Figure 3. Influence of 1,3,5-benzenetriol and indoleacetic acid on rooting of hypocotyls of R. apiculata. A, Seawater control. B, 1,3,5-Benzenetriol (1 mg/ml). C, Indoleacetic acid (2 mg/ml). D, 1,3,5-Benzene-triol (1 mg/ml) + IAA (2 mg/ml).

than in root elongation. For instance, increase in number of roots was by 1.9-fold whereas in root length it was only 1.4-fold, as maximum effect of phenol-IAA synergism (Table 1). Of the phenols studied, 1,3,5-benzenetriol had promising synergistic effect with IAA in promoting the number of roots (Table 1) as reported in many fruit trees.2,3 Besides this, either gallic acid (1 mg/l) (Table 1) or IAA (0.5 and 0.05 mg/l) (Figure 1) could be used for efficient rooting of R. apiculata. The rooted seedlings easily got adapted in the soil when planted after the above treatments. Hence the treatments will be beneficial in raising vigorous seedlings in nurseries for conservation and management of the mangroves. This will augment easy adaptation of the seedling which is otherwise difficult.

Cimetidine-induced histopathological changes in testes of albino mice

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Cimetidine [N-cyano-N'-(methyl-N''-2-(5-methyl-1H-imidazol-4-yl)methylthioethylguanidine] is used as antiulcerative drug and is known to inhibit gastric acid secretion and to reduce pepsin output. It was tested on Swiss albino mice. Mice given 15 mg/kg of the drug orally showed damage of testicular elements just after one week of treatment. Testis sections showed coalescence of cells to form multinucleate bodies in most of the tubules whereas Sertoli cells, spermatogonia and spermatocytes appeared in their normal positions. However, after 2 weeks of treatment with the same dose, considerable damage in the tubules was evident. Even after the recovery phase, a significant alteration in the weight of testis with reduction in sperm count was observed.

A recent advance in medical management of peptic ulcer has been the introduction of the histamine H₂ receptor antagonist, Cimetidine. Most of the reports on Cimetidine showed that this antiulcerative drug has been associated with gynaecomastia as a side effect. Early toxicological studies with Cimetidine showed a decrease in prostate and seminal vesicle weights in rats and dogs treated with the drug orally2,3. Occasional reports of Cimetidine-associated gynaecomastia have appeared in the literature.8-6 Other side-effects noted in clinical usage suggested the possibility of interaction with androgen or estrogen receptors, which can affect the target organs. This prompted us to study the histopathological effect of Cimetidine on testes of albino mice.

Swiss albino male mice of Lacca strain, of age 75-100 days and weighing around 26 g, were used. They were

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Table 1. Effect of Cimetidine (15 mg/kg) consumption on ejaculate sperm count of mice.

<table>
<thead>
<tr>
<th></th>
<th>Ejaculate sperm content (×10⁶) (feeding phase)</th>
<th>Ejaculate sperm count (×10⁶) (recovery phase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.00 ± 0.15</td>
<td>5.21 ± 0.19</td>
</tr>
<tr>
<td>1 Week</td>
<td>4.41 ± 0.23</td>
<td>4.80 ± 0.19</td>
</tr>
<tr>
<td>2 Weeks</td>
<td>2.12 ± 0.25</td>
<td>3.50 ± 1.00</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n = 5); Statistically significant difference from controls, *P < 0.001*.

housed in plastic cages and allowed to adjust to the new environment for two weeks. Food pellets (Hindustan Lever) and tap-water were provided *ad libitum*.

A single dose of 15 mg/kg of Cimetidine was given orally using water as a vehicle for 1 and 2 weeks to groups of 10 animals each. The animals were sacrificed after 1 and 2 weeks (5 each) and their testes, seminal vesicles and prostate glands were weighed. Testes were fixed in Bouin’s, sectioned at 7 μm and stained with

Delafield’s haematoxylin/eosin for pathological analysis. Recovery was studied 15 and 30 days after discontinuation of drug to a group of animals (5 each). Testes, seminal vesicles and prostate glands of these animals were also weighed. Testes were then processed for histopathological analysis as described.

