CHAPTER 8  PHASE V STUDY

EFFECT OF FERULIC ACID (FA) AND/OR CISPLATIN ON DALTON’S ASCITES LYMPHOMA IN SWISS ALBINO MICE

8.1. INTRODUCTION

Phase I, Phase II and Phase IV studies of the current research showed that chemosensitizing effects of ferulic acid (FA) in cisplatin therapy. Though the sensitizing effects of FA were studied, it is also worth to record the reduction of dose-limiting side effects induced by cisplatin through the coupling of FA. In this phase, the potential of FA in reducing the side effects of cisplatin is studied through Dalton’s lymphoma tumor xenografted mice.

Chemotherapy is one of the most conventional therapeutic strategies for human cancers. Cisplatin, which is also named cis diamminedichloroplatinum (DDP), is deemed to be the “penicillin of cancer drugs” due to its universal, early, and effective treatment for many cancers (Kelland 2007).

Cisplatin has a number of side-effects that can limit its use. Nephrotoxicity (kidney damage) is a major concern. The dose is reduced when the patient's creatinine clearance (a measure of renal function) is reduced. Adequate hydration and diuresis is used to prevent renal damage. The nephrotoxicity of platinum-class drugs seems to be related to reactive oxygen species and in animal models can be ameliorated by free radical scavenging agents (e.g., amifostine). Nephrotoxicity is a dose-limiting side effect.

Cisplatin can also cause hypomagnesaemia, hypokalaemia and hypocalcaemia. The hypocalcaemia seems to occur in those with low serum magnesium secondary to cisplatin, so it is not primarily due to the Cisplatin.
This agent can also cause profound bone marrow suppression called ‘myelotoxicity’. Hemolytic anemia can be developed after several courses of cisplatin. It is suggested that an antibody reacting with a cisplatin-red-cell membrane is responsible for hemolysis. Besides the above side effects it also caused ototoxicity, neuro toxicity, nausea, vomiting, hair loss and other minor illness. Free radical-mediated reactions are responsible for a wide range of chemotherapy-induced side effects (Weijl et al. 1997).

Cisplatin treatment causes oxidative stress, endoplasmic stress and abnormal changes in the redox environment. Cisplatin causes another important side effect that is, reduction of antioxidant plasma levels and generation of free radicals in normal cells (Masuda et al.1994; Baliga et al.1998; Weijl1998; Wozniak et al. 2004). It is believed to be an important mechanism in the development of Cisplatin toxicity.

Reports have also shown that cisplatin is also a potential human carcinogen which developed secondary malignancies in patients who has been treated with it (Greene 1992). Much effort has been put into reducing the mutagenic side effects of Cisplatin by administration of modulating agents, usually free radical scavenges.

Daltons lymphoma induced ascitic tumor model is a widely accepted experimental model to test the efficacy of any anticancer agent and also its toxic effects to the host. Though cisplatin is a widely used chemotherapeutic drug the side effects as a result of cisplatin treatment makes it unpleasant for continuous use.

The aim of the phase V study was to investigate the side effects from treatment with cisplatin and ferulic acid compared to common cisplatin treatment, to see if a significant treatment gain would be achieved. In detail, to
study the toxicity profile of cisplatin at organ level in mice and to reduce this effect with the help of a plant based chemosensitizer, ferulic acid.

8.2. MATERIALS AND METHODS

8.2.1. Chemicals

Ferulic acid (FA), cisplatin, thiobarbituric acid (TBA), trichloroacetic acid (TCA), phenazine methosulphate (PMS), nitroblue tetrazolium (NBT), 5, 5-dithiobis 2-nitrobenzoic acid (DTNB), glutathione (GSH) and trypan blue were purchased from Sigma Chemicals Co., St. Louis, USA. All other chemicals and solvents of analytical grade were obtained from S.D. fine chemicals, Mumbai, Fisher Inorganic and Aromatic Limited, Chennai and Central Drug House (P) Ltd, New Delhi, India.

8.2.2. Animal care

Male Swiss albino mice in the age of 11–12 weeks, weighing 25–30 g were purchased from Small Animal Breading Station, Agricultural University (Mannuthy, Thrissur, Kerala, India). The animals were housed in groups of four or five in polypropylene cages and provided standard pellet diet and water *ad libitum* and maintained under controlled conditions of temperature and humidity, with a 12 h light/dark cycle. The animals were maintained as per the principles and guidelines of the Institutional ethical committee for animal care in accordance with the Indian National Law on animal care and use (Reg. No. KMCRET/Ph.D./2011).

8.2.3. Induction of Dalton’s lymphoma ascites (DLA) tumor

Ascites Dalton’s lymphoma was maintained *in vivo* by intraperitoneal (i.p.) serial transplantation of approximately $1 \times 10^7$ viable tumor cells in 0.25
mL phosphate buffered saline (pH 7.4) per animal. Tumor-transplanted mice usually survived for 19–21 days (Khynriam and Prasad 2003).

8.2.4. Dosage and preparation of treatment drugs

Ferulic acid (FA) was solubilized in distilled water just before treatment and was administered orally thrice a week in alternate days at a dose of 40 mg/kg body weight from the next of tumor inoculation for 15 days (Alias et al. 2009).

A single dose of cisplatin (8 mg/kg body weight) was administered intraperitoneally (i.p.) in 0.9%NaCl to tumor-bearing mice on the 10th day after tumor inoculation when the tumor was in log phase of growth (Khynriam and Prasad 2003).

8.2.5. Treatment schedule

The animals were divided into 5 groups of 6 animals each. Group 1 served as control. Groups 2-5 were induced with Dalton’s ascites lymphoma on day 0. Treatment with Ferulic acid was started from day 1 (the next day after tumor induction) in groups 3 and 5 and continued till the 14th day. Groups 4 and 5 were treated with a single dose of cisplatin in the day 10 alone. All the treatments were continued till the day 14. The experiment was terminated at the end of 15th day. The brief experimental design of Phase V study is given in table 8.1.
### Table 8.1. Experimental design of Phase V study

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid tumor control</td>
<td>Ascites tumor alone induced mice (untreated)</td>
</tr>
<tr>
<td>Solid tumor + FA</td>
<td>Ascites tumor treated with FA from day 1 - day 14</td>
</tr>
<tr>
<td>Solid tumor + cisplatin</td>
<td>Solid tumor treated with cisplatin on day 10 alone</td>
</tr>
<tr>
<td>Solid tumor + FA + Cisplatin</td>
<td>Solid tumor treated with cisplatin on day 10 and FA</td>
</tr>
<tr>
<td></td>
<td>from day 1 - day 14</td>
</tr>
</tbody>
</table>

8.2.6. Mean survival time (MST)

Another set of animals of all groups treated in similar fashion was maintained for a period of 35 days for calculating mean survival time (MST). After induction, all the groups were checked for mortality and it was recorded in the chart for 35 days. From this, the average survival time (MST) of the animals was calculated.

