RESULTS
CHAPTER 5

RESULTS

5.1 Standardization and validation of rat model of Monocrotaline induced pulmonary hypertension

5.1.1 Standardization of the dose of monocrotaline to induce pulmonary hypertension in rat

5.1.1.1 Effect of different doses and duration of MCT treatment on survival and body weight

There was decrease in percent survival and body weight of the rats after MCT treatment. This loss of body weight was very severe at the dose of 80 mg/kg and all the rats could not survive beyond a fortnight. So, the studies with MCT 80 mg/kg were discontinued because of lethality.

![Figure 5.1](image-url)

**Figure 5.1** Effect of MCT treatment on (a) survival (%) (b) body weight (g). Data values were expressed as Mean ± S.E.M. (n=10). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (a=p<0.05 vs. control 21 days, bbb=p<0.001 vs. control 28 days, ccc=p<0.001 vs. control 35 days, $$=p<0.01 vs. MCT 21 days and # = p<0.05 vs. MCT 28 days).
On the other hand, following the administration of dose of 60 mg/kg MCT, there was a decrease in the percent survival and body weight after 21 days (95% and 386.5 ± 5.45 g; p<0.05) which further went down after 28 days (85% and 367.5 ± 9.02 g; p<0.05). The maximum decrease in percent survival and body weight occurred at 35 days (65% and 330.8 ± 14.88 g; p<0.001). The percentage survival was 100% and their body weight after 21, 28 and 35 days were 372±8.2 g; 393.6± 7.42 g and 411.5± 5.9 g respectively.

5.1.1.2 Effect of MCT administration on right ventricular pressure (RVP) of rats

Increase in the RVP is a characteristic feature of PH and there was a significant time dependent increase in the RVP i.e. 24.16 ± 0.80 mmHg (p<0.05); 29.84 ± 1.84 mmHg (p<0.001) and 44.25 ± 1.76 mmHg (p<0.001) after 21, 28 and 35 days of MCT treatment respectively, as compared to the respective control groups (Figure 5.2).

![Figure 5.2](image-url)  
**Figure 5.2** Effect of MCT treatment on Right Ventricular Pressure (RVP) (mmHg). Data values were expressed as Mean ± S.E.M. (n=10). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (a=p<0.05 vs. control 21 days, bbb=p<0.001 vs. control 28 days, ccc=p<0.001 vs. control 35 days, $$$=p<0.001 vs. MCT 21 days and ###=p<0.001 vs. MCT 28 days).
5.1.1.3 Effect of MCT administration on right ventricular hypertrophy of rats

RVH is a cardiac complication closely associated with PH. There was no RVH after 21 days following MCT administration. However, a time dependent increase in RVH was observed after 28 and 35 days following MCT treatment. Value for RV/LV+S, RV/HW and RV/BW ratio after 28 days were 0.253 ± 0.018 (p<0.05); 0.312 ± 0.009 (p<0.05) and 0.94 ± 0.11 (p<0.05) respectively. There was further increase in the ratios RV/LV+S (0.405 ± 0.027, p<0.001), RV/HW (0.40 ± 0.071, p<0.01) and RV/BW (1.35 ± 0.119, p<0.01) after 35 days following MCT treatment as compared to the control group (Figure 5.3 a-c).

Figure 5.3 Effect of MCT administration on right ventricular hypertrophy (RVH). (a) ratio of RV/LV+S, (b) RV/HW and (c) RV/BW. Data values were expressed as Mean ± S.E.M. (n=10). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (b=p<0.05 vs. control 28 days, cc=p<0.01 vs. control 35 days, ccc=p<0.001 vs. control 35 days, $=p<0.05 vs. MCT 21 days, $$=p<0.01 vs. MCT 21 days, $$$=p<0.001 vs. MCT 21 days, #=p<0.05 vs. MCT 28 days and ###=p<0.001 vs. MCT 28 days). (RV= Right Ventricle, LV+S= Left Ventricle + Septum, HW= Heart Weight and BW= Body Weight).
5.1.1.4 Effect of MCT administration on systemic hemodynamic parameters

There was no significant change in Hemodynamic parameters amongst in various groups after 21, 28 and 35 days after MCT administration.

Table 5.1 Hemodynamic parameters in various groups after 21, 28 and 35 days after MCT administration.

<table>
<thead>
<tr>
<th>Hemodynamic parameters</th>
<th>Control</th>
<th>MCT 21 days</th>
<th>MCT 28 days</th>
<th>MCT 35 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP</td>
<td>114.4±4.92</td>
<td>106.71±5.27</td>
<td>95.45±6.49</td>
<td>103.41±10.15</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>81.9±4.57</td>
<td>79.66±4.33</td>
<td>80.06±8.87</td>
<td>74.22±6.99</td>
</tr>
<tr>
<td>Mean BP</td>
<td>92.79±6.68</td>
<td>88.66±3.85</td>
<td>85.47±6.72</td>
<td>83.81±8.16</td>
</tr>
<tr>
<td>Heart Rate</td>
<td>398±18</td>
<td>406±13</td>
<td>371±10</td>
<td>387±12</td>
</tr>
</tbody>
</table>

MCT- monocrotaline; BP- blood pressure

5.1.1.5. Effect of MCT administration on lung weight to body weight ratio of rats

Lung weight/ body weight ratio (LW/BW) is a marker of pulmonary vascular remodelling and inflammation in lungs. There was no significant
Figure 5.4 Effect of MCT administration on lung weight to body weight ratio (LW/BW). Data values were expressed as Mean ± S.E.M. (n=10). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+p<0.001 vs. control and +++p<0.001 vs. control).

increase in the LW/BW after 21 days following MCT treatment. But after 28 days and 35 days of MCT treatment LW/BW increased significantly to 0.026 ± 0.0013, p<0.01 and 0.029 ± 0.0015, p<0.001 respectively as compared to their time matched control group (Figure 5.4).

From these studies it can be concluded that MCT at a dose of 60 mg/kg established pulmonary hypertension as this dose caused a significant increase in the RVP and RVH.
5.1.2 Validation of monocrotaline model of pulmonary hypertension using different doses of Endothelin receptor antagonist Bosentan in MCT model of pulmonary hypertension

5.1.2.1 Effect of different doses of endothelin receptor antagonist bosentan on survival and body weight

MCT administration caused a decrease in percent survival (66.7%) as compared to control rats (100%) and administration of BOS (100 mg/kg) proved protective as it improved the percent survival (83.33%). The body weight following MCT treatment after 35 days was significantly less (325 ± 7.00g, p<0.01) as compared to the body weight of the control rats (414 ± 8.08 g) and BOS treatment (100 mg/kg) improved body weight in MCT exposed rats (377.3± 13.07 g, p<0.05). Bosentan at a dose of 50 mg/kg was found to be ineffective (Figure 5.5 a and b).

![Figure 5.5](image)

**Figure 5.5** Effect of Endothelin receptor antagonist Bosentan on (a) survival (%) and (b) body weight (g) in MCT treated rats. Data values were expressed as Mean ± S.E.M. (n=10). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (++p<0.01 vs. control, *p<0.05 vs. MCT).
5.1.2.2 Effect of different doses of endothelin receptor antagonist bosentan on right ventricular pressure (RVP)

Increase in the RVP is a marker for the increase in the pulmonary arterial pressure and pulmonary vascular resistance. There was a significant increase in the RVP following MCT treatment (43.41 ± 1.95 mmHg, p<0.01) after 35 days, as compared to the control group (21.60 ± 0.62 mmHg). Administration of BOS (100 mg/kg) in MCT treated group significantly attenuated the increase in the RVP (30.16 ± 3.01 mmHg, p<0.01). However, administration of BOS at the dose of 50 mg/kg following MCT treatment could not lower the increased RVP significantly (Figure 5.6).

![Figure 5.6](image)

**Figure 5.6** Effect of endothelin receptor antagonist Bosentan Right Ventricular Pressure (RVP) (mmHg) in MCT treated rats. Data values were expressed as Mean ± S.E.M. (n=10). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (++p<0.01 vs. control, **p<0.01 vs. MCT).
5.1.2.3. Effect of different doses of bosentan on right ventricular hypertrophy (RVH)

PH leads to RVH and there was a significant increase in the physical signs of RVH- RV/LV+S (0.45 ± 0.016, p<0.001), RV/HW (0.342 ± 0.028, p<0.01) and RV/BW (1.03 ± 0.12, p<0.01) following MCT treatment as compared to the control group (0.20 ± 0.015; 0.153 ± 0.014; 0.465 ± 0.02 respectively). Administration of BOS at the doses of 100 mg/kg in MCT treated group significantly inhibited the RVH (RV/LV+S- 0.33 ± 0.02, p<0.05; RV/HW- 0.233 ± 0.02, p<0.05 and RV/BW- 0.655 ± 0.06, p<0.05). This was also evident from histological analysis of cardiac sections and decrease in the RV wall area and RV to LV+S area % (Figure 5.7).
Figure 5.7 Effect of Endothelin receptor antagonist Bosentan on Right Ventricular Hypertrophy (RVH) in MCT treated rats. (a) RV/LV+S, (b) RV/H and (c) RV/BW. (d) H&E stained cardiac sections showing RVH. (e) RV wall area (mm²) and (f) RV/LV+S wall area (%) in various groups. Data values were expressed as Mean ± S.E.M. (n=10). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (**p<0.01 vs. control, ###p<0.001 vs. control *p<0.05 vs. MCT). (RV= Right Ventricle, LV+S= Left Ventricle + Septum, HW= Heart Weight and BW= Body Weight).
5.1.2.4 Effect of different doses of endothelin receptor antagonist on lung weight/ body weight ratio (LW/BW)

Following MCT treatment there was a significant increase in the lung weight/ body weight (LW/BW) ratio (0.0089 ± 0.00049, p<0.01), a marker of pulmonary vascular remodelling and inflammation in PH, as compared to the control group (0.0052± 0.00017). Administration of Bosentan at the doses of 100 mg/kg in MCT treated group significantly blunted the increase in the LW/BW ratio (0.0066 ± 0.00062, p<0.01) (Figure 5.8).

