Protection of Mouse Testes, Epididymis and Adrenals with Speman against Cadmium Intoxication

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INTRODUCTION
Cadmium is a heavy metal pollutant which is toxic to plants, animals and human beings (Nriagu, 1980). In mammals, cadmium damages the testes, accessory sex organs, sperms and adrenals (Gunn and Gould, 1970). Fortunately sterility and sexual disorders in man have not been reported following cadmium intoxication but osteomalacia, polynephritis, cancer of the nasopharynx and prostate have been reported in men professionally exposed to cadmium (Kjellstrom et al., 1979; Lemen et al., 1976; Yosumura et al., 1980).

In the present investigation an attempt has been made to prevent destruction of mice testes, epididymis and adrenals with Speman (an indigenous drug), which is known to promote spermatogenesis (Parikh, 1971; Subbarao, et al., 1973; Khaleeluddin et al., 1973: Talaulikar and Nagarsekar, 1976: Jayatilak et al., 1976 (a) and (b), Pardanani et al., 1976; Nath, 1982) and is used to cure male sterility (oligozoospermia).

The aim of this investigation was to test the ability of Speman to nullify the toxic effects of cadmium so that in future it can be recommended to cure testicular dysfunction in man if it results following cadmium poisoning.

MATERIALS AND METHODS
Three and half month old male albino Swiss mice were obtained from the Biological Products Division, Veterinary College, Mhow (M.P.) and were used in the present experiments. Ten mice were placed in each propylene cage (290 x 220 x 140 mm) and given standard mice food and tap water ad libitum. CdCl₂ made by BDH was dissolved in distilled water to prepare a solution of 1 mg/ml.

Each Speman tablet (The Himalaya Drug Company, Bombay) contains the following constituents:

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orchis mascula</td>
<td>65 mg</td>
</tr>
<tr>
<td>Lactuca scariola</td>
<td>16 mg</td>
</tr>
<tr>
<td>Hygrophila spinosa</td>
<td>32 mg</td>
</tr>
<tr>
<td>Mucuna pruriens</td>
<td>16 mg</td>
</tr>
<tr>
<td>Exts. Parmelia perlata</td>
<td>16 mg</td>
</tr>
<tr>
<td>Argyreia speciosa</td>
<td>32 mg</td>
</tr>
<tr>
<td>Tribulus terrestris</td>
<td>32 mg</td>
</tr>
<tr>
<td>Leptadenia reticulata</td>
<td>32 mg</td>
</tr>
<tr>
<td>Suvarnavang (Mosaic Gold)</td>
<td>16 mg</td>
</tr>
</tbody>
</table>
Speman is not soluble in water, hence The Himalaya Drug Company supplied a suspension having 10 mg Speman per ml, which was administered orally through injection using a blunt needle.

The mice were divided into four groups:
I. Group A: All mice were injected with 1 mg CdCl₂ intramuscularly only once + placebo given orally daily.

II. Group B: All mice received a single injection of 1 mg CdCl₂ + 0.5 ml suspension containing 5 mg Speman given orally daily.

III. Group C: All mice received a single injection of 1 mg CdCl₂ + 1 ml suspension containing 10 mg Speman given orally daily.

IV. Group D: Controls no treatment at all.

The contents of Speman suspension were as follows:

- Speman powder (200 mesh) 100 gm
- Nipagin Sodium (0.25%) 2.5 gm
- Nipasol Sodium (0.15%) 10 gm
- Distilled water to make 1000 ml

Placebo contained the same constituents as cited above except Speman.

Speman administration was continued for 60 days. The mice were killed on the 15th, 30th and 60th days. Their testes, epididymis and adrenals were fixed in alcoholic Bouin’s fluid and routine microtome sections of 6 to 8 microns were cut and stained in Delafield’s haematoxylin and eosin. The experiments were done thrice.

Observations of permanent slides, camera lucida drawings and photomicrographs have formed the basis of the present results.

