CHAPTER 5
RADIOPROTECTIVE, NEPHROPROTECTIVE AND ANTI-INFLAMMATORY ACTIVITIES OF THE PLANT *RUBIA CORDIFOLIA* LINN
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5.1. INTRODUCTION

Cancer accounts for nearly 25% of all human deaths. Despite a number of medical advances; no sure-fire is available as yet (Balachandran et al., 2005). The most common treatment modality promising a cure appeared to be a combination of radiotherapy and chemotherapy. Following radiotherapy, the risk of normal tissue complication constitutes a significant clinical concern and limits the radiation dose that can be delivered to patients. One of the current problems in radiobiological research pertains to the protection of living cells from radiation induced damage. Although radiotherapy is an effective treatment for malignant diseases, radiation morbidity thus develops in certain patients with above normal radiosensitivity (Nair et al., 2004).

DNA has a limited chemical stability (intrinsic or induced by exogenous agents) and is one of the most biologically important targets of reactive oxygen species. Maintenance of its integrity is a major goal in radiation research. About 100 different kinds of base and sugar damage have been identified. Free-radicals can damage nucleobases and sugar units in DNA either directly, or indirectly. Radiation induced cellular lethality has been ascribed to the formation of aqueous free radicals, which attack vital cellular sites, such as DNA and cell membranes. The development of radioprotective agents has been the subject of intense research in view of their potential for use within a radiation environment; such as space exploration, radiotherapy and even nuclear war. A variety of compounds with different molecular structures, therapeutic activities and metabolic functions are known to have radioprotective action. However no ideal, safe synthetic is available to date, so the search for alternative sources, including plants, has been ongoing for several decades.

The major forms of cellular damage induced by radiation are DNA damage, lipid peroxidation and protein oxidation. Ionizing radiation generates ROS as a result of water radiolysis. In actively metabolizing cells, there is considerable water apart from the target macromolecules. These ROS can induce oxidative damage to vital cellular molecules and structures including DNA, lipids protein and membranes (Nair & Nair, 2010).

In Ayurveda, the traditional Indian system of medicine, several plants have been used to treat free radical-mediated ailments and, therefore it is logical to expect
that such plants may also render some protection against radiation damage. Herbal radioprotection is a multifaceted phenomenon, and thus there is a need to investigate the different modes of radioprotective action of herbal drugs. Systematic screening approach leads to identifying potential new candidate drugs from plant sources, for mitigation of radiation injury (Arora et al., 2005).

Cisplatin is one of the most effective chemotherapeutics against a wide range of cancers including head, neck, ovarian and lung cancers. But its usefulness is limited by its toxicity to normal tissues, including cells of the kidney proximal tubule. Cisplatin may be accumulated in the tubular epithelial cells of proximal kidney tubule, causing nephrotoxicity. The nephrotoxicity induced by cisplatin is characterized by morphological destruction of intra cellular organelles, cellular necrosis, loss of microvilli, alterations in the number and size of the lysosomes and mitochondrial vacuolization, followed by functional alterations including inhibition of protein synthesis, GSH depletion, lipid peroxidation and mitochondrial damage (Kuhlmana et al., 1988).

