CHAPTER VII

EFFECT OF WITHANIA SOMNIFERA ON

CYCLOPHOSPHAMIDE INDUCED TOXICITY
1. Introduction

The non-selective damage to normal cells caused by chemotherapeutic agents has instigated investigations of methods to improve the therapeutic efficacy. One strategy is to increase the sensitivity of neoplastic cells. An alternative tactic would be to increase the resistance of normal cells to the effects of cytoidal therapy. In order that this approach to be successful, the protecting agent invest be selectively excluded from the malignant tissue. Many of the chemotherapeutic agents currently used in therapy are cytotoxic to normal cells and leads to unwanted side effects; mainly mediated through reactive oxygen species (64).

Cyclophosphamide is an alkylating agent activated to cytotoxic metabolites 4-OH- cyclophosphamide and a phosphoramide mustard by mixed function oxidases in hepatic microsomes (65). It has been shown that during this procedure it can produce free radicals. The product of free radicals could attack all the biological molecules leading to cellular death.

Immunosuppression is a major drawback in chemotherapy (66). It has also toxic side effects such as nausea, vomiting, mucosal, ulceration, alopecia; pulmonary fibrosis; cardiac and hepatic toxicity. Drugs that could reduce these side effects as well as stimulate the immunity will be of great help in improving cancer treatment strategies. Present chapter mainly deals with the ameliorative effect of *Withania somnifera* on cyclophosphamide induced toxicity.
II. Materials and Methods

II.1 Animals.

Swiss albino mice were purchased from the National Institute of Nutrition, Hyderabad, India. The animals were kept in air controlled rooms and fed with normal mouse chow and water ad libitum.

II.2 Chemicals.

Cyclophosphamide, Para rosiniline hydrochloride and α-naphyl acetate; Thiobarbituric acid, Giemsa stain and Harris Hematoxylin were used. Glutathione (GSH) and 5-5-dithiobis-2-nitrobenzoic acid (DTNB) (Ellman’s reagent), Sodium 2- mercapto ethane sulphoxide (mesna); total protein and blood urea kits were also used. All other chemicals used were of analytical reagent grade (Chapter 11).

II.3 Determination of the effect of Withania somnifera on the haematological parameters in mice after cyclophosphamide treatment.

Two groups of Swiss albino mice (10 mice/group, 20-25 gm) were used for this study. All the animals were treated with 10 doses of cyclophosphamide (25mg/kg. b.wt; i.p) on consecutive days. One group of animals were treated with 10 doses of Withania extract (20mg/dose/animal, i.p). Blood was collected from the caudal vein and parameters such as total WBC count (haemocytometer), differential count (Leishman’s stain), haemoglobin level (cyanmeth-
aemoglobin method) and the weight of the animals were recorded prior to the CTX treatment and continued on every third day for 30 days.

II.4 Determination of the effect of Withania somnifera on the bone marrow cellularity and \( \alpha \)-esterase positive cells.

Three groups of Swiss albino mice (6 mice/group) were used to carry out this study. Group one mice was treated with CTX (25mg/kg/b.wt.) as given above, group two was treated with CTX and Withania (20mg/dose/animal, i.p). Three mice from each group were sacrificed on 11th day and 15th day of treatment and the bone marrow was collected from the femur into medium containing 2% FCS. The number of bone marrow cells was counted using a haemocytometer and expressed as total live cells/femur.

A smear using the above bone marrow preparation was made on clean glass slides and stained with Harris Haematoxylin to determine the non specific \( \alpha \)-esterase activity according to the method of Bancroft and Cook (50) as described in chapter 11.

II.5 Determination of enzyme levels in serum and liver.

Glutamine pyruvate transaminase level of liver and serum were determined by the method of Bergmayer and Bernt (67); Alkaline phosphatase activity was determined according to King and Armstrong (68) and lipid peroxidation was determined by Yagi’s method (46).
11.6. Determination of the effect of *Withania somnifera* on IFN-γ, IL-2 and GM-CSF in CTX treated animals.