The method of Ratnasooriya was followed for the determination of the effect of Cimetidine ingestion on ejaculate sperm count. The ejaculate sperm content of male mice was determined a day before sacrificing. Male mice were individually paired with a single mature female in the male home cage (8 h/day). If mating had taken place, as determined by the presence of spermatozoa in the vagina, the females were

Figures 5-8. 5, TS of testis after 15 days of recovery following Cimetidine administration for 2 weeks. H&E, (×1000). 6, TS of testis showing normal interstitial elements and arrangements of germinal elements after 30 days of recovery following Cimetidine administration for 1 week. H&E, (×1000). 7, TS of testis showing vacuolation after 30-day recovery period of 2-week-treated mice. H&E, (×1000). 8, TS of testis after recovery for 30 days following 2-week-treatment, showing pyknosis and vacuolation (arrows) H&E, (×100).
Table 2. Changes in the weight of testis, prostate and seminal vesicle after 15 mg/kg of Cimetidine ingestion.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1 Week</th>
<th>2 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding phase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testis wt (g)</td>
<td>0.606 ± 0.021</td>
<td>0.459 ± 0.025</td>
<td>0.380 ± 0.020</td>
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<tr>
<td>Prostate wt (g)</td>
<td>0.125 ± 0.015</td>
<td>0.110 ± 0.014</td>
<td>0.098 ± 0.015</td>
</tr>
<tr>
<td>Seminal vesicle wt (g)</td>
<td>0.055 ± 0.001</td>
<td>0.052 ± 0.001</td>
<td>0.054 ± 0.002</td>
</tr>
<tr>
<td>Recovery phase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testis wt (g)</td>
<td>0.597 ± 0.020</td>
<td>0.551 ± 0.010</td>
<td>0.460 ± 0.015</td>
</tr>
<tr>
<td>Prostate wt (g)</td>
<td>0.130 ± 0.020</td>
<td>0.120 ± 0.015</td>
<td>0.100 ± 0.012</td>
</tr>
<tr>
<td>Seminal vesicle wt (g)</td>
<td>0.060 ± 0.002</td>
<td>0.058 ± 0.003</td>
<td>0.062 ± 0.002</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n = 5). Statistically significant difference from controls, *P* < 0.001 (Student’s *t* test).

Sacrificed via cervical dislocation. The uterine horns, Fallopian tubes and ovaries were exposed and freed from surrounding tissues. Haemostats were clamped on each Fallopian tube and the lower end of the vagina. The entire reproductive tract was removed and transferred to a 10 cm petri dish containing 0.9% saline at 37°C. The clamps were removed and the vagina and uterine walls were scraped gently to remove any embedded spermatozoa. The number of spermatozoa present was determined using an improved Neubauer haemocytometer (white cell dilution). Counts were performed in duplicate and were expressed as sperm count (× 10⁹).

Ejaculate sperm count, as determined during both phases of the investigation, is presented in Table 1. Treated mice showed a significant reduction in ejaculate sperm content (*P* < 0.001) compared to the control mice. Analysis of male ejaculate content after recovery phase also showed significant reduction in sperm content after 30 days.

Table 2 shows a significant decrease in the weights of testis and prostate although seminal vesicle appeared to be normal after Cimetidine ingestion.

Histopathological analysis of the testes revealed significant alterations. Spermatogenesis was observed in some central tubules of 1 week-treated mice (Figure 1) but there was high incidence of multinucleated giant cells in most of the tubules (Figure 3). After 2 weeks of treatment the effect was more deleterious as there were vacuolations and displacements of the germinal elements (Figures 2 and 4). When observed after the recovery periods of 15 and 30 days the damage to the testes appeared to be reversible after 1-week treatment (Figures 6 and 7). However, after 2-week treatment, various forms of architectural disarrangements (vacuolization, karyolysis, pycnosis) were very common in most of the tubules even after the recovery phase (Figures 5 and 8).

The present investigations were undertaken to evaluate the effect of Cimetidine on the testes of male albino mice. In addition to the lowered sperm counts and altered testicular weights following Cimetidine ingestion, histopathological studies revealed irreversible damage to the testes after 2 weeks of treatment. In concurrence with the present results the male rat pups exposed to Cimetidine during late gestation have been reported to appear phenotypically 'feminized' at birth and having smaller testes with lower testosterone level. They even demonstrated reduced sexual performance when studied in later adult life distant from any exposure to the drug.

It has also been observed that Cimetidine interacts with androgen receptors but fails to initiate a post-receptor message, which is the cause for the significant decrease in the weights of testes and prostate. Moreover, the clinical usefulness of Cimetidine is occasionally inhibited as a result of such actions, which account, at least in part, for the impotence, loss of libido, reduction in sperm counts and gynaecomastia seen in some men who use the drug.


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**Serum ferritin and cholesterol levels in B and EB thalassaemia**

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In multitransfused B and EB thalassaemic children, level of serum ferritin, an index of depot iron, is inversely related to serum cholesterol level. The observation suggests that excess body iron has an inhibitory action on the biosynthesis of cholesterol.

Iron overload is a serious problem with multitransfused individuals like B and EB thalassaemias because each unit of 250 ml blood that is transfused adds approximately 150 mg of iron to the body stores. The body's limited ability to get rid of the excess iron results in a...