control rats. Similarly a significant increase (p<0.001) in the activities of lysosomal hydrolases namely acid phosphatase, beta-D-glucuronidase,
Table 11: Level of heart mitochondrial lipids in control and experimental group of rats

Values are expressed as mean ± SD for 6 animals in each group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Group 1)</th>
<th>ISPH induced (Group 2)</th>
<th>Mangiferin alone treated (Group 3)</th>
<th>Mangiferin treated + ISPH Induced (Group 4)</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (nmoles/mg protein)</td>
<td>48.01 ± 0.87</td>
<td>53.01 ± 2.12 <strong>a</strong></td>
<td>47.41 ± 1.16 aNS</td>
<td>50.18 ± 1.76 b***</td>
<td>15.75</td>
</tr>
<tr>
<td>Triglycerides (nmoles/mg protein)</td>
<td>30.16 ± 0.40</td>
<td>35.20 ± 1.68 <strong>a</strong></td>
<td>29.18 ± 2.48 aNS</td>
<td>32.82 ± 2.06 b*</td>
<td>11.29</td>
</tr>
<tr>
<td>Phospholipids (nmoles/mg protein)</td>
<td>525.35 ± 32.35</td>
<td>445.68 ± 18.79 <strong>a</strong></td>
<td>529.09 ± 29.38 aNS</td>
<td>493.37 ± 19.68 b***</td>
<td>15.14</td>
</tr>
<tr>
<td>Free fatty acids (nmoles/mg protein)</td>
<td>14.28 ± 1.37</td>
<td>20.62 ± 1.28 <strong>a</strong></td>
<td>13.59 ± 0.79 aNS</td>
<td>16.02 ± 1.14 b***</td>
<td>43.74</td>
</tr>
</tbody>
</table>

Statistical calculation: One way ANOVA followed by posthoc test LSD
a - Compared with Group 1  *** p < 0.001, ** p < 0.01, * p < 0.05, NS - Non significant
b - Compared with Group 2
Table 12: Activity of lysosomal enzymes and LPO in serum and heart (lysosomal sub cellular organelle) of control and experimental group of rats

Values are expressed as mean ± SD for 6 animals in each group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Group 1)</th>
<th>ISPH induced (Group 2)</th>
<th>Mangiferin alone treated (Group 3)</th>
<th>Mangiferin treated + ISPH Induced (Group 4)</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Phosphatase (μm of phenol released / hr · 100 mg protein)</td>
<td>Serum</td>
<td>92.45 ± 6.32</td>
<td>115.66 ± 9.24 ***</td>
<td>91.01 ± 6.39 NS</td>
<td>17.32</td>
</tr>
<tr>
<td></td>
<td>Lysosome</td>
<td>127.52 ± 6.82</td>
<td>109.50 ± 8.57 ***</td>
<td>135.20 ± 7.57 NS</td>
<td>10.78</td>
</tr>
<tr>
<td>β-D-Glucuronidase (μm of p-nitrophenol liberated / hr / 100 mg protein)</td>
<td>Serum</td>
<td>9.29 ± 0.58</td>
<td>14.26 ± 1.10 ***</td>
<td>8.90 ± 0.60 NS</td>
<td>10.34 ± 0.82 b***</td>
</tr>
<tr>
<td></td>
<td>Lysosome</td>
<td>50.29 ± 3.33</td>
<td>27.23 ± 1.91 ***</td>
<td>51.13 ± 2.69 NS</td>
<td>46.79 ± 1.57 b***</td>
</tr>
<tr>
<td>β-D-Glucosidase (μm of p-nitrophenol liberated / hr / 100 mg protein)</td>
<td>Serum</td>
<td>9.79 ± 0.20</td>
<td>16.05 ± 1.12 ***</td>
<td>9.57 ± 0.47 NS</td>
<td>10.93 ± 0.81 b***</td>
</tr>
<tr>
<td></td>
<td>Lysosome</td>
<td>15.70 ± 1.11</td>
<td>12.60 ± 0.85 ***</td>
<td>16.61 ± 1.22 NS</td>
<td>14.47 ± 1.15 b**</td>
</tr>
<tr>
<td>β-D-Galactosidase (μm of p-nitrophenol liberated / hr / 100 mg protein)</td>
<td>Serum</td>
<td>13.18 ± 0.94</td>
<td>23.20 ± 1.55 ***</td>
<td>12.29 ± 0.93 NS</td>
<td>14.89 ± 1.37 b***</td>
</tr>
<tr>
<td></td>
<td>Lysosome</td>
<td>34.33 ± 1.16</td>
<td>23.35 ± 1.29 ***</td>
<td>35.00 ± 2.34 NS</td>
<td>32.16 ± 2.01 b***</td>
</tr>
<tr>
<td>Cathepsin (nmole of tyrosine liberated / hr / 100 mg protein)</td>
<td>Serum</td>
<td>15.43 ± 1.04</td>
<td>21.40 ± 1.71 ***</td>
<td>15.01 ± 1.17 NS</td>
<td>17.36 ± 1.39 b***</td>
</tr>
<tr>
<td></td>
<td>Lysosome</td>
<td>64.58 ± 1.42</td>
<td>53.77 ± 1.74 a***</td>
<td>65.26 ± 1.67 aNS</td>
<td>60.67 ± 3.28 b***</td>
</tr>
<tr>
<td>Protein (mg/g tissue)</td>
<td>Lysosome</td>
<td>23.69 ± 1.63</td>
<td>16.56 ± 0.91 a***</td>
<td>23.16 ± 1.47 aNS</td>
<td>21.42 ± 1.47 b***</td>
</tr>
<tr>
<td>Lipid peroxide (nmole of TBARS reactant/mg protein)</td>
<td>Lysosome</td>
<td>48.13 ± 1.84</td>
<td>68.53 ± 2.99 a***</td>
<td>47.43 ± 1.45 aNS</td>
<td>51.23 ± 2.47 b***</td>
</tr>
</tbody>
</table>