Three groups of animals (3 mice/group) were used for this study. Group 1 animals were kept in normal. Group 2 animals were treated with 10 doses of CTX (25mg/dose/animal i.p.) and group 3 animals were treated with 10 doses of Withania (20mg/dose/animal i.p.) and CTX (25mg/dose/animal i.p.). The animals were sacrificed 24h. after the last dose of drug treatment, blood was collected and serum separated. Cytokines - IFN-γ, IL-2 and GM-CSF were determined using ELISA kits.

11.7. Determination of the effect of *Withania somnifera* on CTX induced urotoxicity.

Three groups of Swiss Albino mice (24 animals/group) were used for the study. All the animals were treated with a single chronic dose of CTX (1.5m moles /kg b.wt.). Group 1 animals were treated with CTX alone and kept as control; GroupII animals were treated with Withania extract (20mg / dose / animal, i.p.) for 5 days prior to CTX administration and Group III animals were treated with a single dose of mesna (4.5m moles/animal) a known protector of urotoxicity. Eight animals from each group were sacrificed after 4h. 24h; 48h. of CTX administration. Body weight of the animals was recorded prior to the CTX administration and at the time of sacrifice. The animals were sacrificed and the urinary bladder and blood were collected. The relative organ weights of liver and bladder were recorded. Urine was collected before sacrificing the animals. Serum and urine were used for estimating total protein content (84) and urea N₂ (83). Morphological analysis of urinary bladder was performed by observing the inflammation,
colouration and size of the bladder by three different persons. A portion of liver and bladder were used for the estimation of GSH content (75) (Chapter II).

II.8 Histopathological analysis.

Urinary bladder and Intestine were fixed in 10% formaldehyde. After several treatment in different concentration of alcohol the dehydrated tissue was embedded in paraffin wax. Sections were cut and stained with hematoxylin and eosin and histopathological analysis was carried out. (Chapter -II)

III. Results

III.1 Effect of Withania somnifera on the body weight of CTX treated mice.

Body weight of the animals treated with CTX alone was found to be significantly reduced (-2.9gm) on 30th day while the animals treated with CTX along with Withania extract, body weight was found to be increased (+2.3g) on the same day (Fig.VII.1).

III.2 Effect of Withania somnifera extract on total WBC count.

Effect of Withania somnifera extract on total WBC count of CTX treated animals is given in Figure VII.2. Initially there was a decrease in the total WBC count of both CTX alone and CTX along with the Withania treated animals but later total WBC was found to be significantly higher in Withania treated group. On 12th day WBC count in the Withania treated group was 6120 cells/mm³ while in the CTX alone treated group it was only 3720 cells/mm³. The total
count in Withania treated group was normalised by 15th day while total WBC count in CTX alone treated group did not regain the normal level even after 30 days. These results indicate that administration of Withania extract could stimulate the haemopoietic system.

### III.3 Effect of *Withania somnifera* on the bone marrow cellularity after cyclophosphamide treatment.

The effect of Withania administration on the bone marrow cellularity is given in table VII.1. The bone marrow cell number on 11th day in the CTX alone treated animals was 5.6x10⁶ cells/femur while that of CTX in the presence of Withania was 10.9x10⁶ cells/femur. Bone marrow cellularity in CTX alone treated animals did not reach the normal value even after 15 days (8x10⁶ cells/femur) whereas Withania and CTX treated group showed a bone marrow cellularity of 13.1x10⁶ cells/ femur on the same day.

### III.4 Effect of *Withania somnifera* on α- esterase positive cells.

Effect of *Withania somnifera* on the number of α- esterase positive cells is given in table VII. 2. The number of α- esterase positive cells in the bone marrow of CTX treated animals was low (883/4000 cells) when compared to normal animals (1189/4000 cells). A significant increase in the α- esterase positive cells was observed in the bone marrow of CTX along with Withania treated group (1130/4000 cells).

### III.5 Effect of Withania extract on relative organ weight of mice.

Administration of Withania extract was shown to enhance the weight of the spleen and
thymus (Table VII.3). The spleen size and weight was significantly increased (P<0.001) in CTX + Withania treated group (1.11g/100 g.b.wt.). Similarly the weight of thymus was also increased (0.20g/100g.b.wt.) by the administration of Withania extract (P<0.001) compared to that of CTX treated animals (0.05g/100g.b.wt.). There was no effect on the weight of kidney or liver after Withania administration.

