Effect of Phenytoin Sodium on Reproductive Parameters in Adult Male Wistar Rats

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ABSTRACT
Objective: To assess the effects of phenytoin sodium on rat sperm morphology, sperm count, motility and histopathological changes in testis
Design: Experimental study
Setting: Kasturba Medical College, Manipal, Karnataka State, India
Materials and Methods: Male Wistar rats (13-14 weeks old) were treated with phenytoin sodium and sacrificed at the end of 2nd, 4th, 5th, 7th, 10th and 15th week after the last exposure to phenytoin sodium. Epididymal sperm count, sperm motility, sperm morphology and histopathology of testes were analysed.
Results: Sperm count and sperm motility were decreased significantly by phenytoin sodium. The percentage of abnormal sperms increased significantly in a time dependent manner. Histopathological study revealed that phenytoin sodium caused sloughing of epithelial cells in the testis.
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KEY WORDS: histopathology, phenytoin sodium, sperm count, sperm morphology, sperm motility

INTRODUCTION
Mainly three factors contribute to sterility in the modern world; the trend for couples to delay having children after marriage, the rise in sexually transmitted diseases and a puzzling drop in sperm production in males. Federal agencies, such as the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) and international bodies like Organization for Economic Co-operation and Development (OECD) provide a number of test protocols and guidelines for identifying the adverse reproductive effects. These protocols and guidelines are used by the industry to test pesticides, industrial chemicals, pharmaceuticals and food additives for potential reproductive toxicity in laboratory animals.

All of this information is critical for performing a risk assessment of the chemical and ultimately, for making regulatory decision about the allowable uses of the substance as well as the labeling requirements. For many years, clinicians have searched for the best end point in semen that could be used to predict the fertility in individual men.

Spermatogenesis is an acyclic, well-organized, highly co-ordinated process that encompasses different cell associations called stages. The maintenance of adult mammalian spermatogenesis is dependent upon the steroid hormone testosterone, which is produced by testicular leydig cells in response to the secretion of pituitary luteinizing hormone. Previous studies of spermatogenesis in the rat have shown that the experimental reduction of intratesticular testosterone to low enough levels results in germ cell loss and that the re-administration of testosterone restores spermatogenesis. Sperm count is one of the most sensitive tests for spermatogenesis, since, it gives the cumulative result of all stages in sperm production, and it is highly correlated with fertility. It is absolutely necessary that epileptic patients receive long-term therapy with antiepileptic drugs. Some antiepileptic drugs such as phenytoin, sodium valproate, primidone and phenobarbitol have been suspected to be gonadotoxic, mutagenic and teratogenic. Antiepileptics diminish sexual potency and fertility in young male epileptics. The mutagenic changes...
have proportional relation with carcinogenesis\textsuperscript{[16]}.
This is alarmingly problematic especially in children, since these effects last longer affecting fertility and / or forming basis for carcinogenesis. Phenytoin is excreted in human semen in small quantities and this may possibly affect the testosterone levels. Reduced plasma concentrations of free testosterone have been detected in male epileptic patients receiving phenytoin.

Meng \textit{et al}\textsuperscript{[17]} observed possible mutagenic effect of phenytoin on human sperm cells. According to Russel and Russell\textsuperscript{[18]}, male germ cells are very ideal and easy for the study of the genotoxicity of drugs since they exist in different phases of cell development and differentiation. Genotoxic effects of the drug would result in morphologically abnormal sperms and therefore the counting and classification of the types of abnormal sperm can determine the presence and extent of genotoxicity\textsuperscript{[19]}. The architectural makeup of stages within seminiferous tubules and atypical cell types within stages varies with the level of efficiency of spermatogenesis, and this variation may reflect differences in yield of early spermatogonial divisions that are responsible for generating the different stages. Increase in tubular length along with diameter seems to be a continuous process until puberty. Testis tubule diameter is directly correlated with testicular weight\textsuperscript{[20]}. Histopathology on reproductive tissues is valuable for male reproductive toxicity assessment. Histological evaluations can be especially useful because they are a relatively sensitive indicators of damage and they provide information on toxicity from a variety of protocols. In addition, histological data can provide information on site (including target cells) and extent of toxicity after short-term testing and can also indicate the potential for recovery. The quality of histological analyses of spermatogenesis is improved by proper fixation and embedding of testicular tissue\textsuperscript{[21-23]}.