**RESULTS**

The results, which are presented in the form of Tables 1 to 5 and photomicrographs, reveal the following facts:

a) **Pattern of Speman plus CdCl₂ alone, induced changes**

   A careful persual of Tables 1 to 4 and all photomicrographs shows the effects of CdCl₂ alone, and in combination with Speman, on the testes, epididymis and adrenals. Typical responses are evident after the 15th, 30th and 60th days.
Table 1: Diameter of seminiferous tubules of mice (in microns) at different intervals after cadmium chloride and Speman treatment (Mean ± SE)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Duration of Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15 days</td>
</tr>
<tr>
<td>A</td>
<td>Single injection of CdCl₂ + Placebo</td>
<td>16.70 ± 0.74</td>
</tr>
<tr>
<td>B</td>
<td>Single injection of CdCl₂ + Speman 5 mg daily</td>
<td>18.51 ± 0.93</td>
</tr>
<tr>
<td>C</td>
<td>Single injection of CdCl₂ + Speman 10 mg daily</td>
<td>15.87 ± 1.52</td>
</tr>
<tr>
<td>D</td>
<td>Control</td>
<td>19.64 ± 1.19</td>
</tr>
</tbody>
</table>

*Statistically significant, based on ‘t’ test at 5% level of significance.

Table 2: Diameter of tubules of epididymis and height of cells lining tubules at different intervals (in microns) after CdCl₂ and Speman treatment (Mean ± SE)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Diameter after</th>
<th>Height of cells after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15 days</td>
<td>30 days</td>
</tr>
<tr>
<td>A</td>
<td>Single injection of CdCl₂ + Placebo</td>
<td>26.90* ± 3.02</td>
<td>32.51* ± 2.04</td>
</tr>
<tr>
<td>B</td>
<td>Single injection of CdCl₂ + Speman 5 mg daily</td>
<td>27.80* ± 0.22</td>
<td>23.80* ± 2.89</td>
</tr>
<tr>
<td>C</td>
<td>Single injection of CdCl₂ + Speman 10 mg daily</td>
<td>19.87* ± 0.66</td>
<td>14.58* ± 0.44</td>
</tr>
<tr>
<td>D</td>
<td>Control</td>
<td>40.38 ± 0.63</td>
<td>–</td>
</tr>
</tbody>
</table>

*Statistically significant, based on student’s ‘t’ test at 5% level of significance.

Table 3: Number of germinal and other cells present in the testes of mice at various intervals following CdCl₂ and Speman treatment (Mean ± SE)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Days</th>
<th>Primary Spermatocytes</th>
<th>Secondary Spermatocytes</th>
<th>Spermatids</th>
<th>Sperms + =Present</th>
<th>Sperms − =Absent</th>
<th>Sertoli cells</th>
<th>Leydig cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Single injection of CdCl₂ + Placebo</td>
<td>15</td>
<td>58.75* ± 10.67</td>
<td>80.37* ± 11.22</td>
<td>40.64* ± 6.54</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>B</td>
<td>Single injection of CdCl₂ + Speman 5 mg daily</td>
<td>15</td>
<td>66.56* ± 3.68</td>
<td>83.50* ± 6.71</td>
<td>60.16* ± 2.24</td>
<td>+</td>
<td>6.55* ± 0.84</td>
<td>19.82* ± 1.26</td>
<td>–</td>
</tr>
<tr>
<td>C</td>
<td>Single injection of CdCl₂ + Speman 10 mg daily</td>
<td>15</td>
<td>75.78* ± 4.89</td>
<td>92.50* ± 6.49</td>
<td>73.12* ± 1.81</td>
<td>+</td>
<td>25.60* ± 0.85</td>
<td>26.00* ± 1.58</td>
<td>18.33* ± 1.80</td>
</tr>
<tr>
<td>D</td>
<td>Control</td>
<td>15</td>
<td>76.66* ± 7.09</td>
<td>76.66* ± 6.68</td>
<td>30.00* ± 2.89</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*Statistically significant, based on ‘t’ test at 5% level of significance.
Table 4: Diameter of cortex and medulla of adrenals in mice at different intervals following CdCl$_2$ and Speman treatment (Values in microns; Mean ± SE)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Duration in days</th>
<th>Cortex</th>
<th>Medulla</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Single injection of CdCl$_2$ + Placebo</td>
<td>15</td>
<td>108.12 ± 3.05*</td>
<td>60.83 ± 3.36*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>93.87 ± 7.30</td>
<td>50.83 ± 4.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>92.45 ± 0.96*</td>
<td>42.12 ± 0.17*</td>
</tr>
<tr>
<td>B</td>
<td>Single injection of CdCl$_2$ + Speman 5 mg daily</td>
<td>15</td>
<td>66.12 ± 1.44*</td>
<td>33.87 ± 1.55*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>85.16 ± 2.14*</td>
<td>43.54 ± 1.82*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>96.64 ± 5.07*</td>
<td>48.06 ± 6.14</td>
</tr>
<tr>
<td>C</td>
<td>Single injection of CdCl$_2$ + Speman 10 mg daily</td>
<td>15</td>
<td>86.90 ± 10.42</td>
<td>48.70 ± 6.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>79.03 ± 10.70</td>
<td>45.41 ± 5.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>79.35 ± 5.52</td>
<td>37.16 ± 7.73</td>
</tr>
<tr>
<td>D</td>
<td>Control</td>
<td>–</td>
<td>79.09 ± 0.31</td>
<td>44.96 ± 1.49</td>
</tr>
</tbody>
</table>

