Protection of Male Reproductive Organs in Mice with Speman against Mercuric Chloride Intoxication

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ABSTRACT
Intraperitoneal administration of mercuric chloride at a dose of 50 mg/mouse/day to male mice weighing 22-24 gms over a period of 60 days, caused weight loss of gonad and accessories, degenerative changes in testes and epididymis, reduction in motile sperm population, loss of alkaline and acid phosphatase activity, total protein and ascorbic acid content.

When a herbal drug ‘Speman’, was only administered at a dose of 25 mg/mouse/day to mice receiving mercuric chloride injections for 60 days, it significantly decreased the damaging effect of mercury. All parameters of the study showed improvement. Sperm motility was found to be 50-60%. The results suggest that this herbal drug may be used to restore Hg-induced impairment of spermatogenesis.

Key words: Mercuric chloride; oligozoospermia; spermatogenesis; histopathological changes; herbal compound

INTRODUCTION
Inorganic mercury-induced testicular damage is a known fact in experimental animals1,2. In humans, mercury induces loss of libido3, hypospermia, astheno-spermia and teratospermia4. A herbal drug, Speman manufactured by The Himalaya Drug Co., Bangalore, India, may be used to cure infertility and oligozoospermia associated with testicular dysfunction. Under laboratory conditions, this drug has been found to protect mammalian testes against the ill-effects of cadmium chloride5, ceric sulphate6, X-ray7 and electrical shock8.

A study was conducted to assess the protective effect of this herbal drug on the reproductive organs of mice, against HgCl₂ intoxication, under laboratory conditions.

MATERIAL AND METHODS
Six-month-old Swiss albino mice weighing 25 gms, obtained from J.N.U., New Delhi, India were divided into the following groups:

Group I: Controls (C): 30 mice of whom each received 0.2 ml distilled-deionised water per day, for 60 days. They were given standard food and tap water ad libitum.
Group II: \( \text{HgCl}_2 \) treatment group (\( P \)): 30 mice of whom each received 0.2 ml 50 mg \( \text{HgCl}_2 \) solution (Ranbaxy 99.9% salt dissolved in distilled-deionised water), per day intraperitoneally. They were given standard food and tap water \textit{ad libitum}.

Group III: \( \text{HgCl}_2 \) Intoxication + Speman therapy group (\( P + D \)): 30 mice of whom each received orally, in addition to injection as administered in the ‘p’ group, 0.3 ml drug suspension in water containing 25 mg Speman.

Each Speman tablet consists of \textit{Orchis mascula} 65 mg, \textit{Lactuca scariola} 16 mg, \textit{Astercantha longifolia} 32 mg, \textit{Mucuna pruiriens} 16 mg, \textit{Parmelia perlata} 16 mg, \textit{Argyreia speciosa} 32 mg, \textit{Tribulus terrestris} 32 mg, \textit{Leptadenia reticulata} 32 mg and Mosaic gold 16 mg. Speman, ground to a fine powder so as to obtain a smooth suspension in distilled water, was administered orally.

On day 61, the mice were sacrificed and their organs were removed in chilled saline. Tests and epididymis were fixed in Bouin’s fluid for their histology. Fresh tests were homogenised in chilled 0.25 M sucrose to prepare 10% (w/v) solution for biochemical study.

Fresh epididymis was placed and punctured in an ice-cold phosphate buffer to extract the contents. Using a haemocytometer, slide sperm was observed for motility and counting.

Biochemical estimation (except ascorbic acid by DNPH-aniline method) and sperm counting were done in a pathological laboratory. Ready-to-use kits and reagents manufactured by Span Diagnostic (India), were used for biochemical estimation.

**RESULTS**
For convenience, the results are described under separate headings.

**Gravimetric** (Table 1):

(a) **Body weight:** No significant change in body weight of mice occurred following \( \text{HgCl}_2 \) or \( \text{HgCl}_2 + \text{Speman} \) treatment, as compared to controls.

(b) **Wet weight of testes, seminal vesicle, epididymis and prostate:** \( \text{HgCl}_2 \) treatment caused significant loss in weight of testes and accessory organs. However, when Speman was administered along with \( \text{HgCl}_2 \), weight of testes and accessories (except prostate), were significantly lower than controls, but the values were significantly
higher than those recorded after HgCl\(_2\) intoxication. This indicates that the drug provided complete protection to the prostate and partial protection to the other organs.