![Figure 5.8](image-url)

*Figure 5.8* Effect of endothelin receptor antagonist on Lung weight /Body weight (LW/BW) ratio in MCT treated rats. Data values were expressed as Mean ± S.E.M. (n=10). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (**p<0.01 vs. control, ***p<0.01 vs. MCT).

5.1.2.5 Effect of endothelin receptor antagonist on pulmonary vascular remodeling

MCT induced PH is characterized by pulmonary vascular remodelling and medial hypertrophy. Similarly, in the present study, quantitative morphometry showed that MCT caused a significant increase in muscularization of distal pulmonary arteries with diameter <100 µm (40.5 ± 1.04%, p< 0.001) and 100-
250 µm (38.50 ± 1.7%, p<0.001) as compared to control animals (21.5 ± 1.32% and 16.75± 1.31% respectively). Quantitative morphometric analysis demonstrated that BOS 100 mg/kg significantly decreased the muscularization of <100 µm (28.25 ± 1.65%, p<0.001) and 100-250 µm (19.75 ± 2.17%, p<0.001) distal pulmonary arteries as compared to MCT treated rats.

![Image of pulmonary arteries with hematoxylin and eosin staining and bar graphs showing medial wall thickness](image)

**Figure 5.9** Effect of endothelin receptor antagonist, bosentan (BOS) on pulmonary vasculature remodeling induced by MCT administration. (a) Hematoxylin and eosin staining of pulmonary arteries. (b) Medial wall thickness of <100µm diameter arteries. (c) Medial wall thickness of 100-250 µm diameter arteries. Mean ± S.E.M. (n=5-7). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+++p<0.001 vs. control and ***p<0.001 vs. MCT).
5.1.2.6 Effect of bosentan on systemic hemodynamic parameters

There was no significant change in hemodynamic parameters amongst various groups (Table 5.2).

**Table 5.2** Effect of Endothelin receptor antagonist on systemic hemodynamic parameters in MCT treated rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>MCT</th>
<th>BOS (50 mg/kg)</th>
<th>BOS (100 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>111.06±10.47</td>
<td>107.31±9.40</td>
<td>116.4±6.1</td>
<td>98.93±1.734</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>69.86±5.54</td>
<td>74.68±7.09</td>
<td>77.7±5.9</td>
<td>76.01±1.224</td>
</tr>
<tr>
<td>Mean Arterial Pressure (mmHg)</td>
<td>83.59±7.43</td>
<td>85.55±6.88</td>
<td>87.57±3.1</td>
<td>83.65±1.247</td>
</tr>
<tr>
<td>Heart Rate (HR) beats/minute</td>
<td>384±26</td>
<td>379±32</td>
<td>364±25</td>
<td>359.1±27.05</td>
</tr>
</tbody>
</table>

MCT- Monocrotaline, BOS- Bosentan
5.2 A study on the involvement of poly (ADP-ribose) polymerase-1 (PARP-1) in pulmonary hypertension

5.2.1 Standardization of the dose of PARP-1 inhibitor, 1,5-Isoquinolinediol (ISO), in MCT model of pulmonary hypertension in rat

5.2.1.1. Effect of different doses of PARP-1 inhibitor, 1,5-Isoquinolinediol (ISO) dose in MCT model of pulmonary hypertension on survival and body weight

MCT administration in rats led to a significant decrease in the percent survival (65%) and delay in the body weight gain (330.8 ± 14.88 g, p<0.001) after 35 days, as compared to the control group (100% and 411.2 ± 9.98 g).

**Figure 5.10** Effect of PARP-1 inhibition by ISO on (a) survival (%) and (b) body weight in MCT treated rats. Data values were expressed as Mean ± S.E.M. (n=10). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+++p<0.001 vs. control, **p<0.01 vs. MCT).

Administration of ISO at the doses of 3 mg/kg and 6 mg/kg in MCT treated group significantly improved the survival (85% and 90%) and increased the body weight of rats (386.5±9.8 g, p<0.01 and 388.3 ± 8.4 g, p<0.01).
respectively. The dose of ISO 1.5 mg/kg could not cause significant improvement in survival or body weight.

5.2.1.2 Effect of different doses of PARP-1 inhibitor, 1,5-Isoquinolinediol (ISO), on right ventricular pressure (RVP)

Increase in the RVP is a marker for the increase in the pulmonary arterial pressure and pulmonary vascular resistance. There was a significant increase in the RVP following MCT treatment (44.25 ± 1.68 mmHg, p<0.001) after 35 days as compared to the control group (18.94 ± 1.19 mmHg). Administration of ISO at the doses of 3 mg/kg and 6 mg/kg in

![Graph showing effect of PARP-1 inhibition by ISO on Right Ventricular Pressure (RVP) (mmHg) in MCT treated rats. Data values were expressed as Mean ± S.E.M. (n=10). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test ++p<0.001 vs. control, ***p<0.001 vs. MCT).](image)

**Figure 5.11** Effect of PARP-1 inhibition by ISO on Right Ventricular Pressure (RVP) (mmHg) in MCT treated rats. Data values were expressed as Mean ± S.E.M. (n=10). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test ++p<0.001 vs. control, ***p<0.001 vs. MCT).

MCT treated group significantly inhibited the increase in the RVP (30.35 ± 1.75 mmHg, p<0.001 and 31.13 ± 2.07 mmHg, p<0.001, respectively). However, administration of ISO at the dose of 1.5 mg/kg following MCT treatment caused mild but not a significant decrease in the RVP. ISO at the doses of 3 mg/kg and 6 mg/kg had similar effect in lowering RVP.
5.2.1.3 Effect of different doses of PARP-1 inhibitor, 1,5- Isoquinolinediol (ISO) on right ventricular hypertrophy (RVH)

PH leads to RVH and there was a significant development in RVH (RV/LV+S-0.40 ± 0.027, p<0.001; RV/HW- 0.40 ± 0.071, p<0.01; RV/BW- 1.16 ±0.102, p<0.01) following MCT treatment as compared to the control group (RV/LV+S-0.18 ± 0.007, RV/HW- 0.15 ± 0.007; RV/BW- 0.48 ± 0.031). Administration of ISO at the doses of 3 mg/kg and 6 mg/kg in MCT treated group significantly lowered the physical parameters of RVH (RV/LV+S- 0.27 ± 0.014, p<0.01 and 0.24 ± 0.014, p<0.001; RV/HW- 0.22 ± 0.006, p< 0.05 and 0.20 ± 0.0068, p<0.05; RV/BW- 0.68±0.11, p<0.05 and 0.63±0.13, p<0.05, respectively) (Figure 5.12). Dose of ISO at 1.5 mg/kg was ineffective in reducing RVH. The protective effect of ISO 3 mg/kg and 6 mg/kg was found to be similar.

**Figure 5.12** Effect of PARP-1 inhibition by ISO on Right Ventricular Hypertrophy (RVH) in MCT treated rats. (a) RV/LV+S, (b) RV/HW and (c) RV/BW. Data values were expressed as Mean ± S.E.M. (n=10). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (++p<0.01 vs. control, +++p<0.001 vs. control, *p<0.05 vs. MCT **p<0.01 vs. MCT). (RV= Right Ventricle, LV+S= Left Ventricle + Septum, HW= Heart Weight and BW= Body Weight).
5.2.1.4 Effect of different doses of PARP-1 inhibitor, 1,5-Isoquinolinediol (ISO), on lung weight/ body weight ratio (LW/BW)

Following MCT treatment there was a significant increase in the lung weight/ body weight ratio (LW/BW) (0.03 ± 0.0015, p<0.001), a marker of pulmonary vascular remodelling and inflammation in lungs, as compared to the control group (0.10 ± 0.0008). Administration of ISO at the doses of 3 mg/kg and 6 mg/kg in MCT treated group significantly inhibited the increase in the LW/BW ratio (0.012 ± 0.002, p< 0.001 and 0.010 ± 0.002, p< 0.001, respectively). No beneficial effect of ISO at 1.5 mg/kg was observed. There was no significant difference between the doses 3 mg/kg and 6 mg/kg (Figure 5.13).

![Figure 5.13](image)

**Figure 5.13** Effect of PARP-1 inhibition by ISO on Lung weight /Body weight (LW/BW) ratio in MCT treated rats. Data values were expressed as Mean ± S.E.M. (n=10). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+++p<0.001 vs. control, ***p<0.001 vs. MCT).
5.2.1.5 Effect of different doses of PARP-1 inhibitor, 1,5- Isoquinolinediol (ISO) on hemodynamic parameters

Table 5.3 Effect of different doses of PARP-1 inhibitor on systemic hemodynamic parameters in MCT treated rats.