*Statistically significant, based on ‘t’ test at 5% level of significance.

Table 5: Recovery pattern of some parameters in mice after a single injection of CdCl$_2$ by Speman treatment for 60 days

<table>
<thead>
<tr>
<th>Group comparison</th>
<th>Diameter of seminiferous tubule</th>
<th>Diameter of tubule of the epididymis</th>
<th>Height of tubular lining of the epididymis</th>
<th>Diameter of the cortex</th>
<th>Diameter of the medulla</th>
<th>No. of primary spermatocytes</th>
<th>No. of secondary spermatocytes</th>
<th>No. of spermatids</th>
<th>No. of Leydig cells</th>
<th>No. of Sertoli cells</th>
<th>Sperms</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Speman 5 mg daily with placebo)</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>B (Speman 10 mg daily with placebo)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Only significant results at 5% level of significance are presented. Recovery means significant betterment towards normal values with the drug when results are compared with those obtained after CdCl$_2$ treatment.

b) **Pattern of maximum recovery with Speman therapy following CdCl$_2$ intoxication.**

Here experiments were conducted for 60 days in order to find out the actual recovery i.e. whether Speman checks and /or reverses the toxic effects of CdCl$_2$. The values of various parameters obtained following 60 days after CdCl$_2$ alone were compared with the values of the same parameters obtained following Speman therapy for 60 days. And ‘t’ test at 5% level of significance was applied to the data for this comparison. The results are presented in Table 5, which shows that Speman at 5 mg/daily dose for 60 days shows a beneficial role by abolishing the toxic effects of CdCl$_2$ on the seminiferous tubules, epididymis, spermatide and medulla of the adrenals. Speman at
10 mg/daily dose is more effective as it removes the toxic effects of CdCl₂ on all the parameters studied. Speman at both doses shows typical relationship for Leydig cells.

**PLATE-I**
Photomicrographs of mice testes with Bouin’s haematoxylin and eosin preparation

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**Fig. 1:**
Control. Normal relationship of intertubular structure to tubules is shown. 100X

**Fig. 2:**
15 days after CdCl₂ injection complete absence of interstitial cells resulted in the intertubular spaces. 100X

**Fig. 3:**
30 days after CdCl₂ injection. Few interstitial cells. 100X

**Fig. 4:**
60 days after CdCl₂ injection. Seminiferous tubules shrink. Few interstitial cells. Extremely few sperms. 100X

**Fig. 5:**
15 days after CdCl₂ injection + 5 mg Speman daily. Interstitial cells are present. 100X

**Fig. 6:**
30 days after CdCl₂ injection + 5 mg Speman daily. No changes are visible. 100X
Fig. 7:  
60 days after CdCl₂ injection + 5 mg Speman daily.  
No change. 100X

Fig. 8:  
15 days after CdCl₂ injection + 10 mg Speman daily.  
Interstitial cells are absent. 100X

Fig. 9:  
30 days after CdCl₂ injection + 10 mg Speman daily. Few interstitial cells are seen in between shrunken seminiferous tubules. 100X.

Fig. 10:  
60 days after CdCl₂ injection + 10 mg Speman daily. Shrinkage of seminiferous tubules and few interstitial cells when compared with Fig. 1 but situation is better when compared with Fig. 4. 100X

Fig. 11:  
Magnified view of a seminiferous tubule showing spermatogenesis and sperms. 400X.
PLATE-III
Photomicrographs of mice caput epididymis, with Bouin’s haematoxylin and eosin. All slides photographed at 50X.