**Histological** (Table 2, Fig. 1-6):

a. *Testes*: In control mice, histology of testes revealed a close-to-normal structure. All the stages of spermatogenesis inside the seminiferous tubules and interstitial cells were visible between them (Figs. 1 and 2). Motile sperm population was 60% and had a normal appearance. Among HgCl\(_2\)-treated mice (50 mg/mouse/day for 60 days), there was a marked shrinkage of seminiferous tubules (Figs. 3 and 4). Population of spermatogonia remained unaffected but the number of primary and secondary spermatocyte, spermatids and interstitial cells showed significant reduction, as compared to corresponding germ cells in the control group. Population of viable spermatozoa appeared to be significantly reduced, i.e. by 10%.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Diameter of seminiferous tubules (m)</th>
<th>No. of spermatogonia</th>
<th>No. of primary spermatocytes</th>
<th>No. of secondary spermatocytes</th>
<th>No. of spermatids (million/ml)</th>
<th>Sperm population</th>
<th>% mobility of sperms</th>
<th>Malformed sperms</th>
<th>Interstitial cells</th>
<th>Height of epididymal epithelial cells (µ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 'C'</td>
<td>222.26 ± 4.83</td>
<td>35.28 ± 2.53</td>
<td>65.50 ± 1.55</td>
<td>85.25 ± 3.39</td>
<td>87.50 ± 3.33</td>
<td>68</td>
<td>60-70</td>
<td>NO</td>
<td>4-6</td>
<td>18.23 ± 8.72</td>
</tr>
<tr>
<td>HgCl(_2)-treated 'P'</td>
<td>112.91* ± 3.47</td>
<td>32.44* ± 2.46</td>
<td>38.1* ± 2.32</td>
<td>15.00* ± 2.14</td>
<td>17.42* ± 2.61</td>
<td>20*</td>
<td>10*</td>
<td>NO</td>
<td>3-4d</td>
<td>17.25* ± 0.30</td>
</tr>
<tr>
<td>HgCl(_2)+ Speman treated (P+D)</td>
<td>203.75* ± 3.44</td>
<td>34.66 ± 3.02</td>
<td>62.60* ± 2.34</td>
<td>110.00* ± 4.24</td>
<td>82.77* ± 3.02</td>
<td>45*</td>
<td>50-60*</td>
<td>No</td>
<td>4-5</td>
<td>9.58* ± 0.68</td>
</tr>
</tbody>
</table>

Statistically significant based on students ’t’ test at 5% level of significance.
*Comparison of ‘C’ versus ‘P’
* Comparison of ‘P’ versus ‘P’ + ‘D’
d Damaged.

Testes of HgCl\(_2\)+ Speman-treated group (P + O) showed a slightly smaller diameter of seminiferous tubules than controls but the size was larger than the HgCl\(_2\)-treated (P) group. The deformity or death of any type of spermatogenic cells and interstitial cells was not seen here. The spermatogenesis appeared to proceed normally (Figs. 5 and 6). The number of primary and secondary spermatocyte was higher than that recorded for the control group, while the number of spermatids did not decrease and remained equal to that of the control group. Motile sperm count was markedly better at 50-60% of the total population, compared to the HgCl\(_2\)-treated group, where it was only 10%.

b. *Epididymis*: Control mice showed typical mammalian structure with sperms (Fig. 7). Treatment resulted in degenerative changes, fully disorganised tubules being visible. The lumen lodged sperm mass (Fig. 8).

Following HgCl\(_2\)+ Speman treatment, the epididymis revealed near-normal structure, as the histopathological signs were mild. Sperm mass was seen in the lumen (Fig. 9).
Biochemical (Table 3): Mice treated with HgCl$_2$, experienced loss of acid and alkaline phosphatase activities, total protein and ascorbic acid contents, even as the levels of cholesterol and glucose rose significantly. HgCl$_2$ administered simultaneously with Speman, showed less severe toxic influence, as evidence by the comparison of values recorded in different groups. This suggests the protective role of Speman.

