Chapter 3

Results
3. Results

3.1 Effect of Antidiabetic Compounds on General Parameters

Changes in various parameters were observed in streptozotocin induced diabetes rats. After induction of diabetes, rats show typical symptoms of diabetes mellitus like polydipsia, polyphagia and polyuria. The diabetic animals have blood glucose level more than 250 mg/dl were consider diabetic and divided into control rats (C), diabetic rats (D), insulin treated diabetic rats (I), vanadate treated diabetic rats (V), Azadirachta indica treated diabetic rats (A), vanadate and Azadirachta indica treated diabetic rats (V+A). The diabetic rats treated with Azadirachta indica in combination with vanadate (V+A) were found to be visibly healthy and they also gained weight compared to the other groups. The mortality rate of the rats given vanadate alone in dose of 0.6 mg/ml was higher than those given vanadate at dose of 0.2 mg/ml along with Azadirachta indica.

3.1.1 Change in Body Weight

The change in general parameters like body weight and tissues weight is given in Table 3.1. Diabetic rats showed reduction in body weight as compared to normal rats. Treatment with insulin for twenty one days effectively improved the weight loss of diabetic rats. Vanadate could not improve the body weight. The Azadirachta indica treatment resulted in improvement in the weight of rats. The combined treatment effectively improved the weight of rats as compared to other groups.
3.1.2 Change in Tissue Weight

As shown in Table 3.1, the weight of liver of the diabetic animals decreased as compared to that of the controls. The kidney weight increased in diabetic animals as compared to normal animals.

Similarly the weight of heart of diabetic animals decreased as compared to diabetic animals.

However there was no significant change in weight of the brain.

Treatment of diabetic rats with insulin, vanadate and *Azadirachta indica* improved the liver and heart weights and the combined treatment with vanadate and *A. indica* was found more effective in improving the alterations.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Diabetic</th>
<th>Insulin</th>
<th>Vanadate</th>
<th><em>A. indica</em></th>
<th>Vanadate + A.indica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt. (g)</td>
<td>258±10.13</td>
<td>146±17.6b</td>
<td>196±11.7b</td>
<td>170±13.2b</td>
<td>205±7.6b</td>
<td>243±4.40</td>
</tr>
<tr>
<td>Liver wt. (g)</td>
<td>6.4±0.25</td>
<td>5.13±0.14b</td>
<td>6.33±0.07</td>
<td>5.9±0.1</td>
<td>5.96±0.07</td>
<td>6.33±0.09</td>
</tr>
<tr>
<td>Kidney wt. (g)</td>
<td>1.7±0.017</td>
<td>1.85±0.027b</td>
<td>1.68±0.014</td>
<td>1.78±0.077</td>
<td>1.66±0.012</td>
<td>1.64±0.01</td>
</tr>
<tr>
<td>Brain wt. (g)</td>
<td>1.66±0.01</td>
<td>1.62±0.01</td>
<td>1.64±0.02</td>
<td>1.62±0.01</td>
<td>1.63±0.01</td>
<td>1.66±0.01</td>
</tr>
<tr>
<td>Heart wt (g)</td>
<td>0.64±0.01</td>
<td>0.44±0.02</td>
<td>0.60±0.01</td>
<td>0.51±0.01</td>
<td>0.53±0.03</td>
<td>0.61±0.01</td>
</tr>
</tbody>
</table>

Table 3.1 Change in body weight and organ weight of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, *Azadirachta indica*-A and combined dose of Vanadate and *Azadirachta indica* –V+A.

All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value

\(^aP<0.01\)  \(^bP<0.05\)
3.1.3 Protein Content

After 21 days of treatment there is no significant change in protein content of various groups of experimental rats in liver, kidney, heart, skeletal muscle and brain (Table 3.2). All the enzyme activities are expressed as specific activity per milligram protein and, therefore, represent true changes under these conditions.