In view of the above findings, the present study was designed to investigate the effects of phenytoin sodium on rat testis.

**MATERIALS AND METHODS**

**Animals**

In the present study, adult male Wistar rats (13 - 14 weeks old) weighing 150-200 g were used. Breeding and maintenance of animals were done according to the guidelines of Committee for the purpose of Control and Supervision of Experiments on Animals; and Animal Welfare Division, Government of India, for the use of laboratory animals. The Institutional Animal Ethical Committee approval was obtained before starting the study. All animals were housed in polypropylene cages using paddy husk bedding at 28 ± 1°C temperature and 50 ± 5% humidity. Animals were fed on laboratory chow and tap water \textit{ad libitum}.

All experimental activities are carried out between 8 - 10 AM.

One hundred and forty-four rats were segregated into 24 groups of six animals each. Six groups each were treated with 0.1 ml of distilled water, gum acacia control, phenytoin sodium 50 mg and phenytoin sodium 100 mg for 60 days. Animals were sacrificed by terminal anesthesia (Pentobarbital sodium, 45 mg/kg, Sigma Chemicals Co.) at the end of 2\textsuperscript{nd}, 4\textsuperscript{th}, 5\textsuperscript{th}, 7\textsuperscript{th}, 10\textsuperscript{th} and 15\textsuperscript{th} week after the last exposure to phenytoin sodium. The animals were weighed before sacrificing. The sacrifice time points – weeks 2, 4, 5, 7, 10 and 15 represent the sampling of spermatozoa in the epididymis and testis, spermatids, secondary spermatocytes, primary spermatocytes, spermatogonia and stem cells during treatment, respectively\textsuperscript{[24, 25]}.

This study was approved by the ethical committee of the hospital.

**Chemicals**

The powdered form of phenytoin sodium was obtained from Cadila Health Care Ltd. The dose and route of administration was based on earlier studies\textsuperscript{[26, 27]}. The required quantity of phenytoin was weighed just before treatment and dissolved in 10 ml of gum acacia (0.2 g gum acacia dissolved in 10 ml of distilled water) and administered orally for 60 days.

**Epididymal sperm count and motility**

The rats were sacrificed and laprotomy was conducted to expose the reproductive system. The latter was removed and placed in phosphate buffered saline, and the epididymis was separated. Both the testes from each animal were removed and weighed on an electronic balance. The sperm suspension was prepared by mincing the cauda epididymis in 1 ml of phosphate buffered saline (pH 7.2). Then the suspension was filtered through 80 µm nylon mesh to remove the tissue fragments. An aliquot (0.05 ml) from the sperm suspension (1 ml) was diluted with 1:40 phosphate buffered saline and mixed thoroughly. After discharging a few drops, a sample of the diluted sperm suspension was introduced into a Neuberger’s improved counting chamber (ROHEM India, depth 0.1 mm). The sperms present in 8 squares except in the erythrocyte area were counted, and then the total count was multiplied by 5 x 10\textsuperscript{4} to obtain the number of sperms per epididymis\textsuperscript{[28, 29]}. A minimum of 100 sperms were observed for motility and percentage of motile sperms was recorded for each animal.

**Sperm morphology assay**

A fine suspension was made and stained with 0.2 ml of 1% aqueous eosin. About one drop of stained suspension was placed on the clean slide. It was dried, cleaned and mounted in DPX. Slides were examined...
for sperm shape abnormality. 1000 sperms / animal were scored. Sperms were classified into normal and abnormal sperms. The abnormal sperms were classified under head abnormalities and tail abnormalities. The head abnormalities were classified as amorphous, hookless, banana shaped, double headed, and bent. The tail abnormalities were classified as coiled / folded and double tailed [9,30].