<table>
<thead>
<tr>
<th>Groups/ Hemodynamic parameters</th>
<th>Systolic BP</th>
<th>Diastolic BP</th>
<th>Mean BP</th>
<th>Heart Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>104.4±7.92</td>
<td>86.71±5.27</td>
<td>92.60±6.49</td>
<td>366±11.13</td>
</tr>
<tr>
<td>MCT</td>
<td>91.9±4.57</td>
<td>79.66±4.33</td>
<td>80.06±8.87</td>
<td>372±8.0</td>
</tr>
<tr>
<td>ISO 1.5 mg/kg</td>
<td>92.79±6.68</td>
<td>88.66±3.85</td>
<td>85.47±6.72</td>
<td>311±14.24</td>
</tr>
<tr>
<td>ISO 3 mg/kg</td>
<td>93.52±7.22</td>
<td>72.11±3.48</td>
<td>74.29±5.12</td>
<td>406±13</td>
</tr>
<tr>
<td>ISO 6 mg/kg</td>
<td>98.01±6.51</td>
<td>76.71±5.34</td>
<td>83.81±8.16</td>
<td>398±18</td>
</tr>
<tr>
<td>DMSO</td>
<td>103.41±10.15</td>
<td>74.22±6.99</td>
<td>83.95±4.11</td>
<td>387±12</td>
</tr>
</tbody>
</table>

MCT- Monocrotaline, ISO- 1,5- Isoquinolinediol, BP- Blood Pressure, DMSO- Dimethylsulfoxide

There was no significant reduction in RVP, RVH and improvement in survival and body weight with 1.5 mg/kg dose of ISO. So, for further studies, this dose of ISO was dropped. Further, there was no significant difference in RVP, RVH and LW/BW at the effective doses of 3 and 6 mg/kg of ISO. Therefore, the lower effective dose of 3 mg/kg of ISO was selected for the further studies. The *per se* effect of standardized dose of ISO was also studied, but there was no effect on any of the studied parameters of PH.
5.2.1.6 Effect of PARP-1 inhibition on arterial oxygen saturation

Oxygen saturation means the percentage of haemoglobin binding sites by oxygen in the bloodstream. The percentage arterial oxygen saturation decreased significantly 35 days following the administration of MCT (81.25 ± 0.62%, p<0.001) as compared to control (92.53 ± 0.49%). Treatment with ISO improved the percentage arterial oxygen saturation (90.97 ± 0.72%, p<0.001). Administration of 10% DMSO following the MCT injection did not have any significant change on percentage arterial oxygen saturation (79.87 ± 1.4 %, p<0.001) (Figure 5.14).

![Figure 5.14](image)

**Figure 5.14** Effect of PARP-1 inhibition by ISO on arterial oxygen saturation (%) in MCT treated rats. Data values were expressed as Mean ± S.E.M. (n=8). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+++p<0.001 vs. control, ***p<0.001 vs. MCT).

5.2.1.7 Effect of PARP-1 inhibition on oxidative stress in lungs

After the treatment with MCT, there was a significant increase in the levels of ROS, MDA, nitrite and a significant decrease in the GSH as compared to the levels of these parameters in the control group in lungs.
Figure 5.15 Effect of PARP-1 inhibition by ISO on levels of (a) reactive oxygen species by fluorimetric method (% Control), (b) reactive oxygen species by FACS (Relative Fluorescence Units; RFU) (c) MDA levels (nmol/ mg protein) (d) Nitrite (nmol/ mg protein) and (e) GSH levels (µg/ mg protein) in lungs. Data values were expressed as Mean ± S.E.M. (n=8). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+p<0.05 vs. control, +++p<0.001 vs. control, *p<0.05 vs. MCT, and **p<0.01 vs. MCT ***p<0.001 vs. MCT).

Administration of ISO in MCT treated group significantly attenuated oxidative stress parameters. However, administration of 10% DMSO (vehicle of ISO) showed no beneficial effect on oxidative stress in MCT treated rats (Figure 5.15).
5.2.1.8 Effect of PARP-1 inhibition on DNA damage

Increased oxidative stress results in DNA damage which is marked by increased expression of g-H2AX (a marker of DNA damage). In MCT treated rats also, there was a significant increase in the expression of g-H2AX after 35 days which was ameliorated by PARP-1 inhibition. Administration of vehicle for ISO (i.e. DMSO) had no protective effect on DNA damage (Figure 5.16).

![Figure 5.16](image)

Figure 5.16 Effect of PARP-1 inhibition by ISO on expression of g-H2AX. Data values were expressed as Mean ± S.E.M. (n=3). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+++p<0.001 vs. control and **p<0.001 vs. MCT).

5.2.1.9 Effect of PARP-1 inhibition on PARP-1 activity, PARP-1 mRNA and PARP-1 protein expression in lungs

After the treatment with MCT, there was a significant increase in the PARP-1 activity (4.06 ± 0.29 % control, p<0.001), PARP-1 mRNA (> 2 folds, p<0.01) and protein expression (~ 3 folds, p<0.001) as compared to
The control group in lungs. Administration of PARP-1 inhibitor (ISO) in MCT treated group significantly reversed the increase in PARP-1 activity (2.59 ± 0.35 % control, p<0.01), PARP-1 mRNA and protein expression. However, administration of 10% DMSO (vehicle of ISO) following MCT treatment had no significant effect in the PARP-1 activity and PARP-1 expression of as compared to MCT treated rats (Figure 5.17).
5.2.1.10 Effect of PARP-1 inhibition on PARylation in lungs

After the treatment with MCT, there was a significant increase in the PARylation of proteins, a marker of increased PARP-1 activity (checked by immunoblotting and immunohistochemistry) as compared to the control group in lungs. Administration of PARP-1 inhibitor (ISO) in MCT treated group significantly reversed the increase in PARylation of various proteins. However, administration of 10% DMSO (vehicle of ISO) following MCT treatment had no effect on reducing PARylation in MCT treated rats (Figure 5.18).

**Figure 5.18** Effect of PARP-1 inhibition by ISO on (PARylation in lungs) (a) PAR expression (immunoblotting) (b) PAR expression (immunohistochemistry). Data values were expressed as Mean ± S.E.M. (n=8). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+++p<0.001 vs. control and **p<0.001 vs. MCT).
5.2.1.11 Effect of PARP-1 inhibition on NAD and ATP levels in lungs

After the treatment with MCT, there was a significant decrease in the NAD and ATP levels as compared to the control group in lungs. Administration of PARP-1 inhibitor (ISO) in MCT treated group significantly improved the levels of NAD and ATP and lungs. However, administration of 10% DMSO (vehicle of ISO) following MCT treatment had no effect on increasing NAD and ATP levels in MCT treated rats.

![Figure 5.19](attachment:image.png)  
**Figure 5.19** Effect of PARP-1 inhibition by ISO on (a) NAD levels (% control) (b) ATP levels (% control). Data values were expressed as Mean ± S.E.M. (n=8). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (++p<0.01 vs. control, +++p<0.001 vs. control, *p<0.05 vs. MCT and **p<0.01 vs. MCT).

5.2.1.12 Effect of PARP-1 inhibition on inflammation in lungs

Inflammation initiates vascular remodelling and participates in the propagation of the disease in the PH. There was a significant decrease in the anti-inflammatory cytokine IL-10 levels (56.18 ± 5.6 pg/mg protein, p<0.001) and increase in the pro-inflammatory cytokine TNF-α levels (179.6 ±19.24, pg/mg protein; p<0.001) in lungs of MCT treated rats as compared to the control group (IL-10- 184.4 ± 16.6 pg/mg protein; TNF-α- 20.33 ± 5.94 pg/mg protein). PARP-1 inhibition in MCT treated group significantly reversed these effects (IL-10- 119.9 ± 14.95, pg/mg protein p<0.01; TNF-α- 98.82 ± 14.19 pg/mg
RESULTS

PULMONARY HYPERTENSION

Figure 5.20 Effect of PARP-1 inhibition by ISO on (a) IL-10 (pg/mg protein) levels (b) TNF α levels (pg/mg protein) (c) p65 subunit of NFκB expression in lungs. Data values were expressed as Mean ± S.E.M. (n=8). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+++p<0.001 vs. control, **p<0.01 vs. MCT and ***p<0.001 vs. MCT).

protein, p<0.01). PARP-1 inhibition also lowered the expression of p65 subunit of NFκB in MCT challenged rats (Figure 5.20).

5.2.1.13 Effect of PARP-1 inhibition on endothelial dysfunction

Endothelial dysfunction is a marked pathophysiological characteristic of PH. Acetylcholine (ACh) induced vasorelaxation was significantly decreased in phenylephrine precontracted pulmonary arteries isolated from MCT treated rats as compared to control group. Pulmonary arteries isolated from ISO treated rats showed a significant increase in the vasorelaxation following phenylephrine induced vasoconstriction as compared to MCT group suggesting improvement in endothelial dysfunction (Figure 5.21). Further, there was significant decrease in the eNOS expression in the lungs of MCT challenged
rats confirming endothelial dysfunction but over expression of the eNOS following ISO treatment proved that PARP-1 inhibition improves endothelial function in MCT induced PH.