<table>
<thead>
<tr>
<th>Protein mg/g</th>
<th>C</th>
<th>D</th>
<th>I</th>
<th>V</th>
<th>A</th>
<th>V+A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>123±2.3</td>
<td>111±10.5</td>
<td>116±5.0</td>
<td>126±2.0</td>
<td>129±8.3</td>
<td>126±1.8</td>
</tr>
<tr>
<td>Kidney</td>
<td>96±4.1</td>
<td>82±6.2</td>
<td>97±0.5</td>
<td>98±0.9</td>
<td>100±1.2</td>
<td>99±2.2</td>
</tr>
<tr>
<td>Heart</td>
<td>43.8±0.6</td>
<td>41.4±0.9</td>
<td>42.5±1.6</td>
<td>43.1±0.3</td>
<td>42.7±1.2</td>
<td>41.5±0.9</td>
</tr>
<tr>
<td>Skeletal</td>
<td>71.2±1.2</td>
<td>67.8±0.40</td>
<td>72.0±0.58</td>
<td>73.5±0.43</td>
<td>72.5±1.1</td>
<td>71.5±1.3</td>
</tr>
<tr>
<td>Brain</td>
<td>57.2±0.49</td>
<td>60.3±0.32</td>
<td>58.2±1.04</td>
<td>59.9±1.38</td>
<td>58±0.11</td>
<td>59.1±0.53</td>
</tr>
</tbody>
</table>

Table 3.2 Protein content in cytosolic fraction of various tissues after 21 days of Diabetic- D, Control-C and Diabetic rats treated with Insulin-I, Vanadate-V, Azadirachta indica-A and Combined treatment with Vanadate and Azadirachta indica V+A.

All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value.
3.2 Glucose Levels in Blood

3.2.1 Change in Plasma Glucose levels

Diabetic rats showed increase in blood glucose as compared to normal rats. The high glucose levels improved almost in all four treated groups. Figure 3.1 shows that the combined treatment with A. indica and vanadate is more effective in improving the hyperglycemic condition as compared to all other treatments.

On treatment with 0.2 mg/ml and 0.6 mg/ml vanadate in drinking water the body weight of the animals kept on decreasing with increased vanadate concentration (Table 3.3) pointing towards vanadate toxicity at higher doses.

However at higher doses of vanadate (0.6 mg/ml) the plasma glucose levels improved considerably as compared to lower levels (0.2 mg/ml) of vanadate as shown in Table 3.4.

After all four type of treatment of the diabetes rats the body weight increased and blood glucose levels decreased. The results of treatments were comparable to the values of the control group (Figure 3.2).
Figure 3.1 Change in Plasma Glucose level of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, *Azadirachta indica*-A and combined dose of Vanadate and *Azadirachta indica* -V+A.

All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value

*P< 0.01  **P< 0.05
<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>258±10.13</td>
</tr>
<tr>
<td>Diabetic + vanadate (0.2mg/ml)</td>
<td>239±9.23</td>
</tr>
<tr>
<td>Diabetic + vanadate (0.6mg/ml)</td>
<td>151±4.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Table 3.3 Comparison of body weight of control and different doses of vanadate treated rats after 21 days of treatment.**

All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value.

<sup>a</sup><sup>P</sup> < 0.01

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>87.6±5.36</td>
</tr>
<tr>
<td>Diabetic</td>
<td>471.66±8.41</td>
</tr>
<tr>
<td>Diabetic + vanadate (0.2mg/ml)</td>
<td>347.6±16.7</td>
</tr>
<tr>
<td>Diabetic + vanadate (0.6mg/ml)</td>
<td>128.75±6.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Table 3.4 Comparison of plasma glucose levels of control, diabetic and different doses of vanadate treated diabetic rats after 21 days of treatment.**

All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value.

<sup>b</sup><sup>P</sup> < 0.05
Figure 3.2 Change in the body weight and blood glucose of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, *Azadirachta indica*-A and combined dose of Vanadate and *Azadirachta indica* –V+A.

All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value.
3.2.2 Change in Glycosylated Hemoglobin

Diabetic rats showed increase in the glycosylated hemoglobin as compared to normal rats. The increased glycosylated hemoglobin levels were normalized almost in all treated groups. Figure 3.3 shows that the combined treatment with *Azadirachta indica* and vanadate is more effective in improving the increased glycosylated hemoglobin level as compared to all other treatments.