Histopathology of testis
The testes / epididymis were removed and fixed in Bouin's fluid for 24 hrs. After excessive washing in 70% alcohol, the tissue was processed for paraffin embedding and 5µ thick paraffin sections were stained with haematoxylin and eosin (Culling et al, Cellular pathology technique)[28].

The sections were analyzed for the presence or absence of vacuoles, gaps and abnormal cells.

Seminiferous tubular diameter (STD) and epithelial height (SEH): The diameters of 20 transversely cut tubules were measured using ocular micrometer calibrated with the stage micrometer (Erna Opticals, Japan). In each tubule, two measurements were made, one perpendicular to the other and their average is taken. The epithelial height was measured in 10 tubules for each animal. In each tubule, the SEH was measured from the basement membrane to the surface of the epithelium at two different regions and the mean was taken.

Statistical analysis
For each group six animals were used and mean ± SD (standard deviation) was calculated. Results obtained from the present study were correlated and analyzed by Analysis of Variance (ANOVA). Values of p < 0.05 were considered statistically significant.

RESULTS
Effect on body weight
There was no significant difference between the control group and phenytoin treated group on body weight of rats.

Effect of on sperm count
There was a significant decrease in sperm count during the 2nd, 4th, 5th and 7th week sampling time. The recovery was almost the same in both the doses and complete recovery was observed by the 15th week. The least number of sperm count was observed during the 5th week sampling time for both the doses. The time response graph shows a significant decrease in the sperm count in a linear manner up to the 5th week and during the 7th week sampling time it shows a marginal increase in sperm count which reaches the control values by the 15th week sample time (Table 1).

Effect on sperm motility
On the second week of sampling time there was a significant reduction in the motility of sperms in rats treated with 100 mg/kg of phenytoin sodium. Even though reduction of sperm motility was seen

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Table 1. Effect of phenytoin sodium on sperm count (x 10^6)

<table>
<thead>
<tr>
<th>Dose</th>
<th>2 weeks</th>
<th>4 weeks</th>
<th>5 weeks</th>
<th>7 weeks</th>
<th>10 weeks</th>
<th>15 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>53.5 ± 1.85</td>
<td>54.33 ± 1.36</td>
<td>55.5 ± 1.04</td>
<td>56.66 ± 1.36</td>
<td>55.66 ± 2.87</td>
<td>55.5 ± 1.04</td>
</tr>
<tr>
<td>Gum acacia control</td>
<td>53.16 ± 0.98</td>
<td>54.5 ± 1.63</td>
<td>54.66 ± 1.63</td>
<td>51.5 ± 1.87</td>
<td>53.16 ± 2.31</td>
<td>54.83 ± 1.16</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>45.66 ± 3.72***</td>
<td>38.33 ± 2.42***</td>
<td>32.3 ± 1.5***</td>
<td>37 ± 1.09***</td>
<td>50.6 ± 2.94</td>
<td>54.66 ± 1.21</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>40.16 ± 6***a</td>
<td>33.5 ± 1.04*** aa</td>
<td>25 ± 0.89*** aaa</td>
<td>34.16 ± 1.32*** a</td>
<td>50 ± 2.89</td>
<td>53 ± 2.36</td>
</tr>
</tbody>
</table>

Each dose from particular time represents mean ± SD from six animals. Significant values are, normal control Vs. treated *** p < 0.001; 50 mg/kg Vs. 100 mg/kg ap < 0.05, aap < 0.01, aaap < 0.001.