8.2.7. Body weight analysis

All the mice were weighed at the end of every five days, after tumor inoculation. Average gain in body weight was determined and recorded. The percentage (%) increase in body weight was calculated by the formula,

\[
\% \text{ increase in body weight} = \left( \frac{\text{Increase in body weight}}{\text{initial body weight}} \right) \times 100
\]
8.2.8. Determination of tumor volume

At the 15\textsuperscript{th} day prior to sacrifice, the animals were anesthetized and the ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube.

8.2.9. Determination of viable cells by trypan blue dye exclusion

Viable Dalton’s lymphoma (DLA) cells (cell viability) in the ascitic fluid was determined by trypan blue dye exclusion method adopted from Gupta (2002).

**Principle**

The cationic chromosomal dye trypan blue, intercalates with DNA of dead cells giving characteristic blue colour to the nucleus. The uptake of the dye is prevented in the living cell by intact cell membrane. By the appearance under a microscope, the viable and non-viable cells can be counted.

**Materials required**

1. Trypan blue dye : 0.4%
2. Light microscope
3. Haemocytometer
4. cover slip

**Procedure**

After 14 days of treatment, the animals were slightly anaesthetized with diethyl ether and a small amount of ascitic fluid was drawn from the peritoneal cavity. 0.2mL of this cell suspension was mixed with 0.5mL of 0.4% trypan blue, 0.3mL of normal saline or PBS and kept aside for 5 minutes (but not more than 15 minutes). From this, one drop of solution was taken on a
Neubauer chamber and the viable and non-viable cells were counted under 10 X power of microscope. Viable cells did not take colour and appeared white on blue background whereas, the non-viable cells (dead cells) were appeared blue.

The percentage of viable cells was calculated using the formula,

\[
\% \text{ Viability} = \frac{\text{Total number of viable cells}}{\text{Total number of viable and non-viable cells}} \times 100
\]

8.2.10. **Preparation of blood serum and tissue samples for biochemical Studies**

At the end of the 15\textsuperscript{th} day, all the animals were sacrificed under anesthesia (i.p. administration of ketamine hydrochloride, 30 mg/kg body weight), by cervical dislocation between 7.30 am to 9.30 am after an overnight fast. The ascitic fluid, blood and tissue (liver and kidney) samples were collected for various biochemical assays.

Blood was collected for estimation of hematological parameters. The serum was separated from the blood by centrifugation at 1,500 x \text{g} for 15 minutes and used for estimation of total protein, antioxidants and lipid peroxidation.

Immediately after sacrifice, liver and kidney tissues were washed with ice cold saline. The tissues were then cut into fragments and homogenized with 3 volumes (w/v) of the appropriate buffer using a Potter-Elvehjam homogenizer with a Teflon pestle and centrifuged at 12000 x \text{g} for 20 minute at 4\degree C. The supernatant was used for the biochemical estimations. A portion of the tissues was used for histological studies.
8.2.11. Estimation of haematological parameters

8.2.11.1. Enumeration of white blood corpuscles (WBC)

The total white blood cells were enumerated according to the method adopted from Ramnath and Kuttan (2000).

Reagents

Turk’s fluid (WBC diluting fluid)

Procedure

Using a white blood cell pipette of haemocytometer, well mixed blood was drawn up to 0.5 mark and WBC diluting fluid was taken up to the 11 mark (dilution of 1 to 20). The fluid blood mixture was well shaken and transferred onto the counting chamber. The cells were allowed to settle to the bottom of the chamber for 2 minutes. Care should be taken that the fluid does not get dried.

Using 10 X or low power objective, the WBC’s were counted uniformly in the larger corner squares.

The cells were expressed as number of cells x10⁹/L.

8.2.11.2. Enumeration of red blood corpuscles (RBC)

The total red blood corpuscles were enumerated according to the method of Ramnath and Kuttan (2000).

Reagents

RBC diluting fluid
Procedure

Using a red blood cell pipette of haemocytometer, well mixed blood was drawn up to 0.5 mark and RBC diluting fluid was taken up to the 101 mark (dilution of 1 to 200). The fluid blood mixture was shaken and transferred onto the counting chamber. The cells were allowed to settle to the bottom of the chamber for 2 minutes. Care should be taken that the fluid does not get dried. Using 45x or high power objective, the RBC’s were counted uniformly in the larger corner squares.

The cells were expressed as number of cells $\times 10^{12}/L$ or $\times 10^6$ / cu.mm.

8.2.11.3. Differential Leukocyte Count

Differential Leukocyte count was determined by the method of Ramnath and Kuttan (2000).

Reagent

Leishmann’s stain: 150mg of powdered Leishmann’s stain was dissolved in 133mL of acetone free methanol

Procedure

A thin film of blood over a glass slide, stained with Leishmann’s stain was examined under oil immersion and the different types of WBC’s were identified. The percentage distribution of these cells was then determined. Smears were made from anticoagulant blood specimens and stained with Leishmann’s stain. The slides were preserved for counting the number of lymphocytes and neutrophils in 100 cells.

The number of neutrophils was expressed as percentage (%)
8.2.11.4. Estimation of Hemoglobin

Haemoglobin in whole blood was determined by the method adopted from Ramnath and Kuttan (2000).

Principle

Haemoglobin is converted into acid haematin by the action of HCl. The acid haematin solution is further diluted with distilled water until its colour matches with exactly that of permanent standard of comparator block. The Hb concentration is read directly from the calibration tube.