III.6 Effect of Withania somnifera on IFN-γ; IL-2 and GM-CSF in CTX treated animals.

Effect of Withania somnifera on IFN-γ, IL-2 and GM-CSF in CTX treated animals is given in Table VII.5. Administration of CTX lowered the IFN level to 30pg/ml which was significantly (p<0.001) enhanced to 74pg/ml by Withania treatment. When CTX was treated IL-2 level was lowered to 4.5pg/ml which was enhanced to 7.5 pg/ml after Withania administration. The lowered level of GM-CSF in the CTX alone treated animals (19.12pg/ml) were brought back to the normal levels (35.47 pg/ml) by Withania extract administration.

III.7 Effect of Withania on the intestinal architecture of CTX treated animals.

The intestinal villi architecture of mice treated with 10 doses of CTX in the presence and absence of Withania is shown in Fig. a,b,c,d and e. Twentyfour hours after the 10th dose of CTX treatment the intestinal villi of CTX alone treated group looked blunt; shortened and eroded; crypts were (Fig. b) non-uniform, fused and the numbers were reduced. Necrosis of mucous cell was observed. In the Withania treated group the crypts were not fused and no reduction in the number of villi seen (Fig. c). Villi looked slightly shortened; not destorted and there was no
necrosis of mucous cells, throughout the villi (Fig.c). On 15 th day after the first dose of CTX alone (Fig.d) treatment, villi were still eroded and damaged; necrosis in the mucous cells was observed in the CTX treated group. In combination with Withania, the intestinal villi architecture was completely normalised (Fig.e).

III.8 Effect of *Withania somnifera* on enzyme analysis.

There was no significant change in serum and liver GPT, alkaline phosphatase and lipid peroxidation by CTX administration and treatment with Withania extract did not alter the value (Table VII.4).

III.9 Effect of Withania extract on the morphology of urinary bladder after CTX administration.

After 4h of the chronic dose of CTX treatment, control animals showed inflamed bladder with noticeable red colouration whereas the CTX along with Withania treated group showed only a slight inflammation but normal colouration (Table VII.6). After 24h, the bladders showed severe haemorrhage and colouration in the control group whereas the Withania treated group showed normal bladder morphology. Even after 48h, the urinary bladder of CTX alone treated group was severely inflamed and dark coloured whereas those of the CTX along with Withania treated group was similar to that of normal.

III.10 Effect of *Withania somnifera* on total protein content in serum and urine of CTX treated animals.

Withania extract could lower the total protein levels in serum and urine which was
drastically enhanced by CTX administration. (Table VII 7-9). After 24h. serum protein of CTX along with Withania or mesna treated group were 7.1g/l and 5.9g/l respectively. After 24h. the protein content in the urine of CTX alone treated group was 10.2g/l whereas in the CTX along with Withania treated group, it was 7.2 g/l and the mesna treated group it was 5.8 g/l.


Analysis of the serum of the animals treated with CTX along with Withania showed normal blood urea N₂ levels (Table VII.7-9). The mesna treated group also retained a normal level of blood urea N₂ level at there time points. Urea as well as urea N₂ level in the urine of CTX in the presence of Withania treated group and the mesna treated group also retained a normal level of blood urea N₂ level at the three time points. At 48h. blood urea N₂ level of the CTX alone treated group was (50mg/100ml) whereas in the group treated with CTX along with of Withania, it was found to be 27.7 mg/100ml and for the mesna treated group it was 28.3mg/100ml. After 24h. urea N₂ level of CTX along with Withania treated group was 29.7mg/l and that of the mesna treated group was 28.3 mg/100ml.


GSH content in both liver and bladder was drastically reduced after the administration of CTX and it remained the same even after 48h.(table VII.9). Administration of Withania extract along with CTX could significantly enhance the GSH content in liver (7.24 nanomoles/
extract along with CTX could significantly enhance the GSH content in liver (7.24 nanomoles/mg/protein) and bladder (3.06 nanomoles/mg/protein). Similar results were observed in liver (7.16 nanomoles/mg/protein) and bladder (3.52 nanomoles/mg/protein) when mesna, a known uroprotector was administered along with CTX.