![Graph showing change in glycosylated hemoglobin (HbA1c) among different groups](image)

**Figure 3.3 Change in Glycosylated hemoglobin (HbA1c) of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, *Azadirachta indica*-A and combined dose of Vanadate and *Azadirachta indica*-V+A.**

All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value:

* $p<0.01$  ** $p<0.05$
3.3 Change in lipid Content

There was an increase in total cholesterol and triglycerides in the diabetic group of rats. However HDL cholesterol level showed reduction (Figure 3.4). Treatment with insulin, vanadate, Azadirachta indica and lower dose of vanadate with Azadirachta indica resulted in improvement of altered lipid level. Total cholesterol (TC) and triglycerides (TG) were reduced in all the treated groups. The level of HDLC increased in the treated groups. Treatment with A. indica and vanadate combination is more effective.

![Graph showing lipid profiles](image)

**Figure 3.4** Comparison of Plasma lipid profile of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, Azadirachta indica-A and combined dose of Vanadate and Azadirachta indica –V+A. All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value
3.4 Changes in Glycogen Content in Liver and Skeletal Muscles

Diabetic rats showed a decrease in the glycogen content of liver and skeletal muscles as compared to normal rats. The decreased glycogen level in liver and skeletal muscles improved almost in all treated groups. We found that the combined treatment by *A. indica* and vanadate is most effective in improving the decreased glycogen content in liver and skeletal muscle as compared to all other treatments (Figure 3.5).

![Graph showing glycogen content](image)

**Figure 3.5 Comparison of Glycogen level in liver and skeletal muscles of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, **Azadirachta indica**-A and combined dose of Vanadate and **Azadirachta indica** -V+A.**

All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value.
3.5 Effect of Antidiabetic Compounds on Free Radical Scavenging Enzymes

Oxidative stress generated by free radicals in diabetic condition results in diabetic complications (Baynes, 1991). The increase in the oxidative stress in diabetes is related to change in the antioxidant system of the cell. Therefore the activities of some major antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase were measured in various tissues of control and diabetic animals. The activities were measured in five tissues, namely liver, kidney, heart, muscle and brain. The quantitative distribution of antioxidant enzymes (Superoxide dismutase, Catalase, Glutathione peroxidase, Glutathione Reductase) was found to vary in the five tissues. The liver shows the highest activity of these enzymes whereas the brain shows the lowest activity when compared with other tissue of control rats.

3.5.1 Superoxide Dismutase (SOD)

Superoxide dismutase is an important enzyme of antioxidant defense system of cell. The enzyme scavenges superoxide anion which is the first reactive oxygen species produced. Any disturbance in the activity of SOD may result in uncontrolled free radical production and thus contribute to the increase in oxidative stress.
3.5.1.1 Liver

The change in the enzyme activity of SOD in the liver cytosolic fraction of control, diabetic and treated diabetic rats after 21 days of treatment was measured and the activities were expressed as units/mg protein. The activity of SOD decreased in liver of diabetic animals. Treatment with insulin, vanadate, *Azadirachta indica* and combined treatment of *Azadirachta indica* and vanadate brought back the values close to control one (Figure 3.6).

3.5.1.2 Kidney

In kidney the change in the activity of SOD was observed in the cytosolic fraction of control, diabetic and treated diabetic rats after 21 days of treatment was measured and the activities were expressed as units/mg protein. The activity of SOD decreased in kidney. Treatment with insulin, vanadate, *Azadirachta indica* and combine treatment of *Azadirachta indica* and vanadate brought back the values close to control one. The combined treatment is more effective as compared to other treatment (Figure 3.6).

3.5.1.3 Heart

Superoxide dismutase enzyme activity was checked in the heart cytosolic fraction of control, diabetic and treated diabetic rats after 21 days of treatment was measured and the activities were expressed as units/mg protein. The activity of SOD increased in heart of diabetic animals. Treatment with insulin, vanadate, *Azadirachta indica* and combined treatment of *Azadirachta*
*indica* and vanadate brought back the values close to control one. The combined treatment is most effective (Figure 3.7).