Requirements

1. Sahli’s haemoglobinometer (comparator)
2. Diluted hydrocholoric acid (1:10 diluted HCl)
3. Hemoglobin pipette with rubber tubing and mouthpiece
4. Distilled water

Procedure

By using pipette, added 0.1 N HCl in the haemoglobinometer up to the lowest marking. 20µl of blood was drawn up to the 20µl mark in the Sahli’s pipette and the blood column was carefully adjusted without bubbles. The excess of blood on the sides of the pipette was wiped by using a dry piece of cotton. The blood was blown into the diluted acid solution in the graduated tube and mixed well by slowly pipetting in and out. It was placed near the standard in the haemoglobinometer and allowed to stand at room temperature of 10 minutes. Few drops of distilled water was added carefully to this and mixed well until the colour is matched with that of standard in the comparator.

The lower meniscus reading of the fluid was noted and expressed in g/100mL.
8.2.12.  Estimation of lipid peroxidation

8.2.12.1.  Thiobarbituric acid reactive substances (TBARS)

The levels of lipid peroxidation in tissues were determined by the method of Ohkawa \textit{et al.} (1979).

**Principle**

Malondialdehyde and other TBARS are quantitated by their reactivity with thiobarbituric acid (TBA) in acidic conditions. The reaction generates a pink coloured chromophore, which can be read in a colorimeter at 535 nm.

**Reagents**

1. 8.1% Sodium dodecyl sulphate (SDS)
2. 20% Acetic acid, adjusted to pH 3.5 with NaOH
3. 0.8% TBA
4. n-Butanol and pyridine mixture (15:1, v/v)
5. Stock MDA solution: 1, 1’, 3, 3’-tetramethoxypropane (184 µg/mL)

**Procedure**

To 0.1 mL of tissue homogenate, 0.2 mL SDS, 1.5 mL acetic acid solution and 1.5 mL aqueous solution of TBA were added. The mixture was made up to 4 mL with distilled water and heated in a water bath at 95°C for 60 minute. After cooling with tap water, 1 mL of distilled water and 5.0 mL of a mixture of n- butanol and pyridine were added and shaken vigorously. After centrifugation at 4000×g for 10 minute, the organic layer was removed and its absorbance at 532 nm was measured.

Values are expressed as mM/ mg tissue.
8.2.13. Determination of antioxidants

8.2.13.1. Superoxide dismutase (SOD, EC 1.15.1.1)

Superoxide dismutase was assayed by the method of Kakkar et al (1984).

Principle

The assay is based on the inhibition of NADH-phenazine methosulphate nitroblue tetrazolium formazan formation. The reaction is initiated by the addition of NADH. After 90 sec incubation, the reaction is stopped by adding glacial acetic acid. The colour developed at the end of the reaction is extracted into n-butanol layer and the absorbance was measured at 520 nm.

Reagents

1. Sodium pyrophosphate buffer, 0.052 M, pH 8.3
2. Absolute ethanol
3. Chloroform
4. n-butanol
5. Phenazine methosulphate (PMS), 186 µM
6. Nitroblue tetrazolium (NBT), 300 µM
7. Reduced nicotinamide adenine dinucleotide (NADH), 780 µM

Procedure

Tissues were homogenized in sodium pyrophosphate buffer. 0.5 mL serum or tissue homogenate, 1 mL water followed by 2.5 mL ethanol and 1.5 mL chloroform (chilled reagents) were added, shaken for 90 sec at 4°C
and then centrifuged. The enzyme activity in the supernatant was determined as follows. The assay mixture contained 1.2 mL of sodium pyrophosphate buffer, 0.1 mL PMS, 0.3 mL NBT and appropriately diluted enzyme preparation in a total volume of 3 mL. The reaction was started by the addition of 0.2 mL NADH. After incubation at 30°C for 90 seconds, the reaction was stopped by the addition of 1 mL glacial acetic acid. The reaction mixture was stirred vigorously, shaken with 4 mL n-butanol and was allowed to stand for 10 minutes, centrifuged and the n-butanol layer was separated. The color intensity of the chromogen in n-butanol layer was measured in a colorimeter at 520 nm. A system devoid of enzyme served as control.

The enzyme concentration required to produce 50% inhibition of chromogen formation in one minute under standard conditions was taken as one unit. The specific activity of the enzyme was expressed as enzyme required for 50% inhibition of NBT reduction / minute / mg protein.

8.2.13.2. Catalase (CAT, EC 1.11.1.6)

The activity of catalase was determined in serum and tissue homogenate by the method of Sinha (1972).

Principle

Dichromate in acetic acid was reduced to chromic acetate, when heated in the presence of H₂O₂ with the formation of perchromic acid as an unstable intermediate. The CAT preparation is allowed to split H₂O₂ for different periods of time. The reaction is stopped at different time intervals by the addition of dichromate-acetic acid mixture in hot conditions. The remaining H₂O₂ forms H₂O₂-chromic acetate which can be determined colorimetrically at 590 nm.
Reagents

1. Phosphate buffer, 0.01 M, pH 7.0
2. Hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), 0.2 M
3. 5% Potassium dichromate (w/v)
4. Dichromate-acetic acid reagent: 5% Potassium dichromate and glacial acetic acid were mixed in the ratio of 1:3. From this, 1.0 mL was diluted again with 4.0 mL acetic acid.
5. Standard H\textsubscript{2}O\textsubscript{2}: 0.2 mM

Procedure

Tissue homogenate was prepared in phosphate buffer. To 0.9 mL phosphate buffer, 0.1 mL serum or tissue homogenate and 0.4 mL H\textsubscript{2}O\textsubscript{2} were added. The reaction was arrested after 15, 30, 45 and 60 sec by adding 2.0 mL dichromate-acetic acid mixture. The tubes were kept in a boiling water bath for 10 minute, cooled and the colour developed was read at 590 nm. Standards in the concentration range of 20-100 µM were processed for test.

The specific activity of the enzyme was expressed as µmoles µM of H\textsubscript{2}O\textsubscript{2} utilized /minute/mg protein.

8.2.13.3. Glutathione peroxidase (GPx, EC 1.11.1.9)

The activity of GPx was assayed in serum and tissue homogenate by the method of Rotruck et al. (1973).