![Graph showing relaxation vs. ACh Concentration](image)

**Figure 5.21** Effect of PARP-1 inhibition by ISO on (a) pulmonary endothelial dysfunction in extralobar pulmonary arteries and (b) eNOS expression in lungs. Data values were expressed as Mean ± S.E.M. (n=8). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test. (+p<0.05 vs. control +++p<0.001 vs. control, *p<0.05 vs. MCT, **p<0.01 vs. MCT and ***p<0.001 vs. MCT)

### 5.2.1.14 Effect of PARP-1 inhibition on pulmonary vascular remodeling

There was a significant increase in the medial wall thickness in pulmonary blood vessels (<100 µm diameter- 40.30 ± 1.36%, p<0.001; 100 µm – 250 µm diameter- 36.70 ± 1.92%, p<0.001) in MCT challenged rats as compared to the control group (<100 µm diameter- 19.50 ± 1.58%; 100- 250 µm diameter- 10.30 ± 0.91%). Administration of ISO in MCT treated group significantly ameliorated the increase in the medial wall thickness (<100 µm diameter- 32.20 ± 1.38%, p<0.01; 100 µm- 250 µm diameter- 21.20± 1.09%) in pulmonary
vasculature. However, administration of vehicle of ISO (10% DMSO) following MCT treatment did not decrease the medial hypertrophy in pulmonary vessels (Figure 5.22 a-c) of MCT challenged rats. Pulmonary vascular remodelling

![Figure 5.22](image)

**Figure 5.22** Effect of PARP-1 inhibition by ISO on pulmonary vasculature remodeling induced by MCT administration. (a) Hematoxylin and eosin staining of pulmonary arteries. (b) Medial wall thickness of <100µm diameter arteries, (c) Medial wall thickness of 100-250 µm diameter arteries and (d) Expression of PCNA in lungs. Data values were expressed as Mean ± S.E.M. (n=8). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (++p<0.01 vs. control, +++p<0.01 vs. control *p<0.05 vs. MCT, **p<0.01 vs. MCT and ***p<0.001 vs. MCT).

associated with PH is marked by increased proliferating cell nuclear antigen (PCNA), a marker of cell proliferation. Our study also showed an increase in the protein content of PCNA in the lungs of MCT treated group as compared to the control group. Treatment with ISO (3 mg/kg) reduced the expression of PCNA demonstrating its effect in curbing proliferation in lungs (Figure 5.22 d).
5.2.1.15 Effect of PARP-1 inhibition on matrix metalloproteinases- MMP-2, MMP-9 and tissue inhibitor of metalloproteinases (TIMP) expression

Extracellular matrix degradation is an important event in the PH pathophysiology. Following the MCT treatment, there was a significant increase in the expression of MMP-2 (~ 4 folds) and MMP-9 (> 2 folds) along with a decrease in the levels of endogenous MMPs inhibitor, TIMP-2, in lungs. The increase in expression of MMPs was attenuated by the administration of ISO and levels of TIMP-2 (~ 2 folds) were increased so as to restore TIMP/MMP balance (Figure 5.23).

5.2.1.16 Effect of PARP-1 inhibition on apoptosis in lungs

Apart from pulmonary vascular remodelling and proliferation, apoptosis resistance in lung vasculature is the characteristic feature of PH. MCT treated rats showed apoptotic resistance as evident from TUNEL studies as compared...
RESULTS

to control rats in lungs. Treatment with ISO showed an increase in apoptotic cells as evident by numerous TUNEL positive cells in the lung sections (Figure 5.24).

The caspase-3 activity, a marker of apoptosis, was also found reduced in the lungs of MCT treated rats as compared to control rats suggesting apoptosis resistance in PH. Treatment with ISO increased the activity of caspase-3 in lungs thus weakening the apoptosis resistance.

Figure 5.24 Effect of PARP-1 inhibition by ISO on apoptosis in lungs. (a) Representative micrographs of TUNEL (green) and DAPI (blue) staining of lung section. Quantitative analysis of apoptotic cells was done by counting the positively stained cells in three randomized area and then summed to obtain the apoptotic population under florescence microscope. The results were expressed as number of positive cells per 0.5 mm². (b) Caspase-3 activity showing ISO treatment increased the activity of caspase-3 in lungs as compared to MCT challenged rats. Data values were expressed as Mean ± S.E.M. (n=5-7 in each group). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test *** p<0.001 vs. MCT.
5.2.1.17 Effect of PARP-1 inhibition on HIF-1α expression

One of the most important transcription factors involved in the physiological responses to PH is HIF-1. Following the MCT treatment, there was a significant increase in the expression of HIF-1α, a sub-unit of HIF-1, in lungs. The increase in expression of HIF-1α was attenuated by the administration of ISO. But there was no decrease in HIF-1α expression upon administration of DMSO (10%) following MCT treatment (Figure 5.25).

![Figure 5.25](image)

**Figure 5.25** Representative western blot showing the effect of PARP-1 inhibition by ISO on the expression of HIF-1α in the lungs of rats from different experimental groups. The results are expressed as mean ± S.E.M. (n=5 in each group). ++p<0.01 vs. control, and *p<0.05 vs. MCT.

5.2.1.18 Effect of PARP-1 inhibition on GSK 3β expression

Following MCT treatment, there was a significant increase in the expression of phospho- GSK3β in lungs. The increase in expression of phospho-GSK 3β was attenuated by the administration of ISO. But there was no decrease in phospho-GSK 3β expression upon administration of DMSO (10%) following MCT treatment (Figure 5.26).
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**Figure 5.26** Representative western blot showing the effect of PARP-1 inhibition by ISO on the expression of phosphor-GSK3β in the lung of MCT challenged rats. The results are expressed as mean ± S.E.M. (n=5 in each group). +++p<0.001 vs. control, and **p<0.01 vs. MCT.

### 5.2.1.19 Effect of PARP-1 inhibition on VEGF expression

One of the most important transcription factors that gets upregulated because of overexpression of HIF-1α is VEGF. Following the MCT treatment, there was a significant increase in the expression of VEGF in lungs. The increase in expression of VEGF was attenuated by the administration of ISO. But there was no decrease in VEGF expression upon administration of DMSO (10%) following MCT treatment (Figure 5.27).

**Figure 5.27** Representative western blot showing the effect of PARP-1 inhibition by ISO on the expression of VEGF in the lung of MCT treated rats. The results are expressed as mean ± S.E.M. (n=5 in each group). +++p<0.001 vs. control and **p<0.01 vs. MCT.
5.2.2 Effect of PARP-1 inhibition in curative model of pulmonary hypertension

5.2.2.1. Effect of PARP-1 inhibition, on survival and body weight of rat in curative model of pulmonary hypertension

MCT administration in rats led to significant decrease in percent survival (60%) and fall in body weight after 35 days (308.2 ± 7.7g, p<0.001), as Administration of ISO (from 21st day to 35th day) at the dose of 3 mg/kg in MCT treated group significantly improved percent survival (83.33%) and increased the body weight (328.7 ± 2.6 g, p<0.05) of rats in curative model of PH compared to the control group (100% and 396.3 ± 7.4 g respectively).

![Figure 5.28](image)

**Figure 5.28** Effect of PARP-1 inhibition by ISO on (a) survival (%) and (b) body weight in MCT treated rats. Data values were expressed as Mean ± S.E.M. (n=10). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+++p<0.001 vs. control, *p<0.05 vs. MCT).
5.2.2.2 Effect of PARP-1 inhibition on right ventricular pressure (RVP) in curative model of pulmonary hypertension

Increase in the RVP is a marker for the increase in the pulmonary arterial pressure and pulmonary vascular resistance. There was a significant increase in the RVP following MCT treatment after 35 days (44.45 ± 1.1 mmHg, p<0.001), as compared to the control group (19.2 ± 1.2 mmHg). Administration of ISO (in curative model of PH for 2 weeks) at the dose of 3 mg/kg significantly prevented the increase in the RVP (39.1 ± 0.91 mmHg, p<0.05) in curative model of PH. (Figure 5.29).

![Graph showing effect of PARP-1 inhibition on RVP in MCT treated rats](image)

**Figure 5.29** Effect of PARP-1 inhibition by ISO on right ventricular pressure (RVP) (mmHg) in MCT treated rats in curative model of PH. Data values were expressed as Mean ± S.E.M. (n=10). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+++p<0.001 vs. control and *p<0.05 vs. MCT).

5.2.2.3 Effect of PARP-1 inhibition on right ventricular hypertrophy (RVH) in curative model of pulmonary hypertension

PH leads to RVH is and there was a significant increase in the RVH following MCT treatment (RV/LV+S- 0.41 ± 0.014, p<0.01; RV/HW- 0.42 ± 0.04, p<0.01; RV/BW- 1.21 ± 0.062, p<0.001) as compared to the control group (RV/LV+S- 0.18 ± 0.01; RV/HW- 0.16 ± 0.014; RV/BW- 0.47 ± 0.025). Administration of ISO at the dose of 3 mg/kg in MCT treated group significantly inhibited the development of RVH (RV/LV+S- 0.37 ± 0.013,
p<0.05; RV/HW-0.27 ± 0.014, p<0.05; RV/BW- 0.74 ± 0.13, p<0.05) in already progressing PH (Figure 5.30).

**Figure 5.30** Effect of PARP-1 inhibition by ISO on right ventricular hypertrophy (RVH) in MCT treated rats. (a) RV/LV+S, (b) RV/HW and (c) RVBW. Data values were expressed as Mean ± S.E.M. (n=10). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+++p<0.001 vs. control and *p<0.05 vs. MCT). (RV= Right Ventricle, LV+S= Left Ventricle + Septum, HW= Heart Weight and BW= Body Weight).

5.2.2.4 Effect of PARP-1 inhibition on lung weight/ body weight ratio (LW/BW) in curative model of pulmonary hypertension

Following MCT treatment there was a significant increase in the lung weight/ body weight ratio (LW/BW) (0.026 ± 0.002, p<0.001), as compared to the control group (0.011 ± 0.0005). Administration of ISO at the dose of 3 mg/kg in
MCT treated group significantly inhibited the increase in the LW/BW ratio (0.019 ± 0.0008, p<0.05) (Figure 5.31).

**Figure 5.31** Effect of PARP-1 inhibition by ISO on Lung weight /Body weight (LW/BW) ratio in MCT treated rats. Data values were expressed as Mean ± S.E.M. (n=10). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+++p<0.001 vs. control, *p<0.05 vs. MCT).

### 5.2.2.5 Effect of PARP-1 inhibition on hemodynamic parameters in curative model of pulmonary hypertension

There was no significant change in hemodynamic parameters amongst various groups when ISO was administered from day 21 to day 35 (Table 5.4).