### 3.5.1.4 Skeletal Muscle

SOD activity was checked in the muscle cytosolic fraction of control, diabetic and treated diabetic rats after 21 days of treatment. The activity of SOD was seen to be decreased. The activities were expressed as units/mg protein. Treatment with insulin, vanadate, *Azadirachta indica* and combined treatment of *Azadirachta indica* and vanadate improved the altered values. *Azadirachta indica* treatment is most effective in improving the altered values (Figure 3.7).

### 3.5.1.5 Brain

The alteration in the enzyme activity of SOD in the brain cytosolic fraction of control, diabetic and treated diabetic rats after 21 days of treatment was measured and the activities were expressed as units/mg protein. The activity of SOD decreased in brain. Treatment of the diabetic animals with insulin, vanadate, *Azadirachta indica* and combined treatment of *Azadirachta indica* and vanadate brought back the values of SOD activity close to control one. The combined treatment is most effective and seen to be better than even insulin (Figure 3.8).
Figure 3.6 Change in activity of superoxide dismutase (SOD) in liver and kidney of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, *Azadirachta indica*-A and combined dose of Vanadate and *Azadirachta indica* –V+A.

All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value

**P< 0.05**
Figure 3.7 Change in activity of superoxide dismutase (SOD) in heart and skeletal muscles of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, *Azadirachta indica*-A and combined dose of Vanadate and *Azadirachta indica* –V+A. All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value.
Figure 3.8 Change in activity of Superoxide dismutase (SOD) in brain of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, *Azadirachta indica*-A and combined dose of Vanadate and *Azadirachta indica* –V+A.

All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value.
3.5.2 Catalase (CAT)

Catalase is the second line of defense in the cell against free radicals. The enzyme catalase catalyzes the decomposition of hydrogen peroxide (H$_2$O$_2$) to water and oxygen. Most aerobic cells contain CAT and its activity varies in tissues. Any alteration in CAT activity will, therefore result in serious crisis in the oxidative state of the cell.

3.5.2.1 Liver

The change in the enzyme activity of CAT in the liver cytosolic fraction of control, diabetic and treated diabetic rats after 21 days of treatment was measured and the activities were expressed as units/mg protein. The activity of CAT decreased in liver. Treatment with insulin, *Azadirachta indica* and combined treatment of *Azadirachta indica* and vanadate brought back the values close to control one. The combined treatment is most effective (Figure 3.9).

3.5.2.2 Kidney

The activity of CAT enzyme in the kidney cytosolic fraction of control, diabetic and treated diabetic rats after 21 days of treatment was measured. The activity of CAT decreased in kidney. The activities were expressed as units/mg protein. Treatment with insulin, vanadate, *Azadirachta indica* and combined treatment of *Azadirachta indica* and vanadate brought back the values close to control one. The combined treatment is more effective as compared to other treatment (Figure 3.9).
3.5.2.3 Heart

The alteration in the enzyme activity of CAT in the heart cytosolic fraction of control, diabetic and treated diabetic rats after 21 days of treatment was measured. The activities were expressed as units/mg protein. The activity of CAT increased in heart. Treatment with insulin, vanadate, *Azadirachta indica* and combined treatment of *Azadirachta indica* and vanadate brought back the values close to control one. The combined treatment is more effective as compared to four other treatments (Figure 3.10).

3.5.2.4 Skeletal Muscle

When CAT activity was checked in the muscle cytosolic fraction of control, diabetic and treated diabetic rats after 21 days of treatment, it was observed to be increased. The activity of CAT increased in muscle. Treatment with insulin, vanadate, *Azadirachta indica* and combined treatment of *Azadirachta indica* and vanadate brought back the values close to control one. The combined treatment is more effective as compared to other treatment (Figure 3.10). The activities were expressed as units/mg protein.