*Rubia cordifolia* (L) commonly known as Indian madder or ‘Manjishta’ belongs to the family *Rubiaceae*. Most parts of this ethnobotanically important plant have been exploited for its medicinal properties. The roots have an antibacterial action, inhibiting the growth of *Staphylococcus aureus*, *S. epidermidis*, *Pneumococci* etc. They are used to lower the blood pressure. The roots are used internally in the treatment of abnormal uterine bleeding, internal and external haemorrhage, bronchitis, rheumatism, stones in the kidney, bladder and gall, dysentery etc (Chopra et al., 1986; Yeung, 1985; Duke et al., 1985). The chloroform extract of the roots of this plant exhibited significant gastroprotective and ulcer-healing properties under experimental conditions (Deoda et al., 2011). Kalra et al also examined the anti-ulcer potential of the alcoholic extract of the roots of this plant on alcohol, ibuprofen, cols-restraint stress and pyloric ligation-induced gastric lesions. The extract has shown a substantial and significant protection against gastric ulcers in all the models (Kalra et al., 2012). The hydro-alcoholic extract of *Rubia cordifolia* was found to be effective in indomethacin-induced enterocolitis in rats (Pawar et al., 2011). The dried powder of *Rubia cordifolia* possess anti-viral and free radical scavenging activities under *in vitro* conditions (Prajapati & Parmar, 2011). The ethanolic extract of the roots of *Rubia cordifolia* protected the lead
nitrate-induced immune response impairment and kidney oxidative damage in Swiss albino mice by virtue of its in vivo antioxidant property (Iodi et al., 2011).

In the present chapter we have evaluated the possible adjuvant role of *Rubia cordifolia* as radioprotector as well as a chemoprotector during two modalities of cancer treatment viz. radiotherapy and chemotherapy. Also efforts are made to find out the mechanism of protection by determining the antioxidant and anti-inflammatory activities.

### 5.2. MATERIALS AND METHODS

#### 5.2.1. Animals

Swiss albino mice were used for the animal experiments.

#### 5.2.2. Irradiation

Irradiation was carried out using a 60C-Theatron Phoenix teletherapy unit (Atomic energy ltd, Ottawa, Canada) at a dose rate of 1.88 Gy per minute.

#### 5.2.3. Preparation of hydroalcoholic extract of *Rubia cordifolia* Linn

Authenticated dried roots of *Rubia cordifolia* were purchased from Amala Ayurvedic Hospital and Research centre and powdered. The powder was extracted with 70% ethanol at room temperature. Extract was filtered through Whatmann no. 1 filter paper and the supernatant was evaporated using rotary evaporator at 45°C and the final liquid suspension was lyophilized to get a powder with 11% yield, hereafter referred as RCE (*Rubia cordifolia* extract). The powder was dissolved in distilled water at desired concentrations and administered orally by gavage to animals.

#### 5.2.4. Protection of DNA from γ radiation induced damages by RCE

##### 5.2.4.1. Effect of RCE on γ-radiation induced damages to plasmid DNA in vitro

The plasmid pBR322 (150 ng) in phosphate buffer (0.1 M), pH 7.4) was exposed to various doses of γ- irradiation (0 Gy- 25 Gy) in the presence and absence of RCE (0-10 mg/ml) on ice. After irradiation DNA was electrophoresed on 0.8% agarose at 55 V for 2 hours and the DNA damage was analyzed by Digital Gel Documentation and Analysis Software, Biotech R&D Laboratories, Yercaud.
(Maurya et al., 2005). The analysis was also done and the D.M.F (dose modifying factor) was calculated (Sambrook et al., 1989).

5.2.4.2. Effect of RCE on the survival of radiation exposed spleenocytes and thymocytes in vitro

Spleen and thymus of normal Swiss albino mouse were excised out and single cell suspensions of these were prepared in DMEM medium containing 10% serum. The cell suspension (1x10⁶ cells/ml) was exposed to different doses of γ- radiation (0 Gy-50 Gy) with and without the presence of RCE (200 mg/ml). Cell survival was determined using Trypan blue dye exclusion method and the percentage survival was calculated for both spleenocytes and thymocytes.

5.2.4.3. Effect of RCE on radiation induced cellular DNA damage under ex vivo conditions

Three healthy volunteers were selected from age group 25-30 years, non-smokers who were not undergoing any medical treatment. Blood was collected by finger prick and stored on ice in heparinized eppendorf tubes separately. The samples were exposed to 4 Gy γ-radiation with or without the presence of RCE (10 mg/ml), comet assay was performed and the comet parameters were estimated. The procedure is described in chapter 2.