**Table 5.4** Effect of PARP-1 inhibition by ISO on hemodynamic parameters in MCT treated rats.

<table>
<thead>
<tr>
<th>Groups/ Hemodynamic parameters</th>
<th>Systolic BP</th>
<th>Diastolic BP</th>
<th>Mean BP</th>
<th>Heart Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>104.4±7.92</td>
<td>86.71±5.27</td>
<td>92.60±6.49</td>
<td>366±11.13</td>
</tr>
<tr>
<td>MCT</td>
<td>91.9±4.57</td>
<td>79.66±4.33</td>
<td>80.06±8.87</td>
<td>372±8.0</td>
</tr>
<tr>
<td>ISO 3 mg/kg</td>
<td>93.52±7.22</td>
<td>72.11±3.48</td>
<td>74.29±5.12</td>
<td>406±13</td>
</tr>
</tbody>
</table>

MCT- Monocrotaline, ISO- 1,5- Isoquinolinediol, BP- Blood Pressure
RESULTS

5.3 Effect of PARP-1 inhibition on right ventricle hypertrophy in MCT induced pulmonary hypertension in rats

5.3.1 Effect of PARP-1 inhibition on right ventricular pressure (RVP)

Increase in the RVP is a characteristic feature of PH. There was a significant increase in the RVP following MCT treatment (44.25 ± 1.68 mmHg, p<0.001) after 35 days, as compared to the control group (18.94 ± 1.19 mmHg). Administration of ISO at the dose of 3 mg/kg in MCT treated group significantly inhibited the increase in the RVP (30.35 ± 1.75 mmHg, p<0.001). Administration of DMSO had no beneficial effect on raised RVP (Figure 5.32).

![Figure 5.32](image)

**Figure 5.32** Effect of PARP-1 inhibition by ISO on right ventricular pressure (RVP) (mmHg) in MCT treated rats. Data values were expressed as Mean ± S.E.M. (n=10). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+++p<0.001 vs. control, ***p<0.001 vs. MCT).

5.3.2 Effect of PARP-1 inhibition on right ventricular hypertrophy (RVH) (physical parameters)

RVH is secondary complication associated with PH. There was a significant development in RVH (RV/LV+S: 0.40 ± 0.027, p<0.001; RV/HW: 0.40 ± 0.071, p<0.01; RV/BW: 1.16 ± 0.102, p<0.01) following MCT treatment as
compared to the control group (RV/LV+S- 0.18 ± 0.007, RV/HW- 0.15 ± 0.007; RV/BW- 0.48 ±0.031). Administration of

![Image](https://example.com/image.png)

**Figure 5.33** Effect of PARP-1 inhibition by ISO on right ventricular hypertrophy (RVH) in MCT treated rats. (a) RV/LV+S, (b) RV/HW and (c) RV/BW. Data values were expressed as Mean ± S.E.M. (n=10). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (++p<0.01 vs. control, +++p<0.001 vs. control, *p<0.05 vs. MCT **p<0.01 vs. MCT). (RV= Right Ventricle, LV+S= Left Ventricle + Septum, HW= Heart Weight and BW= Body Weight).

ISO at the doses of 3 mg/kg in MCT treated group significantly inhibited the development of RVH (RV/LV+S- 0.27 ± 0.014, p<0.01; RV/HW- 0.22 ± 0.006, p<0.05; RV/BW- 0.68±0.11, p<0.05 respectively) (Figure 5.33).

### 5.3.3 Effect of PARP-1 inhibition on right ventricle hypertrophy (histological studies)

Cardiac remodelling develops in PH primarily as a compensatoryadaptive phenomenon against increased resistance in the pulmonary circulation.
a.

**Figure 5.34** Effects of PARP-1 inhibition by ISO on rat cardiac remodeling induced by MCT administration. (a) Hematoxylin and eosin stained heart sections (b) Right ventricle wall area (mm$^2$) and (c) Right ventricle to left ventricle + septum wall area (%). The results are expressed as mean ± S.E.M. (n=5 in each group). +++p<0.001 vs. control and *p<0.05 vs. MCT.

Following the MCT treatment, there was a significant increase in the RV wall area showing RVH. The increase in the RVH was attenuated by PARP-1 inhibition. But there was no decrease in the RVH upon administration of DMSO (10%) following MCT treatment (Figure 5.34).

### 5.3.4 Effect of PARP-1 inhibition on Matrix Metalloproteinases- MMP-2, MMP-9 and Tissue Inhibitor of Metalloproteinases (TIMP) expression

Extracellular matrix degradation is an important event in the cardiac dysfunction. Following the MCT treatment, there was a significant
Figure 5.35 Representative western blot showing the effect of PARP-1 inhibition by ISO on the expression of genes involved in extracellular matrix deposition in the right ventricle of rats. The results are expressed as mean ± S.E.M. (n=5 in each group). *p<0.05 vs. control, **p<0.01 vs. control, *p<0.05 vs. MCT and **p<0.01 vs. MCT.

increase in the expression of MMP-2 and MMP-9 along with a decrease in the expression of TIMP-2 in RV. The increase in the expression of MMPs and decrease in the expression of TIMP-2 was reversed by the administration of ISO and (Figure 5.35).

5.3.5 Effect of PARP-1 inhibition on oxidative stress in right ventricle

After the treatment with MCT, there was a significant increase in the levels of reactive oxygen species (ROS) and malondialdehyde (MDA, a marker of lipid peroxidation), nitrite and significant decrease in the GSH (an endogenous antioxidant) as compared to the control group in right ventricle. Administration of ISO in MCT treated group significantly attenuated the oxidative stress as evident from various parameters. However, administration of 10% DMSO
(vehicle of ISO) following MCT administration had similar effect as MCT treated rats (Figure 5.36).

**Figure 5.36** Effect of PARP-1 inhibition by ISO on level of (a) reactive oxygen species (% Control) by fluorimetry, (b) reactive oxygen species (Relative Fluorescence Units; RFU) by FACS (c) Nitrite (nmol/ mg protein) (d) MDA levels (nmol/ mg protein) and (e) GSH levels (µg/ mg protein) in right ventricle. Data values were expressed as Mean ± S.E.M. (n=8). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (++p<0.01 vs. control, +++p<0.001 vs. control, *p<0.05 vs. MCT, **p<0.01 vs. MCT).

**5.3.6 Effect of PARP-1 inhibition on DNA damage**

Increased oxidative stress is followed by DNA damage and g-H2AX is taken as a marker of DNA damage. There was significant increase in the expression of g-H2AX in MCT treated rats after 35 days, and, this was ameliorated by PARP-1 inhibition. Administration of vehicle for ISO (i.e. DMSO) had no protective effect on DNA damage (Figure 5.37).
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Figure 5.37 Effect of PARP-1 inhibition by ISO on expression of g-H2AX. Data values were expressed as Mean ± S.E.M. (n=3). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+++p<0.001 vs. control and ***p<0.001 vs. MCT).

5.3.7 Effect of PARP-1 inhibition on PARP-1 activity, PARP-1 expression and PARylation in right ventricle

After the treatment with MCT, there was a significant increase in the PARP-1 activity (306 ± 27.36 %control, p<0.001), as compared to the control, and this was reversed by PARP-1 inhibition (145 ± 14.12 % control, p<0.01). Similarly, PARP-1 expression (both mRNA and protein) and PARylation of proteins were markedly raised following MCT exposure as compared to the control group in right ventricle, which were
inhibited by the treatment with ISO. However, administration of 10% DMSO (vehicle of ISO) following MCT treatment had no significant change in the PARP-1 expression as compared to MCT treated rats (Figure 5.38).
5.3.8 Effect of PARP-1 inhibition on NAD and ATP levels in right ventricle

After the treatment with MCT, there was a significant decrease in the NAD and ATP levels as compared to the control group in RV. Administration of PARP-1 inhibitor (ISO) in MCT treated group significantly improved the levels of NAD and ATP in RV. However, administration of 10% DMSO (vehicle of ISO) following MCT treatment had no effect on NAD and ATP levels in MCT treated rats.

![Figure 5.39](image)

**Figure 5.39** Effect of PARP-1 inhibition by ISO on (a) NAD levels (% Control) (b) ATP levels (% Control) in right ventricle. Data values were expressed as Mean ± S.E.M. (n=8). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (++p<0.01 vs. control, +++p<0.001 vs. control, *p<0.05 vs. MCT and **p<0.01 vs. MCT).
5.3.9 Effect of PARP-1 inhibition on inflammation in right ventricle

Inflammation plays a key role in the development and progression of the PH syndrome and in accordance to that there was a significant decrease in the level of anti-inflammatory cytokine IL-10 levels (188.8 ± 19.51 pg/mg protein, p<0.001) and increase in the level of pro-inflammatory cytokine TNF-α (309.6 ± 36.69 pg/mg protein, p<0.001) and expression of p65 subunit of NFκB (> 3folds) in RV of MCT treated rats as compared to the control group (IL-10- 464.0 ± 25.01 pg/mg protein; TNF-α- 30.6 ± 15.06 pg/mg protein). Administration of ISO in MCT treated group significantly reversed (IL-10- 317.2 ± 28.03, p<0.05; TNF-α- 97.96 ± 26.46, p<0.01) these effects (Figure 5.40).
5.3.10 Effect of PARP-1 inhibition on apoptosis in right ventricle

MCT treated rats showed increased apoptosis in RV as evident from TUNEL studies as compared to control rats. Using PARP-1 inhibitor as pharmacological tool led to decrease in the number of apoptotic cells by in the right ventricle sections (Figure 5.41).

