CHAPTER 7

CHEMOPROTECTIVE EFFECT OF

MESO-ZEAXANTHIN AGAINST CISPLATIN

AND DOXORUBICIN INDUCED TOXICITY
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7.4. DISCUSSION
7.1. INTRODUCTION

Although conventional method of treating cancer using chemotherapy has yielded significant clinical benefits, its full therapeutic effectiveness is masked by severe side effects. Cisplatin [Cis-diammine dichloro platinum (II)] is one of the most potent antitumour agents known, displaying clinical activity against a wide variety of solid tumours including testicular, bladder, lungs, oesophagus, stomach and ovarian cancers as well as sarcomas and lymphomas. The cytotoxic mode of action of cisplatin is mediated by its interaction with DNA to form intra strand crosslink adducts, which activate several signal transduction pathways, like those involving ATR, p53, p73 and MAPK and culminate in the activation of apoptotic pathway (Prestayko et al., 1979). Despite its success, cisplatin produces severe side effects such as nephrotoxicity, neurotoxicity, ototoxicity, myelosuppression, nausea and vomiting. Nephrotoxicity, the chief dose-limiting side effect of cisplatin, occurs mainly due to oxidative stress injury (Zahid, 2003).

Doxorubicin (DOX) is a potent broad-spectrum antitumor anthracycline antibiotic commonly used in the treatment of wide range of cancers including haematological malignancies, many types of carcinomas and soft tissue sarcomas. This compound inhibits topoisomerase II by intercalating DNA with high affinity and stabilizes the DNA double strand breaks. Additionally, the quinone structure of anthracycline enhances the catalysis of oxidation–reduction reactions, thereby promoting the generation of oxygen free radicals. These free radicals produced by electron transfer from the semiquinone to quinone moieties of the anthracycline are responsible for myocardial damage and subsequent doxorubicin induced cardiotoxicity (Sinha and Politi, 1990). A number of agents like dexrazoxane, mercapto ethane sulfonate (MESNA) etc has been tried as chemoprotective agents but the toxicity produced after repeated administration limit their clinical significance.

meso-Zeaxanthin [(3R, 3’S)-β, β-carotene-3, 3’-dial, MZ] is a compound prepared from lutein present in marigold flower. Toxicity studies on this carotenoid showed that it is non toxic (Chang, 2007). In vitro and in vivo antioxidant studies on MZ showed that it has significant antioxidant effects (Firdous et al., 2010). Since oxidative stress is the major etiopathological factor responsible for both cisplatin and
doxorubicin-induced toxicities, the aim of this study is to evaluate chemo protective potential of antioxidant MZ.

7.2. MATERIALS AND METHODS

7.2.1. Chemicals

Nitro blue tetrazolium (NBT), glutathione (GSH) and 5-5’ dithiobis (2-nitro benzoic acid) (DTNB) were purchased from Sisco Research Laboratories, Mumbai. Thiobarbituric acid was obtained from Hi Media Laboratories, Mumbai. Cisplatin was obtained from Dabur Pharma, Himachal Pradesh. Doxorubicin was bought from Naprod Life Sciences Pvt. Ltd, Mumbai. The kits for estimating urea, creatinine SGOT, SGPT, LDH and CPK were supplied by Span Diagnostics Ltd, Surat, India.

7.2.2. Determination of Nephroprotective Potential of MZ against Cisplatin Induced Toxicity

Thirty six male Swiss albino mice were divided into 6 groups of 6 animals each.

Group I: Normal

Group II: Cisplatin alone (Cisplatin control)

Group III: Cisplatin + sunflower oil (Vehicle control)

Group IV: Cisplatin + MZ (50 mg/kg b.wt)

Group V: Cisplatin + MZ (100 mg/kg b.wt)

Group VI: Cisplatin + MZ (250 mg/kg b.wt)

Animals in groups II-VI received a single dose (i.p) of cisplatin (16mg/kg b.wt). Oral administration of MZ was started five days prior to cisplatin injection and was continued till the animals were sacrificed by diethyl ether-anaesthesia after three days. Blood and kidneys were collected from all the animals for various biochemical analyses.
7.2.2.1. Effect of MZ on Haematological Profile of Mice during Cisplatin Induced Nephrotoxicity

Blood was collected by heart puncture and was stored in heparinised vials to analyse various haematological parameters. Haemoglobin was estimated as cyanmethaemoglobin formation using Drabkin’s method. Total white blood cells (WBC) were measured after diluting the blood in Turk’s fluid and counting them using a haemocytometer (Chapter 2, section 2.2.7.1 and 2.2.7.2).

7.2.2.2. Effect of MZ on Bone Marrow Cellularity of Mice during Cisplatin Induced Nephrotoxicity

Femurs of all animals were taken and bone marrow cells from both femurs were flushed in to phosphate buffered saline containing 10% goat serum to determine bone marrow cellularity (Chapter 2, section 2.2.8).