5.2.4.4. Effect RCE on radiation induced cellular DNA damage under under in vivo conditions

Male Swiss Albino mice weighing 20-25 g were divided into four groups having 6 animals in each group and the following treatments were given.

Group 1. DDW + sham irradiation
Group 2. DDW +4 Gy γ- irradiation
Group 3. 200 mg/kg body wt RCE + sham irradiation
Group 4. 200 mg/kg body wt RCE + 4 Gy γ- irradiation

Animals were sacrificed by cervical dislocation 1 hour after irradiation; bone marrow cells were collected by flushing the femur bones of each animal with FBS (Fetal Bovine serum) containing PBS (Phosphate Buffered Saline). Spleen was excised out and made into single cells (10⁶ cells/ml). Alkaline comet was
performed using the method given by Nair and Salvi (2008) with minor modifications.

5.2.4.5. Effect of RCE on the survival of mice exposed to whole body lethal dose of \( \gamma \)-radiation (10 Gy)

Swiss Albino male mice (20-25 g body weight) were divided in to 4 groups, each group comprising of 10 animals. Animals in the groups I and II were orally administered with distilled water and those in Group III and IV were orally administered with RCE (200 mg/kg body weight). After one hour of oral administration, Groups II and IV were exposed 10 Gy whole body gamma-radiation. After the radiation exposure, Groups I and II were orally given distilled water and Groups III and IV were orally given RCE for 7 consecutive days. The percentage survival in each group was recorded.

5.2.5. Amelioration of Cisplatin induced nephrotoxicity by RCE

Animals were divided into five groups of six animals each. Group I treated with vehicle (distilled water) was kept as normal. Group II injected with a single dose of cisplatin (12 mg/kg body weight; i.p) was kept as control. Group III was administered with *Rubia cordifolia* extract (RCE) 250 and 500 mg/kg body weight along with cisplatin treatment. Group IV was given RCE at a dose of 500 mg per kg body wt alone. The extract was administered by oral gavage 1 h before and at 24h and 48 h after cisplatin injection. Seventy two hours after the cisplatin injection, animals were sacrificed using ether- anesthesia; blood samples were collected by heart puncture for measuring serum urea and serum creatinine levels. Kidneys were quickly removed and washed with ice-cold normal saline and homogenates (10%, w/v) were prepared in PBS. A part of the homogenate was used for the estimation of reduced glutathione (GSH) and lipid peroxidation. The remaining homogenate was centrifuged at 5000 \( \times \)g for 10 minutes at 4\( ^{\circ} \)C, after removal of the cell debris, supernatant was used for the assay of Super oxide dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) etc.

Total protein was estimated by the method of Lowry *et al* (1951). SOD (McCord & Fridovich, 1969), GSH (Moron *et al*, 1979), and GPx (Hafeman *et al*., 1974) were assayed in brain, liver and kidney. Lipid peroxidation (LPO) in the tissues were estimated using the TBA method of Okhawa *et al*. Methods were described in Chapter 2.
Serum Creatinine is determined by alkaline picric acid method using a diagnostic kit (Agappe Diagnostic Pvt. Ltd; Ernakulam, Kerala, India). Serum urea was determined by diacetylmonoxime (DAM) reagent (modified Berthelot methodology) using a diagnostic kit (Agappe Diagnostic Pvt. Ltd; Ernakulam, India). Histopathological examinations of kidney from the treated groups were evaluated using light microscopy. A portion of the kidney was fixed in 10% formalin solution immediately after sacrifice, passed through ascending grade of alcohol, cleared in xylene impregnated and embedded in paraffin. Sections (5 μm) were made using a microtome, stained with H & E and mounted in DPX. The histopathological examinations were carried out at Sudharma Metropolis Pathological Laboratory, Thrissur, Kerala, India. The slides were observed in light microscope and photographed.

5.2.6. Determination of anti-inflammatory activity of RCE

Acute and chronic anti-inflammatory activities were evaluated. The former was done by the method of carrageenan and the latter by formalin – induced oedema in mice hind paw.