Principle

A known amount of enzyme preparation is allowed to react with H\textsubscript{2}O\textsubscript{2} and GSH for a specified time period. The GSH content remaining after the reaction is measured by Ellman’s reaction.
2 GSH + H$_2$O$_2$ $\xrightarrow{\text{GPx}}$ GSSG + 2 H$_2$O

Reagents

1. Tris-HCl buffer, 0.4 M, pH 7.0
2. Sodium azide solution, 10 mM
3. 10% TCA
4. EDTA, 0.4 mM
5. H$_2$O$_2$, 1.0 mM
6. Reduced glutathione (GSH), 2.0 mM

Procedure

To 0.2 mL of Tris-HCl buffer, 0.2 mL EDTA, 0.1 mL of sodium azide and 0.2 mL enzyme preparation (serum or tissue homogenate) were added and mixed well. To this 0.2mL GSH followed by 0.1 mL of H$_2$O$_2$ were added. The contents were mixed well and incubated at 37°C for 10 minute along with a tube containing all reagents except the homogenate. After 10 minute the reaction was arrested by the addition of 0.5mL of 10% TCA. The tubes were centrifuged and the supernatant was assayed for GSH colorimetrically at 340 nm.

The activities are expressed as µmoles of GSH utilized/minute/mg protein for tissues.

8.2.14. Estimation of protein

The protein content of tissue homogenate and serum were estimated by the method of Lowry et al. (1951).
Principle

Proteins react with Folin-Ciocalteau reagent to give a coloured complex. The colour so formed is due to the reaction of alkaline copper with protein and the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. The intensity of the colour depends on the amount of these aromatic amino acids present.

Reagents

1. 10% TCA
2. NaOH, 0.5N
3. Alkaline copper reagent
   Solution A: 2% sodium carbonate in 0.1N NaOH
   Solution B: 0.5% copper sulphate in water
   Solution C: 1% sodium potassium tartarate in water
   50 mL of solution A was mixed with 0.5 mL of solution B and 1 mL of solution C just before use
4. Folin - Ciocalteau reagent: 100 g sodium tungstate, 25 g sodium molybdate, 700 mL water, 50 mL 85% O-phosphoric acid and 100 mL conc. HCl were taken in a 1500 mL round bottomed flask. The mixture was refluxed gently for 10 h. To this, 150 g lithium sulphate, 50 mL water and a few drops of bromine were added. The mixture was boiled for 15 minute to remove excess bromine. This was diluted in the ratio 1:2 with double distilled water just before use
5. Stock standard: A stock solution was prepared by dissolving 100 mg bovine serum albumin (BSA) in 100mL water in a standard flask. Small quantities of NaOH were added to complete the dissolution of BSA.
6. Working standard: 10 mL of the stock was diluted to 100 mL to obtain a working standard of concentration 100 µg/mL

Procedure

An aliquot of serum or tissue homogenate was diluted to 1.0 mL with saline, then 1.0 mL TCA was added. The mixture was centrifuged, supernatant discarded and the precipitate was dissolved in 1 mL NaOH. From this, aliquots were taken for the estimation. 4.5 mL alkaline copper reagent was added and the contents were allowed to stand at 37°C for 10 minutes. Then 0.5 mL dilute Folin-Ciocalteau reagent was added and mixed. A series of standards of concentration ranging from 20-100 µg and a blank were processed in the same way as that of the test. The blue colour developed was read at 620 nm after 20 minutes.

The amount of total protein is expressed as mg/g tissue.

8.2.15. Histopathological observations

The liver and kidney segments were dissected, fixed immediately in 10% formalin and paraffin-embedded, sectioned and stained with hematoxylin and eosin (H&E) for various histopathological observations. Histological changes were observed in the stained sections of liver and kidney under a light microscope at 40x magnification.

8.2.16. Statistical analysis

All the data were expressed as means ± SD. Data were analyzed by one-way analysis of variance (ANOVA) and the significant difference among treatment groups were evaluated by Duncan’s Multiple Range Test (DMRT). The results were considered statistically significant at p<0.05. All statistical analysis were made using SPSS 11.0 software package (SPSS, Tokyo, Japan).
8.3 RESULTS

The chemoprotective effect of ferulic acid (FA) during cisplatin chemotherapy was studied in Dalton’s lymphoma bearing mice.

Figure 8.1 shows morphological variation of tumor control and FA and/or cisplatin treated mice. The body size of the DLA (group 2) control mice was found to be increased with an increase in the peritoneal size compared to the normal mice. In group 3 (FA treated) and group 4 (cisplatin treated) mice, the size of the peritoneum was decreased due to treatment. A further decrease in size of the animal was observed in group 5, which was treated with both FA and cisplatin.
Figure 8.1. Effect of FA and/or cisplatin on morphological changes of mice induced with Dalton’s lymphoma
Table 8.2. Effect of FA and/or cisplatin on increase in body weight of control and experimental mice

Table 8.2 summarizes the effect of FA on the body weight of DLA induced mice treated with cisplatin. It was found that the percentage increase in body weight of DLA alone induced mice (DLA control) increased significantly \((p<0.05)\) when compared to the control mice, whereas treatment with cisplatin and FA decreased the body weight significantly \((p<0.05)\) when compared to the DLA control mice. A more pronounced effect on body weight decline was observed in mice treated with both FA and cisplatin, which was close to that of normal group mice.
### Table 8.3. Effect of FA and/or cisplatin on mean life span of control and experimental mice

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>MEAN LIFE SPAN (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLA Control</td>
<td>18.78 ± 1.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA + FA</td>
<td>24.66 ± 2.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA + Cisplatin</td>
<td>22.14 ± 2.25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA + Cisplatin + FA</td>
<td>35.04 ± 1.46&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are presented as the means ± SD; n=6
<sup>a-c</sup> p<0.05 the values not sharing a common superscript letter are significantly different

### Table 8.4. Effect of FA and/or cisplatin on peritoneal ascitic fluid volume of control and experimental mice

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>PERITONEAL ASCITIC FLUID VOLUME (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLA Control</td>
<td>9.59 ± 1.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA + FA</td>
<td>6.20 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA + Cisplatin</td>
<td>4.49 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA + Cisplatin + FA</td>
<td>1.99 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are presented as the means ± SD; n=6
<sup>a-d</sup> p<0.05 the values not sharing a common superscript letter are significantly different
Table 8.3 showed the effect of FA and/or cisplatin on mean life span in DLA induced ascitic tumor. The average life span of DLA tumor control was found to be between 18-19 days. Treatment with either FA or cisplatin significantly (p<0.05) increased life span of about 22-25 days, when compared to DLA control mice. It was observed that when DLA mice were treated with both FA and cisplatin, the average life span was increased more than 35 days, which was significantly (p<0.05) higher when compared to other group mice.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>CELL VIABILITY (x10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLA Control</td>
<td>65.99 ± 4.75^a</td>
</tr>
<tr>
<td>DLA + FA</td>
<td>8.01 ± 0.38^b</td>
</tr>
<tr>
<td>DLA + Cisplatin</td>
<td>7.05 ± 0.45^b</td>
</tr>
<tr>
<td>DLA + Cisplatin + FA</td>
<td>4.45 ± 0.28^c</td>
</tr>
</tbody>
</table>