3.5.2.5 Brain

After 21 days of treatment the CAT enzyme activity in the brain cytosolic fraction of control, diabetic and diabetic rats treated with insulin, vanadate, *Azadirachta indica* and combine treatment of *Azadirachta indica* and vanadate was measured. The activities were expressed as units/mg protein. The activity of CAT decreased in brain. *Azadirachta indica* improved the altered
values. Treatment with insulin, vanadate and combined treatment of *Azadirachta indica* and vanadate brought back the values close to control one. The combined treatment is most effective (Figure 3.11).

**Figure 3.9** Change in activity of catalase (CAT) in liver and kidney of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, *Azadirachta indica*-A and combined dose of Vanadate and *Azadirachta indica* –V+A.

All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value

*P< 0.01    **P< 0.05
Figure 3.10 Change in activity of catalase (CAT) in heart and skeletal muscles of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, Azadirachta indica-A and combined dose of Vanadate and Azadirachta indica –V+A. 

All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value.
Figure 3.11 Change in activity of catalase (CAT) in brain of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, *Azadirachta indica*-A and combined dose of Vanadate and *Azadirachta indica* –V+A.

All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value.
3.5.3 Gluthathione Peroxidase (GPx)

Gluthathione peroxidase detoxifies H$_2$O$_2$ or any other hydroperoxide, utilizing reduced gluthathione and forms H$_2$O and oxidized Gluthathione (GSSG). It plays an important role in the enzymatic defense system against oxygen derived free radicals and its activity depends upon its concentration in the tissues. Thus in diabetes the disturbance in oxidative state is reflected in the change in the activity of GPx enzyme.

3.5.3.1 Liver

The alteration in the enzyme activity of GPx in the liver cytosolic fraction of control, diabetic and treated diabetic rats after 21 days of treatment was measured. The activity of GPx decreased in liver. The activities were expressed as units/mg protein. Treatment with insulin, vanadate, Azadirachta indica and combined treatment of Azadirachta indica and vanadate improved altered values. Azadirachta indica treatment is most effective in improving the altered values (Figure 3.12).

3.5.3.2 Kidney

The change in the enzyme activity of GPx in the kidney cytosolic fraction of control, diabetic and treated diabetic rats after 21 days of treatment was measured and the activities were expressed as units/mg protein. The activity of GPx increased in kidney. Treatment with insulin, vanadate and combined treatment of Azadirachta indica and vanadate brought back the values close to control one (Figure 3.12).
3.5.3.3 Heart

GPx activity was checked in the heart cytosolic fraction of control, diabetic and treated diabetic rats after 21 days of treatment was measured and the activities were expressed as units/mg protein. The activity of GPx increased in heart. Treatment with insulin, vanadate, *Azadirachta indica* and combined treatment of *Azadirachta indica* and vanadate improved the changed values (Figure 3.13).

3.5.3.4 Skeletal Muscle

The enzymatic activity of GPx in the muscle cytosolic fraction of control, diabetic and treated diabetic rats after 21 days of treatment was measured. The activity of GPx decreased in muscle. The activities were expressed as units/mg protein. Treatment with insulin, vanadate, *Azadirachta indica* and combined treatment of *Azadirachta indica* and vanadate brought back the values close to control one. *Azadirachta indica* treatment is most effective in improving the altered values (Figure 3.13).

3.5.3.5 Brain

Gluthathione peroxidase activity in the brain cytosolic fraction of control, diabetic and treated diabetic rats after 21 days of treatment was measured. The activity of GPx increased in brain and the activities were expressed as units/mg protein. Treatment with insulin, vanadate, *Azadirachta indica* and combined treatment of *Azadirachta indica* and vanadate brought back the values
close to control one. The combined treatment is more effective as compared to other treatment (Figure 3.14).

Figure 3.12 Change in activity of glutathione peroxidase (GPx) in liver and kidney of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, *Azadirachta indica*-A and combined dose of Vanadate and *Azadirachta indica* –V+A.

All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value

*P* < 0.01    **P** < 0.05
Figure 3.13 Change in activity of glutathione peroxidase (GPx) in heart and skeletal muscles of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, *Azadirachta indica*-A and combined dose of Vanadate and *Azadirachta indica* –V+A.

All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value.
Figure 3.14 Change in activity of glutathione peroxidase (GPx) in brain of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, *Azadirachta indica*-A and combined dose of Vanadate and *Azadirachta indica* –V+A.