5.2.6.1. Carrageenan induced acute paw oedema

Paw oedema was induced by injecting 0.02 ml of 1% carrageenan in physiological saline on the sub plantar tissues of the left hind paw of each mouse (Winter et al., 1962). Aqueous solution of RCE (200 mg/kg body wt) was administered orally 1hr prior to carrageenan administration. The paw thickness was measured using vernier calipers before and after carrageenan injection and thereafter at every hour and continued for 6 hours. Increase in paw thickness as a measure of inflammatory oedema was calculated using the formula Pt-P₀, where P₀ is the initial paw thickness and Pt is the thickness at time t (3h). Per cent inhibition of paw oedema was calculated by the formula (1-Pt/Pc) x 100, where Pt is the increase in paw thickness of the treated, Pc is that of control. The percentage inhibition of paw thickness in drug treated group was compared with the control group. The decrease in paw thickness in drug treated group was compared with the control group. Diclofenac (10 mg/kg body wt) was used as the standard reference.
5.2.6.2. Formalin induced chronic paw oedema

The inflammation was produced by sub plantar injection of freshly prepared 2% formalin in the right hind paw of mice (Chaw, 1989). The paw thickness was measured using vernier calipers 1hr before and after formalin injection. The drug treatment was continued for 6 consecutive days. The increase in paw thickness and the percent inhibition was calculated as above.

5.2.7. Statistical Analysis

Statistical analysis on the results was performed using Microsoft Excel and Microcal Origin soft wares. The data were expressed as mean ± SD. The error bars in the figures indicate the standard error of mean. Statistical analyses of the results were performed using ANOVA with Tukey-Kramer multiple comparisons.

5.3. RESULTS

Scheme 5.1 depict the HPTLC profile of the plant extract Rubia cordifolia.

![HPTLC profile of Rubia cordifolia](image)

Scheme 5.1. HPTLC profile of the plant extract Rubia cordifolia Linn

5.3.1. Protection of DNA from γ radiation induced damages by RCE

5.3.1.1. Effect of RCE on γ‐radiation induced damages to plasmid DNA in vitro

Figure 5.1a represents the effect of RCE on the protection of plasmid DNA under in vitro conditions. Exposure to ionizing radiation induces strand breaks in
plasmid DNA. As can be evidenced from the figures 5.1a, 5.1b and 5.2, it is clear that the presence of RCE prevents the conversion of supercoiled form of DNA to open circular to some extent. The dose modifying factor at 50 % protection was calculated and was found to be 1.38.

Figure 5.1a. Effect of different doses of γ-radiation on plasmid pBR 322 DNA in presence and absence of RCE

Figure 5.1b. Effect of various concentrations of HAE on pBR 322 DNA exposed to 25 Gy γ- radiation. I-0 Gy, 0 RCE, II-0 Gy, 10 mg/ml RCE, III- 25 Gy, 0 RCE, IV-25 Gy, 2 mg/ml RCE, V-25 Gy, 4 mg/ml RCE, VI-25 Gy, 6 mg/ml RCE, VII- 25 Gy, 10 mg/ml RCE
Figure 5.2. Effect of various concentrations of RCE on pBR 322 DNA exposed to 25 Gy \( \gamma \)-radiation.

### 5.3.1.2. Effect of RCE on the survival of radiation exposed spleenocytes and thymocytes in vitro

The survival rate of spleenocytes and thymocytes were decreased in a dose dependent manner when they were subjected to different doses of \( \gamma \)-radiation (0-50 Gy) under \textit{in vitro} conditions. Figures 5.3a and 5.3b demonstrate that the presence of RCE has a protective role in both the cases of spleenocytes and thymocytes when exposed to different doses of \( \gamma \)-radiation. The protective effect was more pronounced in the case of higher doses of radiation exposure.
5.3.1.3. Effect of RCE on damages to cellular DNA of human peripheral leucocytes under ex vivo conditions