Data are presented as the means ± SD ; n=6

a-c p<0.05 the values not sharing a common superscript letter are significantly different

**Table 8.5. Effect of FA and cisplatin on DLA Cell viability by Trypan blue staining**

Table 8.5 shows the effect of FA and/or cisplatin on cell viability in DLA induced ascitic tumor. The viability of DLA cells was significantly (p<0.05) decreased on mice treated with either FA or cisplatin or both when compared to DLA control mice.
<table>
<thead>
<tr>
<th>GROUPS</th>
<th>RBC (1x10^6/mm³)</th>
<th>Total WBC (1x10^3/mm³)</th>
<th>Hb (g%)</th>
<th>Platelets (10^3/mm³)</th>
<th>Packed Cell Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.65 ± 0.97a</td>
<td>10.99 ± 0.63a</td>
<td>13.79 ± 0.39a</td>
<td>180.13 ± 13.62a</td>
<td>0.25 ± 0.01a</td>
</tr>
<tr>
<td>DLA Control</td>
<td>4.22 ± 0.15b</td>
<td>14.32 ± 0.36b</td>
<td>8.62 ± 0.52b</td>
<td>132.00 ± 11.11b</td>
<td>0.92 ± 0.07b</td>
</tr>
<tr>
<td>DLA + FA</td>
<td>7.99 ± 0.63c</td>
<td>11.65 ± 0.47a,c</td>
<td>10.50 ± 0.38c</td>
<td>183.04 ± 18.72a</td>
<td>0.50 ± 0.03c</td>
</tr>
<tr>
<td>DLA + Cisplatin</td>
<td>6.65 ± 0.36d</td>
<td>12.32 ± 0.36c</td>
<td>9.35 ± 0.46d</td>
<td>151.67 ± 14.60c</td>
<td>0.65 ± 0.05d</td>
</tr>
<tr>
<td>DLA + Cisplatin + FA</td>
<td>10.01 ± 0.63a</td>
<td>11.26 ± 0.97a,c</td>
<td>12.65 ± 0.81e</td>
<td>186.22 ± 16.74a</td>
<td>0.33 ± 0.02e</td>
</tr>
</tbody>
</table>

Data are presented as the means ± SD; n=6

\(^{a-e} p<0.05\) the values not sharing a common superscript letter are significantly different

**Table 8.6. Effect of FA and/or cisplatin on hematological parameters of control and experimental mice**
As shown in table 8.6, RBC and Hb were significantly (p<0.05) decreased whereas, WBC was significantly (p<0.05) increased in the DLA control group (group 2) when compared to the normal group (group1). Treatment with FA and/or CDDP (groups 3-5) significantly increased the Hb content and RBC but significantly decreased the WBC count to near normal levels.

The platelet counts were significantly (p<0.05) decreased and the packed cell volume (PCV) was significantly (p<0.05) increased in DLA control mice when compared to the control mice which clearly indicated the diseased condition. On cisplatin alone treatment (group 4), the platelet counts were slightly increased. But in the presence of FA (in groups 3 and 5), the platelet counts were significantly (p<0.05) increased and were close towards normal values. This pattern of changes was reversed for PCV.
<table>
<thead>
<tr>
<th>GROUPS</th>
<th>LYMPHOCYTES (%)</th>
<th>NEUTROPHILS (%)</th>
<th>EOSINOPHILS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>69.44 ± 2.39a</td>
<td>35.63 ± 2.99a</td>
<td>6.51± 0.40a</td>
</tr>
<tr>
<td>DLA Control</td>
<td>28.37 ± 9.56b</td>
<td>57.23 ± 3.40b</td>
<td>3.42 ± 0.28b</td>
</tr>
<tr>
<td>DLA + FA</td>
<td>66.72 ± 4.67a</td>
<td>41.74 ± 3.62c</td>
<td>6.00 ± 0.53a</td>
</tr>
<tr>
<td>DLA + Cisplatin</td>
<td>64.00 ± 5.66a</td>
<td>38.24 ± 1.90a</td>
<td>6.37 ± 0.43a</td>
</tr>
<tr>
<td>DLA + Cisplatin + FA</td>
<td>65.83 ± 6.66a</td>
<td>36.90 ± 2.35a</td>
<td>6.51 ± 0.36a</td>
</tr>
</tbody>
</table>

Data are presented as the means ± SD; n=6

a–c p<0.05 the values not sharing a common superscript letter are significantly different

Table 8.7. Effect of FA and/or cisplatin on WBC differential count of control and experimental mice

In DLA alone induced mice, lymphocyte and eosinophil counts were decreased whereas the neutrophil count was increased significantly (p<0.05) when compared to control mice. On treatment of DLA induced mice with cisplatin alone, the condition was reversed slightly but when treated with both FA and cisplatin, the levels of lymphocytes, eosinophils and neutrophils were significantly (p<0.05) altered to that of the normal values. Moreover, treatment with FA and cisplatin showed enhanced effects than treatment with cisplatin alone in table 8.7.
### Table 8.8. Effect of FA and/or cisplatin on total protein levels of control and experimental mice

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>LIVER</th>
<th>KIDNEY</th>
<th>SERUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>$16.42 \pm 1.20^a$</td>
<td>$15.47 \pm 1.09^a$</td>
<td>$9.69 \pm 0.69^{a,c}$</td>
</tr>
<tr>
<td>DLA Control</td>
<td>$10.27 \pm 1.08^b$</td>
<td>$11.37 \pm 1.06^b$</td>
<td>$14.99 \pm 0.09^b$</td>
</tr>
<tr>
<td>DLA + FA</td>
<td>$13.56 \pm 1.14^c$</td>
<td>$14.92 \pm 1.08^a$</td>
<td>$10.25 \pm 1.58^c$</td>
</tr>
<tr>
<td>DLA + Cisplatin</td>
<td>$14.68 \pm 1.21^c$</td>
<td>$14.37 \pm 1.15^a$</td>
<td>$4.12 \pm 0.07^d$</td>
</tr>
<tr>
<td>DLA + Cisplatin + FA</td>
<td>$14.87 \pm 1.09^c$</td>
<td>$15.02 \pm 1.32^a$</td>
<td>$8.92 \pm 0.46^a$</td>
</tr>
</tbody>
</table>

Data are presented as the means ± SD; n=6

a-d p<0.05 the values not sharing a common superscript letter are significantly different

The effect of FA and cisplatin on total protein content in serum, liver and kidney tissues of DLA induced mice was shown in table 8.8. The total protein content in the liver and kidney tissues of DLA alone induced mice was significantly (p<0.05) decreased than the control group mice. Treatment with both FA and cisplatin (group 5) significantly (p<0.05) increased the protein value when compared to cisplatin alone treatment (group 4).