III. 13. Effect of *Withania somnifera* on the histopathology of the bladder of CTX treated animals.

Administration of *Withania somnifera* extract along with CTX could normalise the bladder pathology (Fig. a). Analysis of bladder after 4h. of CTX treatment showed mild nuclear formation as well as pleomorphism of giant cells in the epithelium (Fig. b). But in combination with Withania, there was no necrosis of cells and it looked like normal epithelium (Fig. c). After 24h in the CTX alone treated group showed epithelium completely replaced by metaphasic epithelium similar to squamous epithelium with prominent vascular nucleus and mitotic activity (Fig. d). But in the presence of Withania it was normalised and numerous folds or rugae were also present (Fig. e). Bladder pathology after 48h. of CTX alone treatment showed the lining epithelium was completely replaced by necrotic cells and numerous acute and chronic inflammatory cells (Fig. f). Muscle layers did not show much change. Bladder pathology was completely normalised in the animals treated with Withania extract along with CTX (Fig. g).

IV. Discussion

One of the major side effects of chemotherapy is the damage of the immune system. Use of plants as the source of immunomodulatory materials is still in its infancy. Present study
was carried out mainly to determine the immunomodulatory activity of *Withania somnifera*, an important plant in Indian traditional medicine, so as to evaluate it as an adjuvant during chemotherapy. The plant extract has been used as a health tonic and produced anti-inflammatory (30) activities.

Administration of *Withania somnifera* extract significantly reduced the leucopenia induced by sublethal dose of cyclophosphamide in mice. The WBC count attained a normal value in *Withania* treated mice at the end of the treatment (10 days) whereas regenerative capacity in animals treated with CTX was very low and did not regain a normal value even after 30 days.

A significant increase in the number of bone marrow cells and α-esterase positive cells was observed in *Withania* treated group compared to CTX alone treated animals indicating the ability of *Withania* to enhance the proliferation of stem cells. Moreover, there was an increase in the size and weight of spleen as well as thymus of *Withania* treated animals, which indicate that the drug may stimulate spleen and thymus cells and thus enhance the immune system. The exact mechanism of action of *Withania* in the amelioration of cyclophosphamide induced toxicity is not clear. It may be due to an enhanced production of growth factors such as GM-CSF which may be involved in its activity. The present study indicates that *Withania somnifera* could alleviate the myelosuppression and subsequent leucopenia induced by CTX in mice.

One of the major side effects of cyclophosphamide administration is urotoxicity. The deleterious effect of CTX on the bladder includes mucosal oedema, hemorrhage and ulceration and sub endothelial telangiectasia and in severe cases fibrosis of the bladder (85). The toxicity of CTX is due to the metabolites of the drug. These metabolites which include chloethyl azaridine
and acrolein are activated by liver microsomes and excreted in the urine. These metabolites react with urothelium of the bladder. Since bladder is the primary storage organ for urine, the content of these metabolites is higher than other areas of the urinary tract, which increase the sensitivity of the bladder to the damage. Present study was aimed to analyse the reversal of urotoxicity induced by CTX using Withania extract.

Administration of Withania extract showed a significant increase in the body weight of mice treated with CTX compared to the CTX alone treated group. Morphological appearance of bladder of CTX and Withania treated group was normal compared to CTX alone, which was dark in colour and severely inflamed. Proteins and other metabolites accumulated in the urinary bladder cause oedema formation and severe damages. But in the presence of Withania, bladder pathology showed no damage showing a normal architecture.

Similarly, blood urea N\textsubscript{2} level as well as urea N\textsubscript{2} of CTX alone treated group was higher when compared with CTX in the presence of Withania and mesna treated group. GSH content of liver and bladder was normalised within 48 h in the Withania treated group but in CTX alone treated group it was drastically low. GSH is essential to maintain the structural and functional integrity of the cells. GSH depletion altered level of intracellular calcium, lowered threshold to oxidative stress, enhancement of DNA-cross linking and alteration in DNA repair. Glutathione is known to protect against CTX induced bladder damage (59).