<table>
<thead>
<tr>
<th>Group</th>
<th>Acid phosphatase (KA unit)</th>
<th>Alkaline phosphatase (KA unit)</th>
<th>Cholesterol (mg/100 gm)</th>
<th>Glucose (mg/100 gm)</th>
<th>Protein (gm/100 ml)</th>
<th>Ascorbic acid (mg/100 gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ‘C’</td>
<td>18.08 ± 1.75</td>
<td>34.37 ± 1.38</td>
<td>230 ± 9.64</td>
<td>12.75 ± 0.85</td>
<td>0.77 ± 0.03</td>
<td>33.00 ± 0.84</td>
</tr>
<tr>
<td>HgCl$_2$-treated ‘P’</td>
<td>1.90* ± 0.45</td>
<td>13.74* ± 0.61</td>
<td>633.00* ± 14.66</td>
<td>40.00* ± 4.85</td>
<td>0.51* ± 0.01</td>
<td>15.00 ± 0.15</td>
</tr>
<tr>
<td>HgCl$_2$-treated + Speman administered (P+D)</td>
<td>15.87* ± 1.46</td>
<td>21.12* ± 0.33</td>
<td>309* ± 12.20</td>
<td>21.75* ± 0.98</td>
<td>1.24* ± 0.11</td>
<td>21.54* ± 0.73</td>
</tr>
</tbody>
</table>

Statistically significant based on students ‘t’ test at 5% level of significance.
* Comparison of ‘C’ versus ‘P’
* Comparison of ‘P’ versus ‘P’ + ‘D’

Fig. 1: Control, compact, well-differentiated seminiferous tubules showing active spermatogenesis. 150X

Fig. 2: Magnified view of previous figure shows spermatogenic cells and sperms in side seminiferous tubule and interstitial cells between them. 600X

Fig. 3: HgCl$_2$ (50 mg, i.p./day/mouse) treatment for 60 days caused significant shrinkage and disorganisation of the seminiferous tubules with arrest of spermatogenesis at secondary spermatocyte stage. Interstitial cells show atrophy (↑). 150X.
**Fig. 4:** Magnified view of the earlier figure showing toxic effect of HgCl₂. Accumulation and disintegrating secondary spermatocytes (SS), atrophy of interstitial cells. 600X.

**Fig. 5:** HgCl₂ (50 mg, i.p./day/mouse) + Speman 25 mg/day/mouse for 60 days well developed seminiferous tubules without showing deformity and death of any type of spermatogenic cell. 150X.

**Fig. 6:** Magnified view of earlier figure, active spermatogenesis is evident as sperms are seen inside the seminiferous tubule and interstitial cells are differentiated. 600X.

**Fig. 7:** Epididymal tubule made-up of intact columnar epithelia cells with prominent nuclei; lumen filled with sperms. 150X.

**Fig. 8:** HgCl₂ treatment caused severe degenerative changes i.e., cytoplasmic dissolution and nuclear disintegration. Hence fusion of adjacent tubules is evident. At some places fully disorganised tubules are also visible as cellular debris (α). 150X.

**Fig. 9:** HgCl₂ + Speman treatment shows fairly better histology (similar to that of controls). Pathological changes are very mild when compared with previous figure. 150X.
DISCUSSION

Present as well as previous results report that inorganic Hg causes morphological distortion, biochemical alteration and functional impairment of testes and epididymis\textsuperscript{1,2,9-12}.

HgCl\textsubscript{2} caused degenerative changes in rat testes and epididymis by lowering the level of 3β-hydroxy 5-steroid dehydrogenase\textsuperscript{13}. This finding is acceptable, as optimal levels of androgens are necessary for maintaining normal structure and functioning of the gonads and accessories\textsuperscript{14}. In the present study, Hg-induced damage was significantly less in the presence of the multiherbal drug, Speman. This reflects its androgen-like activity, as supply of exogenous androgens can restore impaired spermatogenesis by maintaining normal structure and function of the gonads and accessories\textsuperscript{15,16}. Earlier studies indicate similar action of the drug. When Speman was administered to adult rats for 30 days, an improvement in spermatogenesis associated with enhanced sexual desire, was observed\textsuperscript{17}. It is reported that this drug caused significant increase in the circulating levels of blood testosterone in young bulls\textsuperscript{18}. Distinct androgen-like action of the drug was confirmed in castrated mice by studying androgen-dependent parameters\textsuperscript{19}. The drug was found to exert beneficial effects without affecting the pituitary. High cholesterol and low ascorbic acid concentration can be attributed to defective mitochondrial conversion of cholesterol to pregnenolone\textsuperscript{20}.

Hg impairs mitochondrial function as it lowers the ATP content by inactivating SH group-bearing enzymes (ATPase, SDH and others)\textsuperscript{21,22}. Therefore, increased glucose consumption results in high glucose content in testes homogenate. Hg also affects the activities of alkaline phosphatase and transaminases (GOT and GPT) in mammalian kidney\textsuperscript{23}. On the other hand, Speman restores activities of AP, GOT, GPT, SDH and enhances oxygen consumption, which are lowered following irradiation in rat testes\textsuperscript{7}.

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REFERENCES