Whereas the serum of DLA alone induced mice (group 2) showed a significant increase in total protein level when compared to the normal control mice (group 1). Treatment of DLA induced mice with FA and/or cisplatin (groups 3, 4 and 5), significantly reversed this effect.
<table>
<thead>
<tr>
<th>GROUPS</th>
<th>LIVER</th>
<th>KIDNEY</th>
<th>SERUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.17 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.02 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.74 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA Control</td>
<td>1.88 ± 0.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.33 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.34 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA + FA</td>
<td>4.04 ± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.77 ± 0.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.77 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA + Cisplatin</td>
<td>3.44 ± 0.37&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.97 ± 0.14&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.63 ± 0.16&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA + Cisplatin + FA</td>
<td>3.79 ± 0.21&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>3.13 ± 0.21&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.76 ± 0.30&lt;sup&gt;a,c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as 50% inhibition of Nitroblue tetrazolium / minute / mg protein.
Data are presented as the means ± SD; n=6
<sup>a-d</sup> p<0.05 the values not sharing a common superscript letter are significantly different.

**Table 8.9. Effect of FA and/or cisplatin on the activities of superoxide dismutase (SOD) in control and experimental mice**
<table>
<thead>
<tr>
<th>GROUPS</th>
<th>LIVER</th>
<th>KIDNEY</th>
<th>SERUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>54.87 ± 4.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.74 ± 1.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.84 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLAControl</td>
<td>26.39 ± 1.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.22 ± 1.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.33 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA + FA</td>
<td>52.99 ± 1.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.33 ± 2.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.38 ± 0.27&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA + Cisplatin</td>
<td>47.29 ± 2.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.47 ± 1.97&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.66 ± 0.20&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA + Cisplatin + FA</td>
<td>51.69 ± 3.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.97 ± 1.36&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.86 ± 0.24&lt;sup&gt;a,c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as µmoles of H<sub>2</sub>O<sub>2</sub> utilized / minute / mg protein.

Data are presented as the means ± SD; n=6

<sup>a-d</sup> p<0.05 the values not sharing a common superscript letter are significantly different

**Table 8.10. Effect of FA and/or cisplatin on the activities of catalase (CAT) in control and experimental mice**
<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Glutathione peroxidase (GPx)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LIVER</td>
</tr>
<tr>
<td>Control</td>
<td>6.25 ± 0.54&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA Control</td>
<td>3.14 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA + FA</td>
<td>6.15 ± 0.71&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA + Cisplatin</td>
<td>4.78 ± 0.31&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA + Cisplatin + FA</td>
<td>6.01 ± 0.40&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as µM of glutathione utilized / minute / mg protein)
Data are presented as the means ± SD; n=6
<sup>a-c</sup> p<0.05 the values not sharing a common superscript letter are significantly different

Table 8.11. Effect of FA and/or cisplatin on the activities of glutathione peroxidase (GPX) in control and experimental mice
<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Reduced glutathione (GSH)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LIVER</td>
<td>KIDNEY</td>
<td>SERUM</td>
</tr>
<tr>
<td>Control</td>
<td>3.52 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.96 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.36 ± 0.09&lt;sup&gt;a,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA Control</td>
<td>0.92 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.73 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.09 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA + FA</td>
<td>3.00 ± 0.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.65 ± 0.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.45 ± 0.29&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA + Cisplatin</td>
<td>1.75 ± 0.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.87 ± 0.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.34 ± 0.10&lt;sup&gt;a,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA + Cisplatin + FA</td>
<td>2.66 ± 0.14&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.14 ± 0.17&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.38 ± 0.26&lt;sup&gt;a,c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mM / mg protein, Data are presented as the means ± SD; n=6
<sup>a–c</sup> p<0.05 the values not sharing a common superscript letter are significantly different

Table 8.12. Effect of FA and/or cisplatin on the levels of reduced glutathione (GSH) in control and experimental mice
Figures 8.9 – 8.12 shows the effect of FA and/or CDDP on the levels of enzymic (SOD, CAT and GPx) and non-enzymic (GSH) antioxidants in the circulation (serum) and tissues (liver and kidney). The inoculation of DLA cells in mice caused a significant (p<0.05) decrease in the levels of SOD, CAT, GPx and GSH in both circulation and tissues. Treatment of DLA induced mice with cisplatin alone (group 4) significantly (p<0.05) increased the levels of enzymic and non-enzymic antioxidants when compared to DLA control mice (group 2). Whereas treatment of DLA control mice with both FA and cisplatin further increased the circulatory and tissue antioxidant levels significantly (p<0.05) when compared to tumor control (group 2) and cisplatin alone treated (group 4) mice. Tumor control mice treated with FA alone significantly (p<0.05) increased the antioxidant levels when compared to tumor control mice and other treatment groups with values near to that of the normal values.