Morphological, Histopathological, Biochemical and Enzyme analysis of urinary bladder showed that Withania could alleviate the severe urotoxicity induced by CTX. These studies indicate that Withania administration could reduce the toxic side effects of chemotherapy.
Figure VII.1 Effect of *Withania somnifera* on body weight of mice treated with cyclophosphamide
Figure VII.2 Effect of *Withania somnifera* on total WBC Count in Cyclophosphamide treated mice
Table VII - 1

Effect of *Withania somnifera* on bonemarrow cellularity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>11th day</th>
<th>15th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>15.0 x 10^6 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>5.6 x 10^6 ± 0.7</td>
<td>8.0 x 10^6 ± 2.0</td>
</tr>
<tr>
<td>CTX + <em>Withania somnifera</em></td>
<td>10.9 x 10^6 ± 1.2*</td>
<td>13.1 x 10^6 ± 2.1*</td>
</tr>
</tbody>
</table>

*P<0.001

All the animals were treated with 10 doses (25 mg/kg b.wt.) of CTX. Treated animals were given 10 doses of (20mg/dose/animal, i.p.) *Withania* extract.
Table VII - 2

Effect of *Withania somnifera* on α-esterase positive cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>11th day</th>
<th>15th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1189 ± 27.0</td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>687 ± 99.33</td>
<td>883 ± 49.5</td>
</tr>
<tr>
<td>CTX + <em>Withania Somnifera</em></td>
<td>1068 ± 31.8*</td>
<td>1130 ± 33.75*</td>
</tr>
</tbody>
</table>

*P<0.001

All the animals were treated with 10 doses (25 mg/kg b.wt.) of CTX. Treated animals were given 10 doses of (20mg/dose/animal, i.p.) *Withania* extract.
Table VII - 3

Effect of *Withania somnifera* on relative organ weights

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Spleen</th>
<th>Thymus</th>
<th>Liver</th>
<th>Kidney</th>
<th>Bladder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.35 ± 0.03</td>
<td>0.07 ± 0.002</td>
<td>3.08 ± 0.03</td>
<td>0.51 ± 0.04</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>CTX</td>
<td>0.28 ± 0.01</td>
<td>0.05 ± 0.005</td>
<td>3.01 ± 0.02</td>
<td>0.48 ± 0.03</td>
<td>0.36 ± 0.01</td>
</tr>
<tr>
<td>CTX+<em>Withania somnifera</em></td>
<td>1.11 ± 0.02*</td>
<td>0.20 ± 0.01*</td>
<td>3.38 ± 0.05</td>
<td>0.59 ± 0.06</td>
<td>0.05* ± 0.02</td>
</tr>
</tbody>
</table>

*P<0.001

Animals were treated with 10 doses of *Withania somnifera* extract (20mg/dose/animal). CTX treated group received 10 doses of the drug (25mg/kg/b.wt.).
Table VII - 4

Effect of *Withania somnifera* on Enzyme analysis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum Lipid peroxides (nmols/ml/serum)</th>
<th>Serum SGPT (U/ml)</th>
<th>Serum ALP (K/ml)</th>
<th>Liver Lipid peroxides (nmols of Malonaldehyde/mg Protein)</th>
<th>Liver GPT U/mg protein</th>
<th>Liver ALP KA/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1.21±0.3</td>
<td>362±1.3</td>
<td>14.89±.5</td>
<td>0.367±0.03</td>
<td>635±1.04</td>
<td>1.32±0.01</td>
</tr>
<tr>
<td>11th day cyclophosphamide</td>
<td>1.52±.14</td>
<td>423±2.22</td>
<td>21.86±1.5</td>
<td>0.410±0.03</td>
<td>885±6.5</td>
<td>1.81±0.03</td>
</tr>
<tr>
<td>11th day CTX + Withania</td>
<td>1.19±1.1</td>
<td>393±1.5</td>
<td>18.59±.8</td>
<td>0.396±0.08</td>
<td>732±5.5</td>
<td>1.53±0.04</td>
</tr>
<tr>
<td>15th day CTX</td>
<td>1.71±.02</td>
<td>412±2.5</td>
<td>19.28±.1.1</td>
<td>0.418±.05</td>
<td>792±1.6</td>
<td>1.86±0.03</td>
</tr>
<tr>
<td>15th day CTX + Withania</td>
<td>1.39±.03</td>
<td>381±3.3</td>
<td>16.83±1.2</td>
<td>0.383±.06</td>
<td>756±1.9</td>
<td>1.49±0.03</td>
</tr>
</tbody>
</table>