Figure 5.41 Effect of PARP-1 inhibition by ISO on apoptosis in right ventricle. Representative micrographs of TUNEL (green) and DAPI (blue) staining of lung section. ISO treatment decreased MCT-induced increase in number of apoptotic cells. The results are expressed as mean ± S.E.M. (n=5 in each group). +++p<0.001 vs. control and +++p<0.001 vs. MCT.
5.3.11 Effect of PARP-1 inhibition on mitochondrial membrane potential (▲Ψm) in right ventricle

Loss in (▲Ψm) was checked in JC-1 stained single cell suspension of RV. As displayed in dot plots (Figure 5.42), single cell suspension from RV of MCT treated rats showed a marked increase of fluorescence of green indicating loss of mitochondrial membrane potential as compared to the control rats plot. In contrast, in the single cell suspension of ISO treated rats, red fluorescence was maintained at a high level than in the MCT treated group showing a better preservation of mitochondrial membrane potential.

Further, there was marked increase in the expression of mitochondrial PAR in the RV of MCT challenged rats and this was reversed by PARP-1 inhibition.

**Figure 5.42** Effect of PARP-1 inhibition by ISO on (a) loss of mitochondrial membrane potential in right ventricles of MCT treated rats by JC-1 FACS analysis. (b) Quantitative analysis of the dot plots. (c) Representative western blots showing the effect of PARP-1 inhibition on mitochondrial PAR expression in the right ventricle. The results are expressed as mean ± S.E.M. **p<0.01 vs. control, +++p<0.001 vs. control, *p<0.01 vs. MCT and **p<0.001 vs. MCT.
5.3.12 Effect of PARP-1 inhibition on release of cell death factors from mitochondria

There is translocation of AIF from mitochondria to nucleus and cytochrome c from mitochondria to cytosol during apoptosis. In our study, there was mitochondrial to nuclear translocation of AIF and release of cytochrome c from mitochondria to cytosol in right heart of MCT treated rats. AIF translocation and cytochrome c release was reversed by PARP-1 inhibition. All these results indicate a proapoptotic scenario in the right heart of MCT treated rats (Figure 5.43).

**Figure 5.43** Effect of PARP-1 inhibition by ISO on release of cell death factors from mitochondria in right ventricle. Representative western blot results showing effect of ISO treatment on AIF and cytochrome c expression in various cellular fractions in various groups. (n=5-7 in each group). +++p<0.001 vs. control, **p<0.01 vs. MCT and ***p<0.001 vs. MCT.
5.3.13 Effect of PARP-1 inhibition on PARP-1 cleavage and caspase 3 activity

After the treatment with MCT, there was cleavage of PARP-1 and increased caspase 3 activity as compared to the control rats. Administration of PARP-1 inhibitor (ISO) in MCT treated group inhibited PARP-1 cleavage and also attenuated caspase 3 activity in RV. However, administration of 10% DMSO (vehicle of ISO) following MCT treatment had no these MCT mediated effects.

a.

![Cleaved PARP-1 and β-Actin](image)

b.

![Caspase 3 activity](image)

**Figure 5.44** Effect of PARP-1 inhibition by ISO on (a) PARP cleavage and (b) caspase 3 activity in right ventricle. Data values were expressed as Mean ± S.E.M. (n=5-8). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (++p<0.01 vs. control, +++p<0.001 vs. control, *p<0.05 vs. MCT and ***p<0.001 vs. MCT).

5.3.14 Effect of PARP-1 inhibition on genes involved in pathophysiology of cardiac hypertrophy

MCT treatment led to significant decrease in the expression of eNOS and PCNA and increase in the expression of HIF-1α in the right ventricle, however, administration of ISO attenuated these effects of MCT. The
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Figure 5.45 Representative western blots showing the effect of PARP-1 inhibition by ISO on the expression of eNOS, HIF-1α and PCNA expression in the right ventricle. The results are expressed as mean ± S.E.M. (n=5 in each group). ++p<0.01 vs. control, +++p<0.001 vs. control, *p<0.05 vs. MCT and **p<0.01 vs. MCT.

administration of 10% DMSO had no effect on MCT induced effects on eNOS, HIF-1α and PCNA expression in right ventricle (Figure 5.45).

5.3.15 Effect of PARP-1 inhibition on left ventricle

5.3.15.1 Effect of PARP-1 inhibition on left ventricular hypertrophy

There was no significant change in the left ventricle hypertrophy amongst various groups (Figure 5.46).

Figure 5.46 Effect of PARP-1 inhibition by ISO on left ventricular hypertrophy in MCT treated rats. (a) Left ventricle weight to heart weight (b) left ventricle weight to body weight. Data values were expressed as Mean ± S.E.M. (n=8) (LV= Left ventricle weight, HW= Heart weight and BW=Body weight).
5.3.15.2 Effect of PARP-1 inhibition on oxidative stress in left ventricle

There was no significant change in oxidative stress parameters in left ventricle amongst various groups (Figure 5.47).

![Graphs showing oxidative stress parameters](image)

**Figure 5.47** Effect of PARP-1 inhibition by ISO on level of (a) reactive oxygen species by fluorimetry (% Control), (b) reactive oxygen species by FACS (Relative Fluorescence Unit; RFU) (c) MDA levels (nmol/ mg protein) (d) Nitrite levels (nmol/ mg protein) and (e) GSH levels (µg/ mg protein) in left ventricle. Data values were expressed as Mean ± S.E.M. (n=8). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test.
5.3.15.3 PARP-1 activity, PARP-1 expression and PARylation in right ventricle

There was no DNA damage and as a consequence no change in PARP-1 activity and expression of PARP-1 and PAR in left ventricle amongst various groups (Figure 5.48).

Figure 5.48 Effect of PARP-1 inhibition by ISO on (a) PARP-1 activity (% Control) (b) PARP-1 mRNA expression (c) g-H2AX, PARP-1, PAR expression (immunoblotting) in left ventricle. Data values were expressed as Mean ± S.E.M. (n=3). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+++p<0.001 vs. control, **p<0.001 vs. MCT and ***p<0.001 vs. MCT).
5.3.15.4 Effect of PARP-1 inhibition on inflammation in left ventricle

There was no significant change in inflammatory parameters amongst various groups (Figure 5.49).

**Figure 5.49** Effect of PARP-1 inhibition by ISO on (a) IL-10 (pg/mg of protein) and (b) TNF-α levels (pg/mg of protein) and (c) expression of p65 subunit of NFκB in lungs in left ventricle. Data values were expressed as Mean ± S.E.M. (n=8). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test.
5.4 Modulation of PARP-1 by an herbal, *Withania somnifera*, in monocrotaline induced pulmonary hypertension in rats

5.4.1 Standardization of the commercially available *Withania somnifera* (Ashwagandha) powder.

The HPLC method provided well resolved and symmetrical peaks for Withaferin A and Withanolide A with baseline separation. No interference was observed with the other constituents of the plant material. The retention time of withaferin A and withanolide A were about 4.6 and 6.1 min respectively. Concentrations of withaferin A and withanolide A in the dried powder of *Withania somnifera* were found to be 1.91 ± 0.09 mg/g and 0.54 ± 0.03 mg/g respectively (Figure 5.50).

![Figure 5.50](image)

*Figure 5.50* Chromatograms of marker compounds and *Withania somnifera* powder. (A) Representative HPLC chromatographic resolution of standard markers Withaferin A and Withanolide A (B) Resolution of Withaferin A and Withanolide A in *Withania somnifera* powder.
5.4.2 Effect of *Withania somnifera* on survival and body weight

Following the treatment with MCT, there was a significant decrease in survival along with a decrease in the body weight of rats over the period of 35 days as reported previously. However, WS treatment improved the survival and caused the increase in the body weight of MCT treated rats at both the preventive doses of 50 mg/kg and 100 mg/kg (Figure 5.51).

![Figure 5.51](image1)

*Figure 5.51* Effect of *Withania somnifera* on (a) percent survival and (b) body weight. Data values were expressed as Mean ± S.E.M. (n=8). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (++p<0.01 vs. control, +++p<0.001 vs. control, **p<0.01 vs. MCT and ***p<0.05 vs. MCT).

5.4.3 Effect of *Withania somnifera* on right ventricular pressure

All MCT-treated rats showed a significant increase in the RVP as compared with control animals. The five week treatment with WS at the doses of 50 mg/kg and 100 mg/kg significantly decreased the RVP as compared to the MCT challenged rats (Figure 5.52).
Figure 5.52 Effect of *Withania somnifera* on right ventricular pressure (RVP) (mmHg) in MCT treated rats. Data values were expressed as Mean ± S.E.M. (n=8). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+++p<0.001 vs. control, **p<0.01 vs. MCT).

5.4.4 Effect of *Withania somnifera* on hemodynamic parameters

There was no significant change in systemic hemodynamic parameters amongst various groups.

Table 5.5 Effect of *Withania somnifera* treatment on hemodynamic parameters in curative model of pulmonary hypertension in MCT treated rats.

<table>
<thead>
<tr>
<th>Hemodynamic Parameters</th>
<th>Control</th>
<th>MCT</th>
<th>MCT + WS 50</th>
<th>MCT + WS 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (mmHg)</td>
<td>104.2±6.25</td>
<td>106.2±6.27</td>
<td>105.5±7.49</td>
<td>102.4±9.1</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>71.2±5.23</td>
<td>73.6±6.33</td>
<td>69.1±7.87</td>
<td>75.4±5.99</td>
</tr>
<tr>
<td>Mean BP (mmHg)</td>
<td>82.3±5.4</td>
<td>84.4±5.85</td>
<td>81.1±7.72</td>
<td>83.3±9.16</td>
</tr>
<tr>
<td>Heart Rate (beats/min)</td>
<td>375±8</td>
<td>357±9</td>
<td>373±16</td>
<td>362±12</td>
</tr>
</tbody>
</table>

MCT- Monocrotaline, BP- blood pressure
5.4.5 Effect of *Withania somnifera* on oxidative stress in lungs

DCF-DA treatment of tissues showed that there was a significant increase in the level of ROS in lungs of the MCT treated rats. However, administration of WS 50 mg/kg and 100 mg/kg for five weeks lowered the ROS level in lungs (Figure 5.53).