The comet parameters such as % DNA in tail, tail length, tail moment and olive tail moment were found to be increased when human blood leucocytes were exposed to $\gamma$-radiation. In the control irradiated group % DNA in tail was found to be 18.61 ± 2.40, tail length 20.72 ± 2.13, tail moment 3.61 ± 0.97 and olive tail moment 6.72 ± 1.16. The presence of RCE could brought down these levels to
15.27 ± 2.86, 14.32 ± 2.81, 2.24 ± 0.34 and 4.89 ± 0.74 respectively as presented in the figure 5.4.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>RCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Gy</td>
<td>% DNA in Tail</td>
<td>ns 15.27 ± 2.86</td>
</tr>
<tr>
<td>4 Gy</td>
<td>% DNA in Tail</td>
<td>*** 21.64 ± 3.34</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>RCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Gy</td>
<td>Tail Length</td>
<td>ns 5.0 ± 0.5</td>
</tr>
<tr>
<td>4 Gy</td>
<td>Tail Length</td>
<td>*** 7.5 ± 1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>RCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Gy</td>
<td>Tail Moment</td>
<td>ns 1.5 ± 0.2</td>
</tr>
<tr>
<td>4 Gy</td>
<td>Tail Moment</td>
<td>*** 3.0 ± 0.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>RCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Gy</td>
<td>Olive Tail Moment</td>
<td>ns 0.5 ± 0.1</td>
</tr>
<tr>
<td>4 Gy</td>
<td>Olive Tail Moment</td>
<td>*** 3.0 ± 0.5</td>
</tr>
</tbody>
</table>

Figure 5.4. Comet parameters presenting the effect of RCE administration on γ-radiation (4 Gy) induced DNA strand breaks in the human peripheral blood leucocytes ex vivo. Mean of the percentage DNA in tail, tail length, tail moment and olive tail moment are presented as mean± sd. (ns indicate not significant) p>0.05 and *** indicate p <0.001 when compared with respective control arbitrary units (AU))

5.3.1.4. Effect of RCE on damages to cellular DNA following γ-radiation under in vivo conditions

Alkaline comet assay was performed in murine tissues such as spleen cells and bone marrow cells in order to examine the protective effect of RCE against γ-radiation induced changes under in vivo conditions. Exposure to ionizing radiation (4 Gy) induced strand breaks in cellular DNA which can be understood by the increased levels of comet parameters. The administration of RCE (200
mg/kg body wt) prevented the breakage of cellular DNA to a certain extent. All the comet parameters were seen to be decreased both in the bone marrow cells and spleen cells in the RCE administered animals as can be observed from the figures 5.5 and 5.6.

Figure 5.5. Comet parameters presenting the effect of RCE administration on γ-radiation (4 Gy) induced DNA strand breaks in the murine bone marrow cells in vivo. Mean of the percentage DNA in tail, tail length, tail moment and olive tail moment are presented as mean± sd. (ns indicate not significant) p>0.05 and *** indicate p <0.001 when compared with respective control arbitrary units (AU))
Figure 5.6. Comet parameters presenting the effect of RCE administration on γ - radiation (4 Gy) induced DNA strand breaks in the murine spleen cells in vivo. Mean of the percentage DNA in tail, tail length, tail moment and olive tail moment are presented as mean±sd. (ns indicate not significant) p>0.05 and *** indicate p <0.001 when compared with respective control arbitrary units (AU)).

5.3.1.4. Effect of RCE on the survival of mice exposed to whole body lethal dose of γ radiation (10 Gy)

The mortality rate of control irradiated group (10 Gy) was found to be very high as can be seen from the figure 5.7, on the 10th day, 100% mortality was obtained in the control irradiated group. However administration of RCE (200 mg/kg body wt) to animals had significant effect in extending the life span following 10 Gy radiation. The administration of RCE could brought down this mortality rate to some extent. When the animals were given one dose of the drug prior to radiation exposure and a single dose of drug each day, resulted in 30% survival on the 10th day. The mortality seen was 100 % in the RCE treated group in the 14th day. This implies that
administration of RCE could mitigate some deleterious effects of ionizing radiation under experimental conditions.