Animals were treated with 10 doses of *Withania somnifera* extract (20mg/dose/animal). CTX treated group received 10 doses of the drug (25mg/kg/body wt.).
Table VII - 5

Effect of *Withania somnifera* on cytokine Production in cyclophosphamide treated mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cytokine (concentration pg/ml)</th>
<th>IFNγ</th>
<th>IL-2</th>
<th>GM-CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>40±0.7</td>
<td>7.4±15</td>
<td>32.6±13</td>
</tr>
<tr>
<td>CTX</td>
<td></td>
<td>30±0.8</td>
<td>4.5±2</td>
<td>19.2±15</td>
</tr>
<tr>
<td>CTX + Withania</td>
<td></td>
<td>74.87±0.9*</td>
<td>7.5±3**</td>
<td>35.5±12**</td>
</tr>
</tbody>
</table>

*P<0.001
**P<0.01

CTX treated animals received 10 doses of the drug (25mg/kg/b.wt.i.p.) and CTX + Withania treated group received same dose of CTX as above and 10 doses of Withania (20mg/dose/animal i.p).
Table VII - 6

Morphological analysis of urinary bladder after CTX treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>4th</th>
<th>24th</th>
<th>48th</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTX</td>
<td>Bladder inflamed</td>
<td>Bladder inflamed</td>
<td>Severe inflammation of bladder and dark colouration</td>
</tr>
<tr>
<td></td>
<td>Noticeable colouration</td>
<td>severe haemorrhage colouration</td>
<td></td>
</tr>
<tr>
<td>CTX + Withania</td>
<td>Slight inflammation</td>
<td>No Inflammation and looked normal</td>
<td>Bladder looked like normal.</td>
</tr>
<tr>
<td></td>
<td>Normal colouration</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CTX treated animals received a single chronic dose of CTX (1.5 m moles). Treated animals received same dose of CTX and Withania (20 mg/dose/animal, i.p) for 5 days.
Table VII - 7

Effect of Withania somnifera on the Biochemical Parameters 4h after cyclophosphamide treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Change in b.wt (g/100g b.wt.)</th>
<th>R.b.wt (g/100g b.wt.)</th>
<th>Serum Protein (g/100ml)</th>
<th>Blood UreaN(_2) (mg/100ml)</th>
<th>Urine Protein (g/l)</th>
<th>Urea N(_2) (g/l)</th>
<th>Liver GSH (nmol/mg Protein)</th>
<th>Bladder GSH (nmol/mg Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTX</td>
<td>-.58</td>
<td>.08±.1</td>
<td>6.05±.15</td>
<td>28±.05</td>
<td>4.15±.13</td>
<td>12.5±.11</td>
<td>7.25±.15</td>
<td>3.05±.16</td>
</tr>
<tr>
<td>CTX+Withania</td>
<td>+.53</td>
<td>.31±.11</td>
<td>11.44±.12</td>
<td>136.78±.09</td>
<td>7.9±.12</td>
<td>23.1±.1</td>
<td>1.21±.15</td>
<td>0.52±.06</td>
</tr>
<tr>
<td>CTX+mesna</td>
<td>+.55</td>
<td>.07±.11**</td>
<td>7.62±.08**</td>
<td>43.34±.12**</td>
<td>5.8±.12**</td>
<td>15.1±.12**</td>
<td>5.56±.11**</td>
<td>3.39±.11</td>
</tr>
</tbody>
</table>

*P< 0.001
**P< 0.01

The animals treated with Withania extract received 5 doses of the drug (20mg/dose/animal, i.p.) and a single chronic dose of CTX (1.5 mmoles). Mesna treated animals received the same dose of CTX and a single dose of the drug (4.5 mmoles).
Table VII - 8