All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value.
3.5.4 Gluthathione Reductase (GR)

Gluthathione reductase converts the oxidized Gluthathione formed by GPx into reduced Gluthathione so that the intracellular GSH/GSSG ratio is maintained in the cells. Change in the ratio towards either side can cause an imbalance in the redox state causing oxidative stress. As diabetes is characterized by increase in oxidative stress, GR is an important parameter to monitor and study the effect of an antioxidant compound and its role in the control and maintenance of the redox state of the cell.

3.5.4.1 Liver

The alteration in the enzyme activity of GR in the liver cytosolic fraction of control, diabetic and treated diabetic rats after 21 days of treatment was measured and the activities were expressed as units/mg protein. The activity of GR decreased in liver. Treatment with insulin and combined treatment of *Azadirachta indica* and vanadate improved the altered values. The combined treatment is more effective as compared to other treatments (Figure 3.15).

3.5.4.2 Kidney

The change in the enzyme activity of GR in the kidney cytosolic fraction of control, diabetic and treated diabetic rats after 21 days of treatment was measured. The activity of GR increased in kidney. The activities were expressed as units/mg protein. Treatment with insulin, vanadate, *Azadirachta indica* and combined treatment of *Azadirachta indica* and vanadate results in
improvement in altered values. The combined treatment is more effective (Figure 3.15).

### 3.5.4.3 Heart

The enzymatic activity of GR in the heart cytosolic fraction of control, diabetic and treated diabetic rats after 21 days of treatment was measured. The activity of GR increased in heart. The activities were expressed as units/mg protein. Treatment with insulin, vanadate, *Azadirachta indica* and combined treatment of *Azadirachta indica* and vanadate brought back the values close to control one. The combined treatment is more effective as compared to other treatments (Figure 3.16).

### 3.5.4.4 Skeletal Muscle

GR activity was checked in the muscle cytosolic fraction of control, diabetic and treated diabetic rats after 21 days of treatment was measured and the activities were expressed as units/mg protein. The activity of GR decreased in muscle. Insulin and vanadate treatment slightly increased the values. Treatment with *Azadirachta indica* and combined treatment of *Azadirachta indica* and vanadate improved the altered values. *Azadirachta indica* is most effective in improving the altered values (Figure 3.16).

### 3.5.4.5 Brain

Glutathione reductase activity was measured in the brain cytosolic fraction of control, diabetic and treated diabetic rats after 21 days of treatment.
The activities were expressed as units/mg protein. The activity of GR increased in brain. Treatment with insulin, vanadate, *Azadirachta indica* and combined treatment of *Azadirachta indica* and vanadate brought back the values close to control one. The combined treatment is most effective (Figure 3.17).

![Graph of enzyme activity](#)

**Figure 3.15** Change in activity of glutathione reductase (GR) in liver and kidney of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, *Azadirachta indica*-A and combined dose of Vanadate and *Azadirachta indica* –V+A.

All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value.
Figure 3.16 Change in activity of glutathione reductase (GR) in heart and skeletal muscles of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, *Azadirachta indica*-A and combined dose of Vanadate and *Azadirachta indica* –V+A. All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value.
Figure 3.17 Change in activity of glutathione reductase (GR) in brain of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, *Azadirachta indica*-A and combined dose of Vanadate and *Azadirachta indica* –V+A.

All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value.
3.6 Effect of Antidiabetic Compounds on Lipid peroxidation

Diabetes results in significant change in lipid structure and its metabolism particularly in patients with vascular complications. The structural changes are oxidative in nature. Thus the lipid peroxidation is an important parameter in measuring the antidiabetic effects of any compounds on a diabetic animal.