Table 8.13 shows the effect of FA and/or cisplatin on lipid peroxidation in circulation (serum) and tissues (liver and kidney) of DLA induced mice. DLA control (group 2) mice showed a significant (p<0.05) increase in lipid peroxidation indices when compared to control mice. Treatment with FA and/or cisplatin (in groups 3-5) showed a significant decrease in TBARS level when compared to DLA control mice (group 2).
<table>
<thead>
<tr>
<th>GROUPS</th>
<th>LIVER</th>
<th>KIDNEY</th>
<th>SERUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.91 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.57 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.10 ± 0.02&lt;sup&gt;a,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA Control</td>
<td>2.59 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.13 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.24 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA + FA</td>
<td>1.00 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.83 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.08 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA + Cisplatin</td>
<td>1.82 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.02 ± 0.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.14 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>DLA + Cisplatin + FA</td>
<td>1.29 ± 0.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.98 ± 0.08&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>0.11 ± 0.01&lt;sup&gt;a,c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table 8.13. Effect of FA and/or cisplatin on the levels of Thiobarbituric acid reactive substances (TBARS) in control and experimental mice

Values are expressed as M/ mg protein; Data are presented as the means ± SD; n=6
<sup>a-d</sup> p<0.05 the values not sharing a common superscript letter are significantly different
Figures 8.2. and 8.3. shows changes in the histology of liver and kidney of control and experimental mice. In DLA induced mice the liver and kidney histology was distorted indicating liver and kidney damage. Treatment with FA and/or cisplatin reversed the histological pattern towards that of control tissues.

In figure 8.2, the liver histology is distorted and abnormal cells in the Daltons lymphoma control. FA or cisplatin treatment shows high lymphocyte aggregation with hepatocytes reverting back to normalcy. The FA and cisplatin treatment reveals the liver histology close to that of the normal one.

In figure 8.3, control sections showed normal renal parenchyma with glomeruli and tubules. No atypia or neoplasm made out in the samples studied. The tumor control animals exhibited atrophied glomeruli and dilated renal tubules. The treatment brought back the glomeruli and renal tubules to normal. In particular, the FA and cisplatin treatment showed normal histological appearance with no frank renal damage.
Figure 8.2. Microscopic images on changes in the liver histology of FA and/or cisplatin treated DLA induced mice (40x)
Figure 8.3. Microscopic images on changes in the kidney histology of FA and/or cisplatin treated DLA induced mice (40x)
8.4. DISCUSSION

The use of chemotherapeutic drugs in cancer therapy involves the risk of life threatening host toxicity. This is one of the important limitations of cancer chemotherapy using cytotoxic chemotherapeutic drugs like cisplatin. Introduction of plant based anticancer substances like polyphenols in conventional cisplatin therapy may reduce the side effects of it. The protective effects of FA introduced along cisplatin therapy in the present study are discussed here.

The morphological changes of the experimental mice showed an increase in the size of the DLA tumor bearing mice when compared to control mice. This may be due to the accumulation of ascitic fluid in its peritoneal space. Ascitic fluid is the direct nutritional source for tumor cells and a rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells (Prasad and Giri 1994). Treatment with FA and cisplatin showed a decrease in the size of the mice when compared to the tumor bearing mice and cisplatin alone treated mice which may be due to the reduction in the accumulation of ascitic fluid in the peritoneum.

Moreover, the number of viable tumor cells was also found to be decreased significantly in FA and cisplatin treated groups than the cisplatin alone treatment. FA alone treatment also reduced cell viability which confirmed its anticancer effect. Reduction in the number of viable DLA cells led to the reduction in ascitic fluid and hence the body weight and size of the mice. This indicated a direct cytotoxic effect of FA and cisplatin on DLA tumor cells as evidenced by the in vitro studies (Phase II). The effects of both FA and cisplatin treatment was better when compared to FA or cisplatin alone treatment which may be due to the synergistic effect of FA and cisplatin.
The reliable criterion for judging the value of any anticancer drug is the prolongation of lifespan of the animal (Clarkson and Burchenal 1965). The average life span of tumor bearing mice was found to be around 18 - 19 days. Treatment with FA and cisplatin increased the survival time (more than 30 days) of DLA mice, when compared to FA or cisplatin alone treatment (21 - 25 days). This may be due to the enhanced anticancer activity of FA and cisplatin. Reduction in the number of viable cells or increased cytotoxicity of DLA cells by FA and cisplatin would also have contributed to the increased life span.

Hemolytic anemia is one of the side effects of cisplatin which may be due to the abnormal decrease in RBCs due to breakdown. Dalton’s ascites lymphoma caused a significant reduction in RBC, Hb and Platelet levels and increase in WBC and packed cell volume. Treatment with cisplatin alone slightly reversed these effects but to a lesser effect than in FA alone treatment. This may be due to the fact that cisplatin affects the hematological parameters during treatment. Anemia is a common complication in cancer and the situation aggravates further during chemotherapy since a majority of antineoplastic agents including cisplatin exert suppressive effects on erythropoiesis (Hoagland 1982). But treatment with both FA and cisplatin resulted a significant reversal on the levels of the hematological parameters when compared to cisplatin alone treatment and DLA tumor control. The significant restoration in the hematological profile of cisplatin treated and tumor bearing mice could be possibly due to the protective action of FA on the haemopoietic system (Alias et al. 2009). Reports have shown that our body’s immune system can be benefitted from FA (Srinivasan et al. 2007). Hence, the significant improvement in the total WBC and WBC differential counts by FA in cisplatin treated tumor mice suggested the immunomodulatory role of FA.
The cytotoxicity of cisplatin and other chemotherapeutic agents is effective on malignant as well as all the rapidly dividing cells. Hair loss (‘Alopecia’) is one of the side effects of cisplatin. The morphology of animals revealed signs of hair loss (figure 8.1) which may be due to the effect of cisplatin on actively dividing hair follicles (Yanez et al. 2003). Treatment with FA and cisplatin reduced weight gain reduced the hair loss caused by cisplatin treatment and produced a normal hair texture (figure 8.1). This suggested the protective effect of FA over actively diving normal cells of the host. Thus, from the above results it is clear that the side effects of cisplatin over the haematopoietic system during therapy can be balanced by FA.