Statistical calculation  One way ANOVA followed by posthoc test LSD
a - Compared with Group 1  *** p < 0.001, ** p < 0.01, * p < 0.05. NS - Non significant
b - Compared with Group 2
glucosidase, galactosidase and cathepsin D were observed in serum whereas all these enzymes were significantly reduced (p<0.001) in lysosome (native) of heart tissue in Group 2 isoproterenol administered rats when compared to Group 1 control rats.

In Group 4 mangiferin pretreated rats, the lipid peroxide levels were reduced significantly (p<0.001) with an increase in protein levels (p<0.001) as compared to Group 2 rats. Similarly, serum lysosomal enzyme levels such as acid phosphatase (p<0.01) and other hydrolysing enzymes (p<0.001) were significantly decreased and heart lysosomal (native) enzyme activities namely acid phosphatase (p<0.05), glucosidase (p<0.01) and other hydrolases (p<0.001) were significantly increased in Group 4 mangiferin pretreated rats when compared to Group 2 isoproterenol induced rats. No significant changes were observed (NS) in lysosomal protein, lysosomal LPO and lysosomal hydrolase enzyme activities of serum and lysosome subcellular organelle in Group 3 rats when compared to Group 1 control animals.

**Hematological parameters**

The hematological parameters such as RBC, WBC, differential count, platelet count, fibrinogen, Hb, PCV and ESR of control and experimental animals are tabulated in Table 13.

The level of RBC counts, haemoglobin amount and packed cell volume were significantly increased (p<0.001) and ESR showed significant (p<0.001) reduced level in Group 2 isoproterenol induced rats. The level of
Table 13: Level of haematological parameters in control and experimental group of rats