3.6.1 Liver

The concentration of lipid peroxides, in the form of malondialdehyde (MDA) formation was measured in the whole liver homogenate fraction of control, diabetes and diabetic rats after 21 days of treatment with insulin, vanadate, *Azadirachta indica* and combined with *Azadirachta indica* and vanadate. After 21 days of diabetes induction, the diabetic groups showed considerable increase in lipid peroxides formation. Since one of the end products of lipid peroxidation is malonaldehyde, results were calculated as nanomoles of malonaldehyde (MDA) formed/mg protein and are shown in Figure 3.18. The treatment with insulin, vanadate, *Azadirachta indica* and combined treatment resulted in improvement in the altered value. Results showed that the combined treatment is more effective in improving the increased lipid peroxidation in liver as compared to all other treatments.
3.6.2 Kidney

The lipid peroxidation in kidney was measured as formation of malondialdehyde (MDA) in control, diabetes and diabetic rats after 21 days of treatment with insulin, vanadate, Azadirachta indica and combined with Azadirachta indica and vanadate. After 21 days of diabetes induction the diabetic groups showed increase in lipid peroxides formation. Results were calculated as nanomoles malondialdehyde (MDA) formed/mg protein and are shown in Figure 3.18. The treatment with insulin, vanadate, Azadirachta indica and combines treatment result in improvement in the altered value.

3.6.3 Heart

After 21 days of treatment with insulin, vanadate, Azadirachta indica and combined with Azadirachta indica and vanadate the concentration of lipid peroxides, in the form of malondialdehyde (MDA) formation was measured in the heart of control, diabetes and diabetic rats. After 21 days of diabetes induction the diabetic groups showed a marked increase in lipid peroxides formation. Results were calculated as nanomoles malondialdehyde (MDA) formed/mg protein and are shown in Figure 3.19. The treatment with insulin, vanadate, Azadirachta indica and combines treatment result in improvement in the altered value. Results showed that the combined treatment is more effective in improving the increased lipid peroxidation in heart as compared to all other treatments.
3.6.4 Skeletal Muscle

Lipid peroxidation was estimated by determining malondialdehyde (MDA) formation in the skeletal muscle fraction of control, diabetes and diabetic rats after 21 days of treatment with insulin, vanadate, Azadirachta indica and combined with Azadirachta indica and vanadate. After 21 days of diabetes induction the diabetic groups shows increased in lipid peroxides formation. Results were calculated as nanomoles malondialdehyde (MDA) formed/mg protein and are shown in Figure 3.19. The treatment with insulin, vanadate, Azadirachta indica and combines treatment result in improvement in the altered value. Results showed that the combined treatment is more effective in improving the increased lipid peroxidation in skeletal muscle as compared to all other treatments.

3.6.5 Brain

The change in the concentration of lipid peroxides, in the form of malondialdehyde (MDA) formation was measured in the brain of control, diabetes and diabetic rats after 21 days of treatment with insulin, vanadate, Azadirachta indica and combined with Azadirachta indica and vanadate. After 21 days of diabetes induction the diabetic groups shows increased in lipid peroxides formation. Results were calculated as nanomoles malondialdehyde (MDA) formed/mg protein and are shown in Figure 3.20. The treatment with insulin, vanadate, Azadirachta indica and combined treatment results in improvement of altered value.
Figure 3.18 Change in the malondialdehyde levels in liver and kidney of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, Azadirachta indica-A and combined dose of Vanadate and Azadirachta indica –V+A.

All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value

*P< 0.01  **P< 0.05
Figure 3.19 Change in the malondialdehyde levels in heart and skeletal muscles of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, Azadirachta indica-A and combined dose of Vanadate and Azadirachta indica –V+A.

All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value.
Figure 3.20 Change in the malondialdehyde levels in brain of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, *Azadirachta indica*-A and combined dose of Vanadate and *Azadirachta indica* –V+A.

All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value.
3.7 Changes in the Level of GLUT4 by Immunoblotting

Glucose homeostasis is maintained by the uptake of Glucose into the cell for its metabolism by glucose transporter (GLUT) molecule in the plasma membrane via facilitated diffusion (Elmendrof and Pessin, 1999). Insulin hormone function is to increase the expression of GLUT4 in the cell and stimulate the trafficking of GLUT4 from intracellular storage vesicle to the plasma membrane. GLUT 4 is mainly expressed in tissues like skeletal, cardiac muscle and adipose tissue which are insulin sensitive (James et al, 1993). Defect in the GLUT4 trafficking or function in skeletal muscle are thought to be most importance in the development of insulin resistance (Garvey et al, 1998).