![Graph showing survival rates](image)

**Figure 5.7.** Effect RCE on 10 Gy γ- radiation induced mortality

### 5.4. Amelioration of Cisplatin induced nephrotoxicity by RCE

The extent of nephrotoxicity is manifested by the levels of serum urea and creatinine. The values of serum urea and creatinine were significantly elevated in cisplatin treated group as can be evidenced from the data presented in table 5.1. Cisplatin treatment results in two fold increase of these values compared to normal levels. They were found to be about two fold that of normal mice (treated with DW alone) and the administration of RCE could restore these back to normal levels.
Table 5.1. Effect of hydroalcoholic extract of RCE on serum urea and creatinine levels in mice treated with cisplatin (**p <0.001 when compared with cisplatin alone treated group)

<table>
<thead>
<tr>
<th>Treatments (mg/kg body wt)</th>
<th>Urea (mg/dl)</th>
<th>Creatinine(mg/ dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (DDW)</td>
<td>56.69 ±1.79***</td>
<td>0.72 ± 0.064***</td>
</tr>
<tr>
<td>Control (cisplatin treated)</td>
<td>85.52 ± 5.87</td>
<td>2.18 ± 0.098</td>
</tr>
<tr>
<td>Cisplatin+RCE [500mg/kg body wt] mg /kg body wt]</td>
<td>47.42 ± 4.25***</td>
<td>1.37 ± 0.18***</td>
</tr>
<tr>
<td>Cisplatin+ RCE [250 mg /kg b.wt.]</td>
<td>66.74 ± 5.02***</td>
<td>1.00 ±0.16***</td>
</tr>
<tr>
<td>RCE [500mg/kg body wt] mg /kg b.wt.]</td>
<td>49.26 ± 1.27***</td>
<td>0.857 ± 0.072***</td>
</tr>
</tbody>
</table>

Treatment of animals with cisplatin significantly altered the antioxidant status of kidney tissues. The major antioxidant enzymes such as GPx, SOD and catalase were found to be decreased in cisplatin treated animals and per oral administration of RCE could elevate these levels significantly as can be realized from the data presented in table 5.2. The basic effect of cisplatin induced toxicity on the cellular membrane is believed to be peroxidation of membrane lipids. The depletion of the total amount of glutathione at early intervals in treated animals may be due to their utilization in large amount to combat the acute cisplatin induced free radical damage, as glutathione is a major nonenzymatic antioxidant.
Table 5.2. Effect of Cisplatin administration on renal antioxidant enzymes in Swiss albino mice

(* * *p < 0.001 when compared with cisplatin alone treated group)

<table>
<thead>
<tr>
<th>Treatments (mg/kg body wt)</th>
<th>Gpx (U/mg protein)</th>
<th>SOD (U/mg protein)</th>
<th>Catalase (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (DDW)</td>
<td>29.43 ±1.37***</td>
<td>12.23 ±0.47***</td>
<td>15.77±0.86***</td>
</tr>
<tr>
<td>Control (Cisplatin treated)</td>
<td>20.58 ±0.91</td>
<td>6.02 ±0.87</td>
<td>7.87±1.23</td>
</tr>
<tr>
<td>Cisplatin+RCE [500 mg/kg body wt]</td>
<td>25.05 ±1.64***</td>
<td>9.96 ±0.76***</td>
<td>13.2 ±1.17***</td>
</tr>
<tr>
<td>Cisplatin+ RCE [250 mg /kg body wt.]</td>
<td>22.72 ±0.841*</td>
<td>7.13±0.81ns</td>
<td>9.72 ±0.56*</td>
</tr>
<tr>
<td>RCE [500 mg/kg body wt]</td>
<td>27.26 ±1.011***</td>
<td>9.95 ±0.29***</td>
<td>12.95 ±0.414***</td>
</tr>
</tbody>
</table>