Figs. 12 and 13
Epididymis, control. Note the height of the lining epithelium and degree of intervening tissue between tubules.

Fig. 14:
15 days after CdCl₂ injection. Marked widening of the intertubular spaces. Spermatozoa are present. The height of the lining is low.

Fig. 15:
30 days after CdCl₂ injection. Diameter of tubules and height of the lining epithelium are reduced. Sperms are present.

Fig. 16:
60 days after CdCl₂ injection. Diameter of tubules and height of the lining epithelium are reduced. Sperms present.

Fig. 17:
15 days after CdCl₂ injection + 5 mg Speman daily. Diameter of tubules and height of the lining epithelium are reduced. Sperms are present.
Fig. 18: 30 days after CdCl₂ injection + 5 mg Speman daily. Diameter of tubules and height of the lining epithelium are reduced.

Fig. 19: 60 days after CdCl₂ injection + 5 mg Speman daily. Tubular diameter is normal. Height of the lining epithelium is less as compared to controls but more when compared with Fig. 15.

Fig. 20: 15 days after CdCl₂ injection + 10 mg Speman daily. Tubular diameter and height of the lining epithelium are reduced.

Fig. 21: 30 days after CdCl₂ injection + 10 mg Speman daily. Tubular diameter normal but height of the lining epithelium is low.

Fig. 22: 60 days after CdCl₂ injection + 10 mg Speman daily. Tubular diameter and height of the lining epithelium are reduced (However the situation is better when compared with Fig. 15).
PLATE-V
Photomicrographs of mice adrenal gland, with C.S. Bouin’s haematoxylin and eosin preparation.
All slides were photographed at 50X.

Fig. 23: Control adrenals, cortex and medulla normal.

Fig. 24: 15 days after CdCl₂ injection. Hypertrophy of both zones.

Fig. 25: 30 days after CdCl₂ injection. Cortex swells but medulla is normal.

Fig. 26: 60 days after CdCl₂. Cortex swells but medulla is normal.

Fig. 27: 15 days after CdCl₂ injection + 5 mg Speman daily. Cortex and medulla shrink.

Fig. 28: 30 days after CdCl₂ injection + 5 mg Speman daily. Cortex hypertrophies but medulla shrinks.
DISCUSSION
Cadmium-induced changes in the testes, epididymis and adrenals as observed here are in good conformity with the findings of earlier workers (Gunn and Gould, 1970).

The presence of androgens is a must for the maintenance of spermatogenesis (Steinberger and Duckett, 1965) and normal functioning of the epididymis (Prasad et al., 1973; Tuohima and Niema, 1974; Allen, 1958). In the present investigation, when 60 days after Speman administration cadmium-induced changes were nullified significantly, it is logical to think that Speman must have exerted an “androgen-like” activity as earlier reported by Jayatilak et al., (1976), because it is known that exogenous androgens can maintain spermatogenesis in rats and guinea pigs and up to a certain extent in man and monkeys (Steinberger, 1971). Speman’s ability to reverse and/or check cadmium-induced changes further proved its “androgen-like” activity, as CdCl₂ lowers testosterone synthesis in mammals (Saxena et al., 1977) and in fish (Sangland and O’Halloran, 1973).
CdCl₂ lowers oxygen uptake (Rao and Patnaik, 1977) and Speman has been recently reported to enhance oxygen uptake in the testes of rats following irradiation (Sethi et al., 1980). This property of Speman can also be cited for its beneficial role following cadmium intoxication.

Speman (10 mg daily) fully nullified the effects of CdCl₂ on the adrenals and the normal functioning of adrenals can be held responsible for protecting the testes and adrenals at 60 days, as the adrenals also produce androgens (Turner and Bagnara, 1976), and some steroids which influence cell renewal in accessory sex glands (Tullner, 1963). CdCl₂ also causes stress (Singhal and Merali, 1979) and the ability of Speman to maintain the normal structure of the adrenals is reflected in its “antistress” property, as the cortical steroids act against stressors (Selye, 1959).

ACKNOWLEDGEMENTS
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REFERENCES