7.2.2.3. Effect of MZ on α-Esterase Activity of Mice during Cisplatin Induced Nephrotoxicity

Bone marrow cells from the above preparation were immediately smeared on a clear glass slide, air dried and stained with α-naphthyl acetate then counter stained with hematoxyline. α-esterase positive cells were counted and expressed as number of positive cells/4000 bone marrow cells (Chapter 2, section 2.2.9).

7.2.2.4. Effect of MZ on Renal Function of Mice during Cisplatin Induced Nephrotoxicity

Serum separated from the blood was used to analyze various kidney function parameters. Serum creatinine level was determined by alkaline picric acid method using a diagnostic kit (Toro and Ackermann, 1975). Serum urea was determined by diacetylmonoxime (DAM) reagent (modified Berthelot methodology) using a diagnostic kit (Murray, 1984).
7.2.2.5. Effect of MZ on Oxidative Stress Markers in the Kidney of Mice during Cisplatin Induced Nephrotoxicity

Kidneys were quickly removed and washed with ice-cold normal saline and homogenates (10% w/v) were prepared in 0.1M Tris HCl. Lipid peroxidation (LPO) in the homogenate was estimated by thiobarbituric acid method. Formation of conjugated dienes (CD) and tissue hydroperoxides (HP) in the homogenate were determined by the modified method of John and Steven (Chapter 2, section 2.2.11.8 to 2.2.11.10).

7.2.2.6. Effect of MZ on Antioxidant Enzyme Profile in the Kidney of Mice during Cisplatin Induced Nephrotoxicity

Kidney homogenate (10%) was centrifuged at 1000 rpm for thirty minutes at 4°C and was used for the analyses of antioxidant enzymes. Superoxide dismutase (SOD) activity in the homogenate was measured by NBT reduction method (McCord and Fridovich, 1969). Catalase (CAT) activity was estimated by the method of Aebi. Assay of glutathione peroxidase (GPx) was done by the method of Hafeman et al, and the level of glutathione (GSH) in the tissue homogenate was analysed by the method of Moron et al (Chapter 2, section 2.2.11.2 to 2.2.11.5). Estimation of the total protein was carried out by the method of Lowry et al (Chapter 2, section 2.2.11.11).

7.2.2.7. Histopathological Analysis of Kidney

A small portion of kidney was taken for histopathological analysis. Immediately after removal, kidney was washed in PBS, fixed in 10% formaldehyde solution and then embedded in wax. Sections (4µm) were taken and stained with haematoxylin-eosin.

7.2.3. Determination of Cardioprotective Potential of MZ against Doxorubicin Induced Toxicity

Thirty six male Wistar rats were randomly divided in to six groups containing six animals each.

Group I: Normal

Group II: Doxorubicin alone (DOX control)
Group III: Doxorubicin + sunflower oil (Vehicle control),

Group IV: Doxorubicin + MZ (50 mg/kg b.wt)

Group V: Doxorubicin + MZ (100 mg/kg b.wt)

Group VI: Doxorubicin + MZ (250 mg/kg b.wt)

Oral administration of MZ was started 15 days prior to doxorubicin injection. On 16th day one hour after MZ administration, doxorubicin (30 mg/kg b.wt) was injected intra peritoneally to groups II-VI. After 24 hours of doxorubicin injection, rats in all the groups were anesthetized with thiopentone (35 mg/kg; i.p.) and subjected to ECG recording. Finally, all the animals were sacrificed and blood and heart tissues were collected for various biochemical analysis. Dosage and time of sacrifice were decided as per recommended procedure (Mukherjee et al., 2003).

7.2.3.1. Effect of MZ on Electrocardiography (ECG) Alterations of Rats during Doxorubicin Induced Cardiotoxicity

24 hours after DOX injection ECGs of all the animals were recorded using RMS-Digitized polygraph (Poly-Rite D, India). Needle electrodes were inserted under the skin of anesthetized rats for the limb lead at position II. For each ECG tracing ST deviation, PR interval, RR interval, ST interval, QT interval and heart beat rate were measured.

7.2.3.2. Effect of MZ on Serum Biochemical Parameters of Rats during Doxorubicin Induced Cardiotoxicity

Blood was collected by heart puncture and was stored in non-heparinised vials to separate serum. Activities of various cardiac injury marker enzymes in the serum such as lactate dehydrogenase (LDH), creatine phosphokinase (CPK), serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) (Reitman and Frankel, 1957) were determined using diagnostic kits.
7.2.3.3. Effect of MZ on Oxidative Stress Markers in the Cardiac Tissue of Rats during Doxorubicin Induced Cardiotoxicity

A 10 % heart tissue homogenate was prepared in 0.1M Tris HCl. Lipid peroxidation (LPO) in the homogenate was estimated by thiobarbituric acid method. Tissue hydroperoxides (HP) and conjugated dienes (CD) were determined by the modified method of John and Steven (Chapter 2, section 2.2.11.8 to 2.2.11.10).