![Figure 5.53](image)

**Figure 5.53** Effect of *Withania somnifera* treatment on level of levels of reactive oxygen species (% Control) in lungs. Data values were expressed as Mean ± S.E.M. (n=5-7). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (++++p<0.001 vs. control, *p<0.05 vs. MCT, **p<0.01 vs. MCT).

5.4.6 Effect of *Withania somnifera* on DNA damage

Increased oxidative stress results in DNA damage which is marked by increased g-H2AX expression. In MCT treated rats also, there was a significant increase in the expression of g-H2AX after 35 days which was ameliorated WS treatment (Figure 5.54).
RESULTS

Figure 5.54 Effect of Withania somnifera on expression of g-H2AX in lungs. Data values were expressed as Mean ± S.E.M. (n=3). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+++p<0.001 vs. control and ***p<0.001 vs. MCT).

5.4.7 Effect of Withania somnifera on PARP-1 expression in lungs

PARP-1 is one of the most important DNA repair enzymes which get activated upon DNA damage. Following the MCT treatment, there was a significant increase in the expression of PARP-1 in lungs. The increase in expression of PARP-1 was attenuated by the administration of both the doses of WS (50 and 100 mg/kg) (Figure 5.55).

Figure 5.55 Representative western blots showing the effect of Withania somnifera (50 and 100 mg/kg) on the expression of PARP-1 in the lungs of MCT challenged rats. The results are expressed as mean ± S.E.M. (n=5 in each group). (+++p<0.01 vs. control, and ***p<0.05 vs. MCT).
5.4.8 Effect of *Withania somnifera* on pulmonary vascular remodeling

MCT induced PH is characterized by pulmonary vascular remodelling and medial hypertrophy. Similarly, in the present study, quantitative morphometry showed that MCT caused a significant increase in muscularization of distal pulmonary artery of diameter <100 µm and 100-250 µm as compared to control animals. Quantitative morphometric analysis demonstrated that WS at the doses of 50 and 100 mg/kg significantly decreased the muscularization of distal pulmonary arteries and as compared to MCT treated rats (Figure 5.56 a-c).

Pulmonary vascular remodelling associated with PH is marked by increased proliferating cell nuclear antigen (PCNA). There was an increase in the protein content of PCNA, a marker of cell proliferation, in the lungs of MCT treated group as compared to the control group. Treatment with both the doses of WS (50 and 100 mg/kg) reduced the increased expression of PCNA demonstrating antiproliferative action of WS (Figure 5.56 d).
Figure 5.56 Effect of PARP-1 inhibition on pulmonary vasculature remodeling induced by MCT administration. (a) Hematoxylin and eosin staining of pulmonary arteries. (b) Medial wall thickness of <100µm diameter arteries, (c) Medial wall thickness of 100-250 µm diameter arteries and (d) expression of PCNA in lungs. Data values were expressed as Mean ± S.E.M. (n=5-7). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (++p<0.01 vs. control, +++p<0.01 vs. control *p<0.05 vs. MCT and **p<0.01 vs. MCT).
5.4.9 Effect of *Withania somnifera* on lung weight to body weight ratio (LW/BW)

There was a significant increase in LW/BW, a marker of pulmonary remodelling, in MCT treated rats as compared to control rats. The five week treatment with WS at 50 mg/kg and 100 mg/kg significantly reduced the LW/BW as compared to MCT treated rats respectively (Figure 5.57).

![Figure 5.57](image)

**Figure 5.57** Effect of *Withania somnifera* on ratio of lung weight to body weight (LW/BW). Data values were expressed as Mean ± S.E.M. (n=8). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+++p<0.001 vs. control, **p<0.01 vs. MCT and ***p<0.001 vs. MCT) (LW=Lung weight; BW=Body weight).

5.4.10 Effect on *Withania somnifera* on apoptosis in lungs

Apart from vascular remodelling and proliferation, apoptosis resistance is the characteristic feature of PH. MCT treated rats showed apoptotic resistance as evident from TUNEL studies as compared to control rats in lungs. Treatment with WS at doses of 50 mg/kg and 100 mg/kg showed an increase in apoptotic cells as evident by numerous TUNEL positive cells in the lung sections (Figure 5.58 a).

The expression of procapase3 (Figure 5.58 b), a marker of apoptosis, was also found reduced in the lungs of MCT treated rats as compared to control rats demonstrating apoptosis resistance in PH. Treatment with WS increased the expression of procaspase3 in lung thus weakening the apoptosis resistance.
RESULTS

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b. Figure 5.58 Effect of *Withania somnifera* on apoptosis in lungs. (a) Representative micrographs of TUNEL (green) and DAPI (blue) staining of lung section. Quantitative analysis of apoptotic cells was expressed as number of TUNEL positive cells per 0.5 mm². (b) Representative western blot results showing that WS treatment increased the expression of procaspase-3 in lungs as compared to MCT challenged rats (n=5-7 in each group). The results are expressed as mean ± S.E.M. (n=5 in each group). *p<0.05 vs. control,  **p<0.01 vs. MCT and ***p<0.001 vs. MCT. (p<0.05, p<0.01 and p<0.001 one-factor analysis of variance, ANOVA).
5.4.11 Effect of *Withania somnifera* treatment on inflammatory parameters in lungs

Inflammatory conditions prevail in lungs in PH. Similarly, we found an increase in the level of pro-inflammatory cytokine TNF-α and decrease in the level of anti-inflammatory cytokine IL-10 in the lungs following MCT treatment as compared to time matched control rats. The pro-inflammatory effect of MCT was weakened by the administration of WS (50 and 100 mg/kg) with a decrease in TNF-α level and increase in the IL-10 levels in the lung tissue (Figure 5.59 a and b).
One of the most important markers for inflammation is the increased expression of p65 subunit of NFκB. In the present study, there was an increase in the protein levels of p65 subunit of NFκB after five weeks in MCT-challenged rats. This increased p65 subunit of NFκB expression was brought down by WS (50 and 100 mg/kg) treatment, thereby showing its anti-inflammatory effect (Figure 5.59 c).

5.4.12 Effect of Withania somnifera on endothelial dysfunction

Endothelial dysfunction in pulmonary arteries is a marked pathophysiological characteristic of PH. Acetylcholine-induced vasorelaxation was significantly decreased in phenylephrine-contracted extralobar pulmonary arterial rings of MCT-treated rats as compared to control rats. WS treatment at 50 mg/kg and 100 mg/kg caused a significant increase in vasorelaxation in pulmonary arteries as compared to MCT group and showed attenuation of endothelial dysfunction (Figure 5.60 a).

One of the hallmarks of endothelial dysfunction is the decreased expression of endothelial nitric oxide synthase (eNOS) which produces nitric oxide (NO), a potent vasodilator. There was a significant decrease in the expression of eNOS in lungs of MCT-treated rats. This decrease in the expression of eNOS was reversed by the preventive treatment with 50 and 100 mg/kg WS in lungs respectively (Figure 5.60 b).
RESULTS

PULMONARY HYPERTENSION

a.

b.

Figure 5.60 Effect of Withania somnifera on (a) pulmonary endothelial dysfunction in extralobar pulmonary arteries (n = 5aortic rings) and (b) eNOS expression in lungs. Data values were expressed as Mean ± S.E.M. (n=5). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test. (+p<0.05 vs. control +++p<0.001 vs. control, *p<0.05 vs. MCT, **p<0.01 vs. MCT and ***p<0.001 vs. MCT).

5.4.13 Effect of Withania somnifera on HIF-1α in lungs

One of the most important transcription factors involved in the physiological responses to PH is HIF-1. Following the MCT treatment, there was a significant increase in the expression of HIF-1α, a sub-unit of HIF-1, in lungs. The increase in expression of HIF-1α was attenuated by the administration of both the doses of WS (50 and 100 mg/kg) (Figure 5.61).
**Figure 5.61** Representative western blots showing the effect of preventive treatment with *Withania somnifera* (50 and 100 mg/kg) on the expression of HIF-1α in the lungs of rats from different experimental groups. The results are expressed as mean ± S.E.M. (n=5 in each group). **p<0.01 vs. control, and *p<0.05 vs. MCT.

**5.4.14 Effect of *Withania somnifera* on right ventricular hypertrophy in MCT treated rats**

MCT induced PH is associated with right ventricular cardiac hypertrophy. We found a significant increase in RV/LV+S, RV/HW and RV/BW ratios in MCT treated group as compared to control rats. The five weeks preventive treatment with 50 and 100 mg/kg of WS in MCT treated rats showed a significant decrease in RVH (RV/LV+S, RV/HW and RV/BW ratios) as compared to MCT treated rats (Figure 5.62 a and b).
RESULTS

Figure 5.62 Effect of *Withania somnifera* on (a) Hematoxylin and eosin staining of right ventricle (b) RV/LV+S, (c) RV/HW and (d) RV/BW. Data values were expressed as Mean ± S.E.M. (n=8). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+++p<0.001 vs. control *p<0.05 vs. MCT, **p<0.01 vs. MCT and ***p<0.001 vs. MCT) (RV= Right Ventricle, LV+S= Left Ventricle + Septum, HW= Heart Weight and BW= Body Weight).