Values are expressed as mean ± SD for 6 animals in each group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Group 1)</th>
<th>ISPH induced (Group 2)</th>
<th>Mangiferin alone treated (Group 3)</th>
<th>Mangiferin treated + ISPH Induced (Group 4)</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (millions/cu mm)</td>
<td>9.13 ± 0.41</td>
<td>12.00 ± 0.70***</td>
<td>8.88 ± 0.24 aNS</td>
<td>10.00 ± 0.70 b***</td>
<td>39.06</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>20.66 ± 1.86</td>
<td>10.00 ± 0.89***</td>
<td>21.66 ± 0.51 aNS</td>
<td>19.00 ± 1.41 b***</td>
<td>104.6</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>13.01 ± 0.28</td>
<td>20.08 ± 1.28***</td>
<td>12.58 ± 0.80 aNS</td>
<td>14.19 ± 0.81 b***</td>
<td>95.65</td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td>20.11 ± 1.05</td>
<td>38.00 ± 2.12***</td>
<td>18.96 ± 0.76 aNS</td>
<td>22 ± 1.41 b***</td>
<td>232.5</td>
</tr>
<tr>
<td>WBC (cells / cu mm)</td>
<td>7375 ± 440.17</td>
<td>11813 ± 33 ± 1120.47***</td>
<td>7000 ± 494.97 aNS</td>
<td>8249.83 ± 583.86 b***</td>
<td>57.04</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>54.66 ± 4.80</td>
<td>74.83 ± 1.87***</td>
<td>53.21 ± 1.41 aNS</td>
<td>63.25 ± 1.87 b***</td>
<td>82.54</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>39.08 ± 1.42</td>
<td>25.89 ± 4.02***</td>
<td>40.21 ± 4.14 aNS</td>
<td>33.16 ± 5.52 b**</td>
<td>49.9</td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>7 ± 0.48</td>
<td>2 ± 0.14***</td>
<td>7.09 ± 0.46 aNS</td>
<td>5.50 ± 0.35 b***</td>
<td>227.6</td>
</tr>
<tr>
<td>Platelet count (10^5 cells / cu mm)</td>
<td>1.52 ± 0.05</td>
<td>2.81 ± 0.10***</td>
<td>1.5 ± 0.037 aNS</td>
<td>1.90 ± 0.026 b***</td>
<td>601.6</td>
</tr>
<tr>
<td>Fibrinogen count (mg/dl)</td>
<td>227 ± 15.49</td>
<td>340.83 ± 23.54a***</td>
<td>219.5 ± 14.33 aNS</td>
<td>269.16 ± 15.30 b***</td>
<td>60.16</td>
</tr>
</tbody>
</table>

Statistical calculation One way ANOVA followed by posthoc test LSD

a - Compared with Group 1  *** p < 0.001, ** p < 0.01, * p < 0.05. NS - Non significant
b - Compared with Group 2
RBC, Hb and PCV were decreased significantly (p<0.001) and ESR level was significantly increased in Group 4 mangiferin pretreated rats compared to isoproterenol induced Group 2 rats. Group 3 rats showed near normal levels in these parameters (NS) when compared to Group 1 control rats.

The amount of total WBCs and neutrophil showed a significantly increased level (p<0.001) and the percentage of lymphocytes, eosinophil counts were found to be decreased (p<0.001) significantly in Group 2 isoproterenol induced rats when compared to Group 1 control animals. In mangiferin pretreated Group 4 rats, the level of total WBC, neutrophils were significantly decreased (p<0.001) and percentage of lymphocytes, eosinophil counts were significantly increased (p<0.001) when compared to isoproterenol induced Group 2 rats. A non significant change (NS) was observed in Group 3 drug alone treated rats when compared to Group 1 control animals for all these parameters.

The levels of platelet count and fibrinogen contents showed a significantly increased (p<0.001) level in isoproterenol induced Group 2 rats compared to Group 1 control rats. These values were significantly decreased (p<0.001) in Group 4 mangiferin pretreated rats when compared to isoproterenol induced Group 2 rats. There was a non significant change (NS) in Group 3 drug alone treated rats in the above two parameters when compared to Group 1 control animals.

The clotting time, bleeding time and prothrombin time levels are represented in Figure 22.
Figure 22 Level of bleeding time, clotting time and prothrombin in normal and experimental group of rats

Values are expressed as mean ± S.D for 6 animals in each group

- Control
- ISPH Induced
- Mangiferin alone
- Mangiferin + ISPH

Unit (seconds)

Bleeding time

Clotting time

Prothrombin time

Statistical calculation: One way ANOVA followed by posthoc test LSD

a - Compared with Group 1 *** p < 0.001; ** p < 0.01, * p < 0.05
b - Compared with Group 2

NS - Non significant
All these parameters were significantly reduced in Group 2 isoproterenol induced rats (p<0.001) compared to Group 1 control animals whereas Group 4 mangiferin pretreated rats showed a significantly increased (p<0.001) level when compared to Group 2 myocardial damaged rats and showed a non significant change in Group 3 drug control rats as compared with Group 1 control animals.