Effect of *Withania somnifera* on the Biochemical Parameters 24h after CTX treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Change in b. wt (g/100g b. wt)</th>
<th>R. b. wt (g/100g b. wt)</th>
<th>Serum Protein (g/100ml)</th>
<th>Blood UreaN$_j$ (mg/100ml)</th>
<th>Urease N$_j$ (g/l)</th>
<th>Liver GSH (nmole/mg Protein)</th>
<th>Bladder GSH (nmole/mg Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>0.08±1</td>
<td>6.05±14</td>
<td>26.05±05</td>
<td>4.15±13</td>
<td>12.5±11</td>
<td>7.25±15</td>
</tr>
<tr>
<td>CTX</td>
<td>-1.06</td>
<td>0.14±12</td>
<td>10.41±17</td>
<td>53.77±11</td>
<td>10.21±18</td>
<td>27.1±07</td>
<td>1.88±12</td>
</tr>
<tr>
<td>CTX+Withania</td>
<td>+1.26</td>
<td>0.06±11**</td>
<td>7.12±16**</td>
<td>29.57±09**</td>
<td>7.2±16**</td>
<td>17±08**</td>
<td>5.96±13*</td>
</tr>
<tr>
<td>CTX+mesna</td>
<td>+1.5</td>
<td>0.07±13**</td>
<td>5.9±13**</td>
<td>28.57±12**</td>
<td>6.81±14**</td>
<td>15±13**</td>
<td>7.4±12*</td>
</tr>
</tbody>
</table>

The animals treated with *Withania* extract received 5 doses of the drug (20mg/dose/animal, i.p.) and a single chronic dose of CTX (1.5 mmoles). Mesna treated animals received the same dose of CTX and a single dose of the drug (4.5 mmoles).

*P<0.001  
**P<0.01
Table VII - 9

Effect of *Withania somnifera* on the Biochemical Parameters 48h after CTX treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Change in b.wt. (g/100g b.wt.)</th>
<th>R.bl wt (g/100g b.wt.)</th>
<th>Serum Protein (g/100ml)</th>
<th>Blood UreaN₂ (mg/100ml)</th>
<th>Urine Protein (g/l)</th>
<th>Urea N₂ (g/l)</th>
<th>Liver GSH (nmol/ mg Protein)</th>
<th>Bladder GSH (nmol/ mg Protein)</th>
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</thead>
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<tr>
<td>Normal</td>
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<tr>
<td>CTX</td>
<td>-2.13</td>
<td>0.08±1</td>
<td>6.05±16</td>
<td>28±05</td>
<td>4.15±13</td>
<td>12.5±13</td>
<td>7.25±15</td>
<td>3.05±16</td>
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<tr>
<td>CTX+Withania</td>
<td>+1.4</td>
<td>0.13±09</td>
<td>6.75±18</td>
<td>50.06±09</td>
<td>7.35±12</td>
<td>31.4±12</td>
<td>2.93±11</td>
<td>1.19±13</td>
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<tr>
<td>CTX+mesna</td>
<td>+1.8</td>
<td>0.06±0.08**</td>
<td>6.08±13**</td>
<td>27.71±07**</td>
<td>4.3±1**</td>
<td>13.0±11**</td>
<td>7.24±13*</td>
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</tr>
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*P< 0.001  
**P<0.01

The animals treated with *Withania* extract received 5 doses of the drug (20mg/dose/animal, i.p) and a single chronic dose of CTX (1.5 mmoles). Mesna treated animals received the same dose of CTX and a single dose of the drug (4.5 mmoles).
HISTOPATHOLOGY OF C.S. OF INTESTINE

Fig. (a) NORMAL

Fig. (b) CTX - 11TH DAY

Fig. (c) CTX + WITHANIA - 11TH DAY

Fig. (d) CTX - 15TH DAY

Fig. (e) CTX + WITHANIA - 15TH DAY
HISTOPATHOLOGY OF URINARY BLADDER

Fig. (a) NORMAL

Fig. (b) CTX ALONE 4h.

Fig. (c) CTX + WITHANIA 4h.
Figure
HISTOPATHOLOGY OF URINARY BLADDER

Fig. (d) CTX ALONE 24h.

Fig. (e) CTX + WITHANIA 24h.

Fig. (f) CTX ALONE 48 h.

Fig. (g) CTX + WITHANIA 48h.