5.4.15 Effect of *Withania somnifera* on oxidative stress in right ventricle

DCF-DA treatment of tissues showed that there was a significant increase in the level of ROS in RV of the MCT treated rats. However, administration of WS 50 mg/kg and 100 mg/kg for five weeks lowered the ROS level in RV (Figure 5.63).
RESULTS

Figure 5.63 Effect of *Withania somnifera* treatment on level of levels of reactive oxygen species (% Control) in right ventricle. Data values were expressed as Mean ± S.E.M. (n=5-7). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test +++p<0.001 vs. control, **p<0.01 vs. MCT, ***p<0.001 vs. MCT).

5.4.16 Effect of *Withania somnifera* on DNA damage in RV

Increased oxidative stress results in DNA damage which is marked by increased g-H2AX expression. In MCT treated rats also, there was a significant increase in the expression of g-H2AX after 35 days in RV which was ameliorated WS treatment (Figure 5.64).

Figure 5.64 Effect of *Withania somnifera* on expression of g-H2AX in RV. Data values were expressed as Mean ± S.E.M. (n=3). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+++p<0.001 vs. control and ***p<0.001 vs. MCT).
5.4.17 Effect of *Withania somnifera* on PARP-1 expression in right ventricle PH

PARP-1 is one of the most important DNA repair enzymes which get activated upon DNA damage. Following the MCT treatment, there was a significant increase in the expression of PARP-1 in RV. The increase in expression of PARP-1 was attenuated by the administration of both the doses of WS (50 and 100 mg/kg) (Figure 5.65).

![Representative western blots showing the effect of preventive treatment with *Withania somnifera* (50 and 100 mg/kg) on the expression of PARP-1 in the right ventricle of rats from different experimental groups. The results are expressed as mean ± S.E.M. (n=5 in each group). +++ p<0.001 vs. control, ** p<0.01 vs. MCT and *** p<0.001 vs. MCT.](image)

5.4.18 Effect of *Withania somnifera* treatment on inflammatory parameters in right ventricle

There was significant increase in the level of pro-inflammatory cytokine TNF-α and decrease in the level of anti-inflammatory cytokine IL-10 in the right RV following MCT treatment as compared to time matched control rats. The pro-inflammatory effect of MCT was weakened by the administration of WS (50 and 100 mg/kg) with a decrease in TNF-α level and increase in the IL-10 levels in the right ventricle tissue (Figure 5.66 a and b).

In the present study, there was increase in the protein levels of NFκB (p65 subunit) after five week in MCT challenged rats. This increased the
expression of p65 subunit of NFκB was brought down by WS (50 and 100 mg/kg) treatment thereby showing its anti-inflammatory effect (Figure 5.66 c).

Figure 5.66 Effect of Withania somnifera on (a) TNF-α (pg/mg of protein) (b) IL-10 levels (pg/mg of protein) (c) expression of p65 subunit of NFκB in right ventricle. Data values were expressed as Mean ± S.E.M. (n=5-7). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (++p<0.01 vs. control, **p<0.01 vs. MCT and ***p<0.001 vs. MCT).

5.4.19 Effect of Withania somnifera on eNOS expression in right ventricle

Decreased eNOS expression is an important event in cardiac hypertrophy. Following the MCT treatment, there was a significant decrease in the expression of eNOS in RV. The decrease in expression of eNOS was prevented by the administration of both the doses of WS (50 and 100 mg/kg) (Figure 5.67).
Figure 5.67 Effect of *Withania somnifera* on eNOS expression in right ventricle. Data values were expressed as Mean ± S.E.M. (n=5). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman–Keuls multiple comparison test. (++p<0.01 vs. control, *p<0.05 vs. MCT and **p<0.01 vs. MCT).

### 5.4.20 Effect of *Withania somnifera* on HIF-1α expression in right ventricle

One of the most important transcription factors involved in the physiological responses to PH is HIF-1. Following the MCT treatment, there was a significant increase in the expression of HIF-1α, a sub-unit of HIF-1, in RV. The increase in expression of HIF-1α was attenuated by the administration of both the doses of WS (50 and 100 mg/kg) (Figure 5.68).

Figure 5.68 Representative western blots showing the effect of preventive treatment with *Withania somnifera* (50 and 100 mg/kg) on the expression of HIF-1α in the right ventricle of rats from different experimental groups. The results are expressed as mean ± S.E.M. (n=5 in each group). +++p<0.001 vs. control, **p<0.01 vs. MCT and ***p<0.001 vs. MCT.
5.4.21 Effect of Withania somnifera on apoptosis in right ventricle

Apoptosis is involved in the pathogenesis of cardiac remodeling and right ventricular remodelling. MCT treated rats showed increased apoptosis as evident from numerous TUNEL positive cells as compared to control rats (21.00±2.91 cells per 0.5 mm$^2$) in RV. Treatment with WS at doses of 50 mg/kg and 100 mg/kg showed a decrease in apoptotic cells as evident by lesser number of TUNEL positive cells in the RV sections (Figure 5.69 a).

The expression of procapase3 (Figure 5.69 b), a marker of apoptosis, was also found increased in the RV of MCT treated rats as compared to control rats demonstrating increased apoptosis in PH. Treatment with WS decreased the expression of procaspase-3 in RV thus weakening the apoptosis.
RESULTS

a.

Figure 5.69 Effect of *Withania somnifera* on apoptosis in right ventricle. (a) Representative micrographs of TUNEL (green) and DAPI (blue) staining of lung section. Scale bars =50 μm. Quantitative analysis of apoptotic cells was as number of TUNEL positive per 0.5 mm². (b) Representative western blot results showing that WS treatment on the expression of procaspase-3 in right ventricle as compared to MCT challenged rats (n=5-7 in each group). The results are expressed as mean ± S.E.M. (n=5 in each group). +++p<0.001 vs. control, **p<0.01 vs. MCT and ***p<0.001 vs. MCT. (p<0.05, p<0.01 and p<0.001 one-factor analysis of variance, ANOVA).
5.4.22 Effect of treatment of *Withania somnifera* on right ventricular pressure in curative model of PH

All MCT-treated rats showed a significant increase in the RVP as compared with control animals. The two weeks treatment with WS, starting from day 21 to 35, at the doses of 100 mg/kg significantly decreased the RVP as compared to the MCT challenged rats (Figure 5.70). The dose of WS 50 mg/kg was not found to be effective.

![Graph showing effects of treatment on RVP](image)

**Figure 5.70** Effect of *Withania somnifera* on right ventricular pressure (RVP) (mmHg) in MCT treated rats. Data values were expressed as Mean ± S.E.M. (n=8). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (+++p<0.001 vs. control, *p<0.05 vs. MCT).

5.4.23 Effect of *Withania somnifera* on right ventricular hypertrophy in MCT treated rats in curative model of PH

MCT induced PH is associated with right ventricular cardiac hypertrophy. We found a significant increase in RV/LV+S ratio in MCT treated group as compared to control rats. The two weeks curative treatment was done with 50 and 100 mg/kg of WS in MCT treated rats. There was a significant decrease in right ventricular hypertrophy (RV/LV+S) in MCT challenged rats treated with WS 100 mg/kg as compared to MCT treated rats (Figure 5.69 a). The other markers for cardiac hypertrophy such as
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**Figure 5.71** Effect of *Withania somnifera* on (a) RV/LV+S, (b) RV/HW and (c) RV/BW. Data values were expressed as Mean ± S.E.M. (n=8). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (++p<0.01 vs. control, +++p<0.01 vs. control *p<0.05 vs. MCT, **p<0.05 vs. MCT) (RV= Right Ventricle, LV+S= Left Ventricle + Septum, HW= Heart Weight and BW= Body Weight).

The right ventricle to heart weight (RV/HW) and right ventricle to body weight (RV/BW) ratio were also significantly decreased by the treatment with WS 100 mg/kg as compared to MCT treated rats (Figure 5.71 a-c). The dose of WS 50 mg/kg was not found to be effective.

**5.4.24 Effect of Withania somnifera on lung weight to body weight ratio (LW/BW)**

There was a significant increase in LW/BW, a marker of pulmonary remodelling, in MCT treated rats as compared to control rats. Treatment with
WS at 100 mg/kg from day 21 to 35 significantly reduced the LW/BW as compared to MCT treated rats respectively (Figure 5.72).

**Figure 5.72** Effect of *Withania somnifera* on ratio of lung weight to body weight (LW/BW). Data values were expressed as Mean ± S.E.M. (n=8). Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (++p<0.01 vs. control, *p<0.05 vs. MCT) (LW= Lung weight; BW=Body weight).

### 5.4.25 Effect of *Withania somnifera* on hemodynamic parameters

There was no significant change in systemic hemodynamic parameters amongst various groups.

**Table 5.6** Effect of *Withania somnifera* treatment on hemodynamic parameters in MCT treated rats.

<table>
<thead>
<tr>
<th>Hemodynamic Parameters</th>
<th>Control</th>
<th>MCT (60 mg/kg)</th>
<th>MCT + WS (100 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (mmHg)</td>
<td>105.2 ± 4.25</td>
<td>102.4 ± 5.72</td>
<td>101.5 ± 6.4</td>
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<tr>
<td>Diastolic BP (mmHg)</td>
<td>69.2 ± 7.23</td>
<td>73.6±6.33</td>
<td>75.4± 6.61</td>
</tr>
<tr>
<td>Mean BP (mmHg)</td>
<td>82.3±5.4</td>
<td>84.4±5.85</td>
<td>83.3± 9.16</td>
</tr>
<tr>
<td>Heart Rate (beats/min)</td>
<td>365 ± 6</td>
<td>375 ± 14</td>
<td>359 ± 13</td>
</tr>
</tbody>
</table>

MCT- Monocrotaline, BP- Blood Pressure, WS- *Withania Somnifera*