7.2.3.4. Effect of MZ on Antioxidant Enzymes and GSH Level in the Cardiac Tissue of Rats during Doxorubicin Induced Cardiotoxicity

Superoxide dismutase (SOD) activity, catalase (CAT) activity, glutathione peroxidase (GPx) activity and the level of glutathione (GSH) in the cardiac tissue homogenate were analysed (Chapter 2, section 2.2.11.2 to 2.2.11.5).

7.2.3.5. Histopathological Analysis of Cardiac Tissue

A portion of heart was washed in phosphate buffered saline, fixed in 10% formaldehyde solution and then embedded in wax. Sections (4µm) were taken and stained with haematoxylin-eosin.

7.3. RESULTS

7.3.1. Effect of MZ on Cisplatin Induced Nephrotoxicity in Mice

7.3.1.1. Effect of MZ on Haematological Profile of Mice during Cisplatin Induced Nephrotoxicity

Total WBC count and haemoglobin content were considerably decreased after cisplatin challenge in both vehicle and cisplatin control groups. Treatment with MZ at different concentrations (50, 100 and 250 mg/kg b.wt) significantly (P< 0.001) increased these levels in the drug-treated groups in a dose-dependent manner (Table 7.1).

Bone marrow cellularity was markedly decreased in both cisplatin control (7.6±2.1x10⁶) and vehicle control (10.2±2x10⁶) groups when compared to normal group animals (15.2 ±0.57x10⁶). MZ treatment at different concentrations significantly (P<0.001) increased the bone marrow cellularity in a concentration
Table 7.1. Effect of MZ on Haematological Parameters of Cisplatin Treated Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total WBC count (cells/ mm³)</th>
<th>Haemoglobin content (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>8430±368</td>
<td>14.24±1</td>
</tr>
<tr>
<td>Cisplatin control</td>
<td>2520±559.7 ***</td>
<td>10.6±1.4 ***</td>
</tr>
<tr>
<td>Cisplatin + Sunflower oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Vehicle control)</td>
<td>2600±406.2 ns</td>
<td>10.5±1.8 ns</td>
</tr>
<tr>
<td>Cisplatin + MZ (50 mg/kg b.wt)</td>
<td>9920±638 ***</td>
<td>16.99±0.88 ***</td>
</tr>
<tr>
<td>Cisplatin + MZ (100 mg/kg b.wt)</td>
<td>11120±421 ***</td>
<td>17.72±0.48 ***</td>
</tr>
<tr>
<td>Cisplatin + MZ (250 mg/kg b.wt)</td>
<td>16080±550.7 ***</td>
<td>18.56±0.93 ***</td>
</tr>
</tbody>
</table>

The values were expressed as mean ± standard deviation (SD); n=6. The mean values were statistically analyzed by one way analysis of variance (ANOVA) followed by appropriate post hoc test (Dunnett’s multiple comparison test) using Graph pad Instat 3 Software.

ns – Not significant (p>0.05), *** p<0.001 significant. Significant level of cisplatin alone treated control group was compared with that of normal group, whereas significant levels of MZ-treated and vehicle control groups were determined by comparing with those of cisplatin alone treated control group.
dependent manner. In 50 mg/kg b.wt MZ treated group, bone marrow cellularity was 18.6±1.2x10^6 while that of 250 mg/kg b.wt MZ treated group was 26.8±1.7x10^6. Numbers of α-esterase positive cells were also significantly reduced after cisplatin treatment. But MZ treatment significantly (P<0.001) increased the numbers of α-esterase positive cells in a dose dependent manner (Table 7.2).

7.3.1.2. Effect of MZ on Serum Marker Enzymes of Kidney Damage in Mice during Cisplatin Induced Nephrotoxicity

Renal function markers like urea and creatinine were found to be markedly elevated in both cisplatin control (i.e. 154.36±2.8 and 2.7±0.04 respectively) and in vehicle control (150.4±0.57 and 2.58±0.9 respectively) animals when compared to that of normal animals (37.2±2.09 and 0.75±0.04 respectively), indicating renal damage. These increased levels were decreased significantly by MZ treatment. In the 250 mg/kg b.wt drug pre-treated group urea level was 50.3±3.04 and creatinine level was 0.9±0.04 (Figure 7.1).