After 21 days of treatment the expression of GLUT4 is investigated by immunoblotting of STZ-diabetic rat skeletal muscle membrane fraction. The diabetic rats showed decrease in GLUT-4 level in membrane fraction of skeletal muscle (Figure 3.21). These results are in correlation with earlier studies (Li et al, 1997; Mohammad et al, 2006). The reduction in the expression of GLUT4 disturbs the glucose homeostasis which reflects as hyperglycemia in diabetic rats. Treatment of diabetic animals with insulin, vanadate (0.6mg/ml), *Azadirachta indica* and combined treatment with 0.2mg/ml vanadate and *Azadirachta indica* results in the improvement of membrane GLUT4 content. Combined treatment is most effective.
3.8 Changes in the Level of SOD by Immunoblotting

After 21 days of treatment, the expression of SOD was investigated by immunoblotting of STZ-diabetic rat liver fraction. The diabetic rats showed decrease in SOD level in cytosolic fraction of liver (Figure 3.21). The reduction in the expression of SOD results in increased oxidative stress. Treatment of diabetic animals with insulin, vanadate (0.6mg/ml), *Azadirachta indica* and combined treatment with 0.2mg/ml vanadate and Azadirachta indica results in improvement of membrane SOD content. Combined treatment is most effective.

3.9 Changes in the Level of PKCβ2 by Immunoblotting

Protein kinase C is involved in mediating cellular response to extracellular stimuli leading to number of biological process like apoptosis, proliferation, differentiation and exocytotic release in various non-neuronal systems such as islet cells. Protein kinase C represents a family of second messenger-dependent protein kinase that are stimulated by Ca\(^{2+}\) and phospholipid. Reports suggest that many cardiovascular and microvascular abnormalities observed in diabetic patients could be the result of the activation of PKC β isoform.

Figure 3.21 shows after 21 days of treatment increase in PKCβ2 level in membrane fraction of skeletal muscle. This increase due to intracellular hyperglycemia increases the amount of DAG from glycolytic intermediate dihydroxyacetone phosphate. Increase de novo synthesis of DAG activate PKCβ2. Treatment of diabetic animals with insulin, vanadate (0.6mg/ml), *Azadirachta*
"indica and combined treatment with 0.2mg/ml vanadate and *Azadirachta indica* results in the improvement in membrane PKCβ2 content. Combined treatment is most effective.

![Image showing GLUT, SOD, and PKC β2 results](image)

**Figure 3.21** Change in the GLUT, SOD and PKC β2 protein level in Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, *Azadirachta indica*-A and combined dose of Vanadate and *Azadirachta indica*-V+A. All values are expressed as mean ± SEM. The significant comparisons of each experiment group shown are with the control value.

### 3.10 Diabetes Induced Degradation of Genomic DNA

Diabetes generated oxidative stress due to imbalance in ROS and H₂O₂ concentration has deleterious effect on cell. Increase in H₂O₂ concentration disturbs the integrity of the cell and one of the manifestations of this is apoptosis. Fragmentation of DNA is one of the changes of apoptosis.

We assessed the apoptosis by DNA laddering method. The liver genomic DNA was extracted from control and treated rats. Control group of rats showed no sign of fragmentation of DNA in liver genomic DNA. However, liver
genomic DNA extracted from STZ-diabetic rats showed the smearing pattern, which is a feature of fragmentation of DNA and apoptosis (Sandberg et al., 2004; Nagata, 2000). The treatment of diabetic rats with insulin, vanadate, *Azadirachta indica* and lower dose of vanadate with *Azadirachta indica* prevented genomic DNA fragmentation. Result has been shown in Figure 3.22

![DNA Marker C D I V A V+A](image)

Figure 3.22 DNA degradation study of Control-C, Diabetic-D and Diabetic rats treated with Insulin-I, Vanadate-V, *Azadirachta indica*-A and combined dose of Vanadate and *Azadirachta indica* –V+A.