The measurement of lipid peroxidation as thiobarbituric acid reacting substances (TBARS) is a convenient method to monitor oxidative damage in tissues. Reactive oxygen species causes peroxidation of membrane lipids with devastating effect on functional state. The preservation of cellular membrane integrity depends on protection or repair mechanisms capable of neutralizing oxidative reactions. From the figures 5.8a and 5.8b, it can be seen that administration of RCE results in inhibition of cisplatin induced peroxidation of lipids in tissues such as liver and kidney of mice. The protection against cisplatin induced toxicity could stem from the potent antioxidant activity of RCE. Inhibition of LPO in biomembranes has been caused by antioxidants present in the plant extract. From the above results, it can be inferred that the hydro-alcoholic extract of *Rubia cordifolia* render protection against cisplatin induced oxidative stress in renal tissues.
Figure 5.8a. Effect of administration of RCE on cisplatin induced lipid peroxidation in liver.

A. Normal control, B. Control cisplatin treated (12 mg/kg body wt)
C. Cisplatin + RCE (500mg/kg body wt), D. Cisplatin + RCE (250 mg/kg body wt), E. RCE (500 mg/kg body wt)

Figure 5.8b. Effect of administration of RCE on cisplatin induced peroxidation in kidney.

A. Normal control, B. Control cisplatin treated (12 mg/kg body wt)
C. Cisplatin + RCE (500mg/kg body wt), D. Cisplatin + RCE (250 mg/kg body wt), E. RCE (500 mg/kg body wt)

Figure 5.9 presents the histopathology of the renal tissues of mice following various treatments. Histopathological investigation showed that, the normal renal tissue architecture of the untreated mice (Figure 5.9.a) was unaffected with normal
glomeruli. In cisplatin-treated mouse kidney, there is a decreased cellularity of the glomeruli and oedema of the lining of epithelial cells in the renal tubules. Moreover, the nuclei of the lining cells show vaculation. The interstitial tissue also showed edema as can be evident from figure 5.9.b. The renal tissues of cisplatin-treated mice, when administered with RCE after the cisplatin treatment, showed normal glomerular, renal tubule and interstitial tissue appearance (Figures 5.9.c). The nephroprotective action exhibited by RCE implies that it can be used as a chemoprotector for mitigating the adverse effects of drugs used in chemotherapy.

Figure 5.9. Micrograph of kidney of mice (A) Normal, (B) 72 h of Cisplatin injection, (C) Cisplatin and RCE( 250 mg/kg body wt), (D) Cisplatin and RCE( 500 mg/kg body wt), The tissues were fixed in 10% formalin, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin-eosin. The histopathological examinations were carried out using light microscopy (100x).
5.5. Determination of anti-inflammatory activity of RCE

The data of acute anti-inflammatory activity of RCE against carrageenan challenge is presented in figure 5.10a. There was 59.34% inhibition in paw thickness at a dose of 200 mg/kg body weight extract treated groups on the 6th hour; compared to control.

Figure 5.10a. Effect of RCE on Carrageenan induced acute inflammation

Similar results were obtained when formalin was used as an anti-inflammatory inducer for chronic inflammation. On the 7th day, there was 56.5% inhibition in paw thickness when a dose of 200 mg/kg body weight RCE was given to the group (figure 5.10b).

Figure 5.10b. Effect of RCE on Formalin induced chronic inflammation
5.6. DISCUSSION

Radiation is used therapeutically for the treatment of various types of malignancies. The severe side effects of radiotherapy resulted from the damage to normal cells. Rapidly dividing cells of gastrointestinal tract, haematopoeitic systems are more prone to radiation induced damages. In the present study, the radiation damage produced as a result of the whole body irradiation is significantly lowered by continuous administration RCE. Also extract was found to be nontoxic at doses up to 1000 mg/kg body weight. The body weight recorded for animals administered with different doses of drug also supported the non toxicity of drug. There was no death even after administering a dose of 1000 mg/kg body weight (data not shown).