7.3.1.3. Effect of MZ on Antioxidant Enzymes and GSH levels of Mice during Cisplatin Induced Nephrotoxicity

The levels of SOD, CAT, GPx and GSH were decreased in the control group of animals. MZ administration at different concentrations (50, 100 and 250mg/kg b.wt) significantly enhanced these antioxidant systems in the kidney in a dose dependent manner (Table 7.3).

7.3.1.4. Effect of MZ on Oxidative Stress Markers in the Kidney of Mice during Cisplatin Induced Nephrotoxicity

The markers of tissue damage like lipid peroxidation (LPO), tissue hydroperoxides (HP) and conjugated dienes (CD) were high in cisplatin treated control group when compared to normal group. Treatment with MZ at different doses significantly reduced these elevated levels to normal levels (Table 7.4).

7.3.1.5. Histopathology of Kidney

Histopathology of kidney of the control group animals showed many atrophied glomeruli, Bowman’s capsule appear widened, renal tubules showed atrophy in many...
<table>
<thead>
<tr>
<th>Groups</th>
<th>Bone marrow cellularity (cells/femur/ml)</th>
<th>α-esterase positive cells (Number of positive cells/4000 bone marrow cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>15.2 ±0.57x10^6</td>
<td>812±51.6</td>
</tr>
<tr>
<td>Cisplatin control</td>
<td>7.6±2.1x10^6***</td>
<td>636.25±40.3***</td>
</tr>
<tr>
<td>Cisplatin + Sunflower oil (Vehicle control)</td>
<td>10.2±2x10^6 ns</td>
<td>602.5±32.3 ns</td>
</tr>
<tr>
<td>Cisplatin + MZ (50 mg/kg b.wt)</td>
<td>18.6±1.2x10^6***</td>
<td>790.6±30.1***</td>
</tr>
<tr>
<td>Cisplatin + MZ (100 mg/kg b.wt)</td>
<td>21.3±1.4x10^6***</td>
<td>870.2±34.2***</td>
</tr>
<tr>
<td>Cisplatin + MZ (250 mg/kg b.wt)</td>
<td>26.8±1.7x10^6***</td>
<td>950.2±44.8***</td>
</tr>
</tbody>
</table>

The values were expressed as mean ± standard deviation (SD); n=6. The mean values were statistically analyzed by one way analysis of variance (ANOVA) followed by appropriate post hoc test (Dunnett’s multiple comparison test) using Graph pad Instat 3 Software.

ns – Not significant (p>0.05), *** p<0.001 significant. Significant level of cisplatin alone treated control group was compared with that of normal group, whereas significant levels of MZ-treated and vehicle control groups were determined by comparing with those of cisplatin alone treated control group.
Figure 7.1. Effect of MZ on serum marker enzymes during cisplatin induced nephrotoxicity

(a) Serum urea

Values are expressed as mean ± SD; n=6. Values were statistically analysed using one-way ANOVA followed by Dunnett multiple comparison test. *** P< 0.001, ns P> 0.05.
### Table 7.3. Effect of MZ on Antioxidant Enzymes and GSH in the Cisplatin-Treated Kidney

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (U/mg protein)</th>
<th>CAT (U/mg protein)</th>
<th>GPx (n mol/mg protein)</th>
<th>GSH (n mol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1.5 ± 0.15</td>
<td>10.5 ± 1.2</td>
<td>26.1 ± 3.3</td>
<td>11.7 ± 1.2</td>
</tr>
<tr>
<td>Cisplatin control</td>
<td>1.4 ± 0.1*</td>
<td>6.6 ± 1.7**</td>
<td>8.9 ± 0.7***</td>
<td>8.3 ± 0.3*</td>
</tr>
<tr>
<td>Cisplatin + Sunflower oil (Vehicle control)</td>
<td>1.43 ± 0.04 ns</td>
<td>8.2 ± 1.3 ns</td>
<td>9.7 ± 1.1 ns</td>
<td>9.2 ± 0.2 ns</td>
</tr>
<tr>
<td>Cisplatin+ MZ (50mg/kg b.wt)</td>
<td>2.2 ± 0.04***</td>
<td>13.3 ± 0.7***</td>
<td>11.6 ± 1.1 ns</td>
<td>15.0 ± 0.8***</td>
</tr>
<tr>
<td>Cisplatin+ MZ (100mg/kg b.wt)</td>
<td>2.4 ± 0.04***</td>
<td>14.2 ± 0.5***</td>
<td>14.3 ± 1.2**</td>
<td>15.9 ± 0.9***</td>
</tr>
<tr>
<td>Cisplatin+ MZ (250mg/kg b.wt)</td>
<td>2.64 ± 0.06***</td>
<td>15.6 ± 1.4***</td>
<td>15.1 ± 2.1***</td>
<td>17.1 ± 0.72***</td>
</tr>
</tbody>
</table>