It was reported that the presence of tumors in the human body or in an experimental animal is known to affect many functions of the organs like liver and kidney. The increase in the level of TBARS indicated enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defense mechanisms to prevent the formation of excess free radicals. These free radicals damage the lipid components of the cell membrane by peroxidation and denature proteins, which lead to enzymatic inactivation. Free radicals can also cause mitochondrial dysfunction (Yilmaz et al. 2004). Malondialdehyde, the end product of lipid peroxidation was also reported to be higher in carcinomatous tissue than in non-diseased organs. Moreover, cisplatin increases free radical production by inducing glucose-6-phosphate dehydrogenase and hexokinase activity (Yilmaz et al. 2004). Treatment with FA and cisplatin showed a significant decrease in the level of TBARS when compared to tumor bearing mice and cisplatin alone treated mice. This indicated the reduction in free radical yield and subsequent decrease in harm and damage to the cell membrane and decrease in MDA production. The radical scavenging activity of FA would have played a role in reducing the lipid peroxidation of FA and cisplatin treated mice (Sudheer et al. 2007).
Antioxidants level was decreased in the DLA tumor control mice in the present study. As lymphoma progresses, the antioxidants like SOD, CAT, GPx and GSH were seriously affected by the ROS (Ruby et al. 1995 and Ruby et al. 1996). The decrease in activities of SOD, CAT and GPx in liver and kidney may be either due to the direct inactivation of these enzymes (Thomas and Kamat, 1999) or by the decreased aerobic metabolism in lymphoma bearing cells (Greenstein 1954). In the present study, decreased antioxidant levels were observed in the cisplatin treated tumor mice. Reports have shown that the antioxidant enzymes SOD, CAT and GPX are inhibited by cisplatin (Badary et al. 2005; Durak et al. 2002; Yilmaz et al. 2004).

GSH, a strong intracellular antioxidant, is involved in various protective functions in the cells and in the synthesis of nucleic acids (Wang and Ballatori 1998). Decreased levels of anti-oxidants GSH may be due to the damage of the cells (Chatterjee 1998). GSH may act as a safeguarding agent against cisplatin-induced toxic effects. Cisplatin has also been reported to be sufficiently electrophilic to interact with GSH directly (Eastman 1987) and the resulting GSH–platinum conjugates could be actively eliminated from the cells (Ishikawa and Ali-Osman 1993). The involvement of GSH in the metabolism of cisplatin has been reported (Suzuki and Cherian 1990). Previous studies of Khynriam and Prasad (2003) have also confirmed that cisplatin treatment lowered in GSH levels. In the present study we observed decreased levels of GSH in cisplatin treated DLA mice and DLA control mice.

FA is a strong dietary antioxidant (Baskaran et al. 2010). Treatment with FA and cisplatin significantly brought the anti-oxidant system to near normal levels when compared to cisplatin alone treated mice. This may be due to the antioxidant activity of FA employed in the combination. FA possesses distinct structural motifs (Fig. 1) that can also possibly contribute to its antioxidant property. The presence of electron donating groups on the
benzene ring (3-methoxy, and more importantly 4-hydroxyl) of FA gives additional resonance structures of the resulted phenoxy radical which contribute to the stability of this intermediate or even terminating free radical chain reactions (Graf 1992). The next functionality is the carboxylic acid group in FA with adjacent unsaturated double bond that can provide additional attack sites for free radicals and thus prevent them from attacking the membrane. In addition, the carboxylic acid group also acts as an anchor of FA by which it binds to the lipid bilayer providing some protection against lipid peroxidation. Thus the presence of electron donating substituents enhances the antioxidant properties of FA (Kanski et al. 2002). Despite the direct scavenging of ROS, FA can chelate the ferrous ion and decrease the formation of hydroxyl radical via inhibition of iron-dependent Fenton’s reaction (Zhang et al. 2003). Thus, FA effectively quenches the free radicals and prevents them from attacking the membrane and DNA and improves the antioxidant status.

The liver histology is distorted and abnormal cells were presented in the Dalton’s lymphoma control. Signs of liver damage were also observed in cisplatin treated liver. FA and cisplatin treatment brought back the liver histology close to that of the normal one. FA would have played a major role in protecting hepatic cells from therapy induced damage. Previous reports have also shown the protective role of FA in various studies. Rukkumani et al. (2004) showed that FA protected rats from liver damage. Sri Balasubashini et al. (2003) reported protective effects of FA on hyperlipidemic diabetic rats. In a study by Srinivasan et al. (2005), FA protected rats against toxicity induced by carbon tetrachloride. So, in the present study, the liver damage caused by both DLA tumor and cisplatin treatment was prevented by FA and cisplatin treatment because of FA.

One of the main side effects in cisplatin chemotherapy is nephrotoxicity. In the kidney tissues, the control sections showed normal renal
parenchyma with glomeruli and tubules. The tumor control mice exhibited atrophied glomeruli and dilated renal tubules. Cisplatin treated mice kidney showed increased renal damage when compared to other treatments. Oxidative stress injury is actively involved in the pathogenesis of cisplatin-induced acute kidney injury. Reactive oxygen species (ROS) such as superoxide anion (O$_2^\cdot$) (Davis et al. 2001), hydrogen peroxide (H$_2$O$_2$) (Kadikoylu et al. 2004) and hydroxyl radical (\textsuperscript{\cdot}OH) (Shino et al. 2003) which are increased in cisplatin treated kidneys directly act on cell membrane components including lipids, proteins and DNA and destroy their structure (Kawai et al. 2006). Moreover, the kidney accumulates cisplatin to a greater degree than other organs and is the major route for its excretion. The cisplatin concentration in proximal tubular epithelial cells is about 5 times the serum concentration (Kuhlmann et al. 1997). The disproportionate accumulation of cisplatin in kidney tissue contributes to cisplatin-induced nephrotoxicity (Arany and Safirstein, 2003).

The treatment of FA and cisplatin brought back the glomeruli and renal tubules to normal with no frank renal damage. FA because of its radical scavenging activity would have scavenged the active ROS formed due to cisplatin. FA, being an antioxidant would have replenished the antioxidants in kidney cells.

In the present study, administration of FA caused no clinical signs for toxicity, low survival, poor condition, or histological changes suggesting toxicity in the liver and kidney. Hence, it was clear that FA as such does not showed any toxicity or damaging effects on normal mice. Previous studies have also proved that administration of FA to experimental animals did not produce any harmful effects (Lesca et al. 1983; Kawabata et al. 2000; Sri Balasubashini et al. 2003; Srinivasan et al. 2005; Alias et al. 2009; Baskaran et al. 2010). On the whole, introduction of FA in cisplatin therapy protected the normal cells of the host from the side effects of cisplatin by
altering the hematological profile, increasing the life span of mice, increasing the cytotoxicity of tumor cells, decreasing therapy induced hair loss, protecting the haematopoetic system, modulating the immune system, scavenging toxic free radicals, balancing the antioxidant levels and protecting liver and kidney damage.

8.5. CONCLUSION

From the results of the Phase IV studies it was confirmed that ferulic acid protected the host from chemotherapy associated toxicities.