The cellular membrane and DNA are the two main targets of radiation induced lethality and mutagenicity. Studies on plasmid DNA suggest that RCE could protect cellular DNA from radiation induced damages under *in vitro* conditions. Alkaline comet assay is a main indicator of DNA strand breaks. The results obtained for comet assay under *ex vivo* and *in vivo* conditions demonstrate the radioprotective activity of RCE.

The nephrotoxicity is one of the major side effects of cisplatin. Although several studies have been performed to elucidate the molecular mechanisms that cause cisplatin nephrotoxicity, the factors responsible for this are not fully understood. Recently, induction of oxidative free radicals has been implicated in this process. (Baldew *et al.*, 1990) Different strategies have been proposed to inhibit cisplatin induced toxicity. The development of therapies designed to prevent the damaging actions of free radicals may influence the progression of oxidative renal damage induced by cisplatin.

Peroxidation of tissue lipids is a major feature of cisplatin induced nephrotoxicity which may be resulting from cisplatin induced GSH depletion (Kuhlmann *et al.*, 1998). ROS such as hydrogen peroxide, the superoxide anion, and hydroxyl radicals are generated under normal cellular conditions and are immediately detoxified by endogenous antioxidants, like GSH, catalase and super oxide dismutase, but excessive ROS accumulation by cisplatin cause an antioxidant status imbalance and lead to lipid peroxidation and GSH depletion (Saduka *et al.*, 1992). Our data show that cisplatin induced malondialdehyde (MDA) production
was significantly decreased by the p.o. administration of RCE in vivo and it also attenuated cisplatin induced GSH depletion in mice.

It has been suggested that cisplatin is able to generate ROS and that it inhibits the activities of antioxidant enzymes in renal tissues, eg, GPx, SOD and catalase (Naziroglu et al., 2004). In the present study the activities of GPx, SOD and catalase were found to be reduced in kidneys of mice treated with cisplatin. But the RCE administration restored the cisplatin induced impairments to a considerable extent. Thus from the present study, it can be concluded that the hydro-alcoholic extract of Rubia cordifolia (RCE) eliminates the oxidative stress following cisplatin treatment.

Inflammation is body’s response to inactivate or destroy the invading organisms, remove irritants and set stage for tissue repair. Inflammation, a fundamental response may be harmful in conditions such as life threatening and hypersensitive reactions to insect bites, drugs, toxins and in chronic diseases such as rheumatoid arthritis, artherosclerosis, lung fibrosis and cancer (Collins, 1999). An Anti-inflammatory drug activity of which is based on free radical scavenging mechanism is considered as ideal. ROS and free radicals are thought to act directly as cellular messengers to elicit an inflammatory response. ROS and free radicals also activate a series of enzyme systems including protein kinases, protein phosphatases, transcription factors, HSP and increase the extent of inflammation (Trenam et al., 1992). But there is no strong evidence to show that antioxidant supplementation can alleviate inflammation. The antioxidant treatment may become important for the treatment of inflammatory diseases. The hydro alcoholic extract of R. cordifolia significantly inhibited the acute inflammation induced by carrageenan and chronic inflammation induced by formalin at a concentration 200 mg/kg body weight in experimental animals. The significant anti-inflammatory of R.cordifolia could be possibly due to its profound antioxidant activity.

The exact mechanism of action of RCE is not clear at present. The studies on the anti-inflammatory activity presented here demonstrated the potent antioxidant activity of RCE in vivo. The potent antioxidant activity as well as non toxicity suggests that RCE has a great potential for use as radioprotector in human studies. Also the nephroprotective studies demonstrated that RCE can be a good candidate for treating renal failures by implicating its antioxidant power. However further investigations are needed for human applications.