The values were expressed as mean ± standard deviation (SD); n=6. The mean values were statistically analyzed by one way analysis of variance (ANOVA) followed by appropriate post hoc test (Dunnett’s multiple comparison test) using Graph pad Instat 3 Software.

ns - Not significant (p>0.05). * p< 0.05, ** p <0.01 and *** p<0.001 significant. Significant level of cisplatin alone treated control group was compared with that of normal group, whereas significant levels of MZ-treated and vehicle control groups were determined by comparing with those of cisplatin alone treated control group.
Table 7.4. Effect of MZ on Oxidative Stress Markers in Cisplatin Treated Kidney

<table>
<thead>
<tr>
<th>Groups</th>
<th>LPO (n mol/mg protein)</th>
<th>Conjugated dienes (mM/100g tissue)</th>
<th>Tissue hydroperoxides (mM/100g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1.1 ± 0.36</td>
<td>2.1±0.1</td>
<td>4.2±0.2</td>
</tr>
<tr>
<td>Cisplatin control</td>
<td>3.1±0.10***</td>
<td>4.7±0.3***</td>
<td>6.2±0.3***</td>
</tr>
<tr>
<td>Cisplatin + Sunflower oil (Vehicle control)</td>
<td>2.9 ± 0.30ns</td>
<td>4.67±0.3 ns</td>
<td>6.1±0.2 ns</td>
</tr>
<tr>
<td>Cisplatin + MZ (50mg/kg b.wt)</td>
<td>1.3±0.07***</td>
<td>4.1±0.2**</td>
<td>5.7±0.1***</td>
</tr>
<tr>
<td>Cisplatin + MZ (100mg/kg b.wt)</td>
<td>1.2±0.06***</td>
<td>3.12±0.4**</td>
<td>4.7±0.21***</td>
</tr>
<tr>
<td>Cisplatin + MZ (250mg/kg b.wt)</td>
<td>1.2±0.05***</td>
<td>2.4±0.2***</td>
<td>4.3±0.2***</td>
</tr>
</tbody>
</table>

The values were expressed as mean ± standard deviation (SD); n=6. The mean values were statistically analyzed by one way analysis of variance (ANOVA) followed by appropriate post hoc test (Dunnett’s multiple comparison test) using Graph pad Instat 3 Software.

ns - Not significant (p>0.05). ** p <0.01 and *** p<0.001 significant. Significant level of cisplatin alone treated control group was compared with that of normal group, whereas significant levels of MZ-treated and vehicle control groups were determined by comparing with those of cisplatin alone treated control group.
areas and lumen of many of them contained hyalinised material, interstitial tissue also showed scattered lymphocytes and plasma cells. Such changes were found to be minimal in the MZ treated animals, indicating its protective role (Figure 7.2).

7.3.2. Effect of MZ on Doxorubicin Induced Cardio Toxicity in Rats

7.3.2.1. Effect of MZ on ECG Alterations of Rats during Doxorubicin Induced Cardiotoxicity

ECG tracings showed normal cardiac activity in all rats in the untreated normal group with a mean heart beat rate of 401±14 beats/min. Rats in the DOX alone treated group showed several ECG changes (Figure 7.3) including bradycardia (216±11 beats/min), increased ST deviation and prolonged PR, RR, ST and QT interval. Pre-treatment with MZ significantly improved such ECG abnormalities as evidenced by increase in heart beat rate and decrease in DOX-induced increase in ST, PR, RR and QT intervals (Table 7.5).

7.3.2.2. Effect of MZ on Serum Marker Enzymes of Cardiac Injury of Rats during Doxorubicin Induced Cardiotoxicity

Various marker enzymes of cardiac injury in the serum like LDH, CPK, SGOT and SGPT levels were found to be drastically elevated in both DOX alone treated group and vehicle control group animals when compared to those of normal animals, indicating cardio-toxicity produced by DOX. These increased levels were decreased significantly by MZ pre-treatment in a dose dependent manner (Table 7.6).

7.3.2.3. Effect of MZ on Antioxidant Enzymes and GSH Levels in the Cardiac Tissue of Rats during Doxorubicin Induced Cardiotoxicity

The levels of SOD, CAT, GPx and GSH in the heart tissue were significantly decreased in the control group animals when compared to the normal animals and the levels were enhanced by MZ administration in a dose dependent manner (Table 7.7).
Figure 7.2. Histopathological analysis of cisplatin treated kidney

(a) Normal histology of kidney. (b) Cisplatin alone control,

A – indicates widened Bowman’s capsule and atrophied glomerulus,  B – indicates hyalinization of
renal tubules,  → indicates infiltration of plasma cells in the interstitial tissue.

(c) Cisplatin+100mg/kg.b.wt meso-zeaxanthin treated.

(d) Cisplatin+250mg/kg.b.wt meso-zeaxanthin treated.

(Haematoxylin – Eosin staining, x 400).
7.3. Effect of meso-zeaxanthin on ECG alterations of rats during doxorubicin induced cardiotoxicity

(a) ECG tracing of normal group animals showed normal heart rate, ST deviation, PR, RR, ST and QT interval. (b) Rats in the DOX alone treated group showed several ECG changes including bradycardia, increased ST deviation and prolonged PR, RR, ST and QT interval. (c) Rats in DOX+ 100mg/kg b.wt MZ pre-treated group showed decreased ECG abnormalities. (d) Rats in DOX+ 250mg/kg b.wt MZ pre-treated group showed normal ECG tracings.
### 7.5. Effect of MZ on Doxorubicin-Induced ECG Changes

<table>
<thead>
<tr>
<th>Groups</th>
<th>Heart beat rate (beats/min)</th>
<th>RR interval (m sec)</th>
<th>PR interval (m sec)</th>
<th>ST deviation (m volt)</th>
<th>ST interval (m sec)</th>
<th>QT interval (m sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>401±14</td>
<td>153±6</td>
<td>83±3</td>
<td>0.25±0.1</td>
<td>102±5</td>
<td>383±33</td>
</tr>
<tr>
<td>DOX control</td>
<td>216±11***</td>
<td>278±11***</td>
<td>135±9***</td>
<td>0.60±0.24**</td>
<td>368±9***</td>
<td>998±60***</td>
</tr>
<tr>
<td>DOX+ Sunflower oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Vehicle control)</td>
<td>218±10.5**ns</td>
<td>278±11**ns</td>
<td>132±9**ns</td>
<td>0.61±0.23**ns</td>
<td>360±8.8**ns</td>
<td>990±56**ns</td>
</tr>
<tr>
<td>DOX+ MZ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(100mg/kg b.wt)</td>
<td>300±11***</td>
<td>200±9.8***</td>
<td>121±8**</td>
<td>0.4±0.2**</td>
<td>250±7.2***</td>
<td>566±44***</td>
</tr>
<tr>
<td>DOX+ MZ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(250mg/kg b.wt)</td>
<td>345±12***</td>
<td>180±8***</td>
<td>105±6***</td>
<td>0.27±0.18***</td>
<td>120±6***</td>
<td>396±40***</td>
</tr>
</tbody>
</table>

The values were expressed as mean ± standard deviation (SD); n=6. The mean values were statistically analyzed by one way analysis of variance (ANOVA) followed by appropriate post hoc test (Dunnett’s multiple comparison test) using Graph pad Instat 3 Software. **ns** - Not significant (p>0.05). **p <0.01 and ***p<0.001 significant. Significant level of Dox alone treated control group was compared with that of normal group, whereas significant levels of MZ-treated and vehicle control groups were determined by comparing with those of Dox alone treated control group.
Table 7.6. Effect of MZ on Serum Marker Enzymes during Doxorubicin Induced Cardiotoxicity

<table>
<thead>
<tr>
<th>Groups</th>
<th>LDH (IU/l)</th>
<th>CPK (IU/l)</th>
<th>SGOT (IU/l)</th>
<th>SGPT (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>127.2 ±3.2</td>
<td>99±0.6</td>
<td>39±1.1</td>
<td>33±2</td>
</tr>
<tr>
<td>Doxorubicin alone</td>
<td>504.8±2.6***</td>
<td>396±3.2***</td>
<td>375±1.2***</td>
<td>99±2.6***</td>
</tr>
<tr>
<td>Doxorubicin+ Sunflower oil</td>
<td>496±1.3ns</td>
<td>392±3.7ns</td>
<td>372±4ns</td>
<td>97±2.7ns</td>
</tr>
<tr>
<td>(Vehicle control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxorubicin+ MZ (50 mg/kg b.wt)</td>
<td>400.3±6.3***</td>
<td>252±1.3***</td>
<td>220±1.2***</td>
<td>76±3.3***</td>
</tr>
<tr>
<td>Doxorubicin+ MZ (100 mg/kg b.wt)</td>
<td>330.4±19***</td>
<td>184.8±2.6***</td>
<td>168±2.3***</td>
<td>52±2.3***</td>
</tr>
<tr>
<td>Doxorubicin+ MZ (250 mg/kg b.wt)</td>
<td>109.6±5.7***</td>
<td>115.2±3.2***</td>
<td>38±1.3***</td>
<td>41±4***</td>
</tr>
</tbody>
</table>

The values were expressed as mean ± standard deviation (SD); n=6. The mean values were statistically analyzed by one way analysis of variance (ANOVA) followed by appropriate post hoc test (Dunnett’s multiple comparison test) using Graph pad Instat 3 Software.

ns - Not significant (p>0.05), *** p<0.001 significant. Significant level of Dox alone treated control group was compared with that of normal group, whereas significant levels of MZ-treated and vehicle control groups were determined by comparing with those of Dox alone treated control group.
Table 7.7. Effect of MZ on Antioxidant Enzymes and GSH during Doxorubicin Induced Cardiotoxicity

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (U/mg protein)</th>
<th>CAT (U/mg protein)</th>
<th>GPx (n mol/mg protein)</th>
<th>GSH (n mol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3.4±0.4</td>
<td>6.1±0.1</td>
<td>13.6±1.14</td>
<td>9.2±0.84</td>
</tr>
<tr>
<td>DOX control</td>
<td>0.5±0.01***</td>
<td>0.49±0.05 ***</td>
<td>3.5±0.5 ***</td>
<td>3.8±0.84 ***</td>
</tr>
<tr>
<td>Doxorubicin+ Sunflower oil (Vehicle control)</td>
<td>0.49±0.03 ns</td>
<td>0.486±0.04 ns</td>
<td>3.64±0.59 ns</td>
<td>3.96±0.09 ns</td>
</tr>
<tr>
<td>DOX+ MZ (50mg/kg b.wt)</td>
<td>0.72±0.02 ns</td>
<td>2.3±0.25 ns</td>
<td>5.3±0.45 **</td>
<td>4±0.98 ns</td>
</tr>
<tr>
<td>DOX+ MZ (100mg/kg b.wt)</td>
<td>0.84±0.04 *</td>
<td>4.24±0.35 ***</td>
<td>8.47±0.55 ***</td>
<td>4.6±1.14 ns</td>
</tr>
<tr>
<td>DOX+ MZ (250 mg/kg b.wt)</td>
<td>2.34±0.13 ***</td>
<td>6.4±0.50 ***</td>
<td>10.8±0.84 ***</td>
<td>10.2±0.8 ***</td>
</tr>
</tbody>
</table>

The values were expressed as mean ± standard deviation (SD); n=6. The mean values were statistically analyzed by one way analysis of variance (ANOVA) followed by appropriate post hoc test (Dunnett’s multiple comparison test) using Graph pad Instat 3 Software. ns - Not significant (p>0.05). * p<0.05, ** p<0.01 and *** p<0.001 significant. Significant level of Dox alone treated control group was compared with that of normal group, whereas significant levels of MZ-treated and vehicle control groups were determined by comparing with those of Dox alone treated control group.
7.3.2.4. Effect of MZ on Oxidative Stress Markers in the Cardiac Tissue of Rats during Doxorubicin Induced Cardiotoxicity

Formation of conjugated dienes and tissue hydro peroxides as well as LPO levels in the DOX alone treated animals were significantly high compared to normal rats, and these parameters were reduced to normal levels by MZ treatment in a dose dependent manner (Table 7.8).

7.3.2.5. Histopathological Analysis of Cardiac Tissue

Histopathology of heart tissue of the DOX alone treated control group animals showed massive necrosis of heart muscle fibers along with focal loss and marked fragmentation. Disorganized arrangement with no well-defined boundaries or distinct bundles of myocardial fibers was observed. Nuclei were scattered. However pre-treatment with MZ helped to retain the normal histology of heart (Figure 7.4).

7.4. DISCUSSION

Cisplatin continues to be a mainstay chemotherapeutic agent, but dose-limiting nephrotoxicity remains a major side effect. Platinum compounds are believed to mediate their cytotoxic effects through their interaction with DNA. In an aqueous environment, the chloride ligands of cisplatin are replaced by water molecules generating a positively charged electrophile. This electrophile reacts with nucleophilic sites on intracellular macromolecules like DNA to form inter and intra strand cross-links, thereby arresting DNA synthesis and replication in rapidly proliferating cells and in turn induces apoptosis (Thadhani et al., 1996).

Many chemotherapeutic agents including cisplatin cause myelosuppression. This could be understood from the decrease in the levels of total WBC count, haemoglobin content, bone marrow cellularity and α-esterase positive cells of the cisplatin-challenged control group animals. But administration of MZ significantly reversed the myelosuppression produced by cisplatin in a dose-dependent manner, indicating the immunomodulatory potential of carotenoid MZ.

Cisplatin induces the generation of various ROS through inactivation of cellular antioxidant system, disruption of mitochondrial respiratory chain, or interaction with microsomal cytochrome P450 enzymes (Pabla and Dong, 2008).
### Table 7.8. Effect of MZ on Oxidative Stress Markers in the Heart Tissue During Doxorubicin Induced Toxicity.

<table>
<thead>
<tr>
<th>Groups</th>
<th>LPO (n mol/mg protein)</th>
<th>Conjugated dienes (mM/100g tissue)</th>
<th>Tissue hydroperoxides (mM/100g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2.0±0.2</td>
<td>3.1±0.11</td>
<td>5.2±0.2</td>
</tr>
<tr>
<td>DOX control</td>
<td>5.6±0.1***</td>
<td>5.7±0.3***</td>
<td>7.2±0.3***</td>
</tr>
<tr>
<td>DOX+ Sunflower oil (Vehicle control)</td>
<td>5.3±0.2 ns</td>
<td>5.67±0.3 ns</td>
<td>7.1±0.2 ns</td>
</tr>
<tr>
<td>DOX+ MZ (50mg/kg b.wt)</td>
<td>4.2±0.18*</td>
<td>5.1±0.4**</td>
<td>6.8±0.18*</td>
</tr>
<tr>
<td>DOX+ MZ (100mg/kg b.wt)</td>
<td>3.4±0.2***</td>
<td>4.1±0.2**</td>
<td>6.2±0.1***</td>
</tr>
<tr>
<td>DOX+ MZ (250mg/kg b.wt)</td>
<td>1.8±0.1***</td>
<td>3.4±0.2***</td>
<td>5.3±0.2***</td>
</tr>
</tbody>
</table>

The values were expressed as mean ± standard deviation (SD); n=6. The mean values were statistically analyzed by one way analysis of variance (ANOVA) followed by appropriate post hoc test (Dunnett’s multiple comparison test) using Graph pad Instat 3 Software.

ns - Not significant (p>0.05). * p< 0.05, ** p <0.01 and *** p<0.001 significant.

Significant level of Dox alone treated control group was compared with that of normal group, whereas significant levels of MZ-treated and vehicle control groups were determined by comparing with those of Dox alone treated control group.
Figure 7.4. Histopathological analysis of heart tissue of rats

(a) Normal histology of heart

(b) Doxorubicin alone treated control group showed massive necrosis of heart muscle fibers along with focal loss and marked fragmentation. Disorganised arrangement with no well-defined boundaries of myocardial fibers was observed.

(c) DOX+ Sunflower oil treated- Showing massive necrosis.

(d) DOX+ 100mg/kg b.wt meso-zeaxanthin pre-treated group showed minimal cardiac damage.

(e) DOX+ 250mg/kg b.wt meso-zeaxanthin pre-treated group retained normal histology.

(Haematoxylin – Eosin staining, x 400).
Highly potent ROS appear to target multiple cellular components, such as lipids, proteins, and DNA and activate multiple signaling pathways and thereby implicated in the pathogenesis of acute cisplatin-induced renal injury (Kawai et al., 2006). Antioxidant enzymes and glutathione are among the endogenous system that are available for the removal or detoxification of these free radicals and their products. The antioxidant status in the kidney tissue of mice was found to be decreased after cisplatin administration and was significantly improved by the MZ treatment in a dose dependent manner. Various oxidative stress markers in the kidney like LPO, CD, HP, which were markedly elevated by cisplatin-challenge, were decreased to normal levels by treatment with MZ.

Doxorubicin (DOX) is effective in curative and adjuvant chemotherapy of many malignant tumours. Unfortunately, its clinical use is limited by dose-dependent cardiac side effects that lead to degenerative cardiomyopathy, congestive heart failure and death. It is believed that induction of oxidative stress by the oxidation-reduction cycle of DOX, and the rupture of the cell membrane resultant from lipid peroxidation would be key events in the development of cardiomyopathy (Ferreira et al., 2008). In the present study cardio-toxicity, which is a major side effect of DOX, could be observed in DOX-control group animals as there was an elevation in the levels of various serum marker enzymes. MZ could significantly modulate the cardio-toxicity as evident from decrease in the serum marker enzymes in carotenoid treated groups.

The major etiopathological factor in the DOX-induced cardio-toxicity is generation of free radicals (Xu et al., 2001), which in turn impair the antioxidant defense mechanism leading to an increased membrane lipid peroxidation, damage of membrane structure and inactivation of membrane bound enzyme. The antioxidant status in the heart tissues of rats was found to be decreased after DOX administration. But it was significantly improved by the MZ pre-treatment in a dose dependent manner. Various oxidative stress markers were also found to be significantly decreased after MZ pre-treatment when compared to DOX alone treated control animals. The results of the study clearly demonstrated that there was an increase in myocardial injury as indicated by prolonged ST interval, PR interval, RR interval and QT interval and a decrease in heart beat rate of ECG pattern in DOX alone treated group, but MZ pre-treatment significantly restored ECG changes towards normalcy.
MZ can be considered as a good chemo protector, due to its non toxicity profile, acceptable route of administration (oral), antioxidant status and immuno modulatory effects. So a combination therapy of cisplatin/doxorubicin with MZ will have a profound beneficial effect in the treatment of cancer.