Table 2. Effect of phenytoin sodium on sperm motility

<table>
<thead>
<tr>
<th>Dose</th>
<th>2 weeks</th>
<th>4 weeks</th>
<th>5 weeks</th>
<th>7 weeks</th>
<th>10 weeks</th>
<th>15 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>44.16 ± 1.32</td>
<td>44.16 ± 1.16</td>
<td>44.5 ± 1.76</td>
<td>44 ± 1.41</td>
<td>42.5 ± 1.04</td>
<td>42.66 ± 1.36</td>
</tr>
<tr>
<td>Gum acacia control</td>
<td>43.66 ± 1.75</td>
<td>43.33 ± 2.33</td>
<td>42.83 ± 1.16</td>
<td>43.5 ± 1.22</td>
<td>42.83 ± 0.75</td>
<td>43.16 ± 1.72</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>41 ± 1.67*</td>
<td>36 ± 1.41***</td>
<td>32 ± 1.67***</td>
<td>38.66 ± 1.03***</td>
<td>40.33 ± 1.63</td>
<td>43.33 ± 0.81</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>38.33 ± 1.21***</td>
<td>33.83 ± 1.47***</td>
<td>29.16 ± 1.32***a</td>
<td>34.16 ± 1.32*** a</td>
<td>39.66 ± 1.27*</td>
<td>42 ± 1.09</td>
</tr>
</tbody>
</table>

Each dose from a particular time represents mean ± SD from six animals. Significant values are; normal control vs. treated, *p < 0.05, ***p < 0.001; 50 mg/kg vs. 100 mg/kg, ap < 0.05, aap < 0.01.
in the rats treated with 50 mg/kg during the 2nd week sampling time it was not as significant as the decrease in sperm motility observed in rats treated with the higher dose. The sperm motility was least during the 5th week sampling time in both the doses of the drug. The recovery period was slightly faster for the rats treated with 50 mg/kg and complete recovery was seen by the 10th week sampling time (Table 2). However, the recovery period for the rats treated with 100 mg/kg took longer time and reached complete recovery by the 15th week sampling time.

Even though there was reduced motility in the 100 mg/kg treated rats when compared to the 50 mg/kg treated rats, significance was observed only during the 5th and 7th week sampling time.

Effect on sperm morphology
No significant difference in the percentage of abnormal sperms was found between the drug treated and control groups during the 2nd week sampling time. The incidence of abnormal sperms progressively increased from the 4th week sampling time to the 7th week sampling time (Table 3). Coiled or folded sperms were the most commonly seen abnormality in all the sampling times studied. However, the number of abnormal sperms with amorphous head and hookless heads was also common during all the sampling weeks. Phenytoin sodium seems to affect the morphology of sperm in a time dependent manner. The percentage of abnormal sperm was highest during the 7th week sampling time in rats treated with 100 mg/kg as well as with rats treated with 50 mg/kg (Table 3). The recovery period was similar for both 100 mg/kg and 50 mg/kg treated rats. The percentage of abnormal sperms reached closer to the control values in both the doses of the drug during the 10th week sampling time. Complete recovery was observed in the 15th week sampling time (Table 4).

Effect on the microscopic architecture of testes
Sloughing was observed in the treated groups. A phenomenal increase in sloughing was observed in rats treated with 100 mg/kg during the 5th week. The presence of vacuoles was also observed in the 5th and 7th week at both the doses (Figs. 1, 2 and 3).

### Table 3: Effect of phenytoin sodium on incidence of abnormal sperms in seven week sampling time

<table>
<thead>
<tr>
<th>Drug/ Dose</th>
<th>Sample time (weeks)</th>
<th>Normal sperm</th>
<th>Abnormal Sperms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Amorphous</td>
</tr>
<tr>
<td>Normal Control</td>
<td>7</td>
<td>6.16 ± 2.31</td>
<td>3.83 ± 0.75</td>
</tr>
<tr>
<td>Gum acacia</td>
<td>7</td>
<td>4.33 ± 2.06</td>
<td>3.83 ± 1.47</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>7</td>
<td>16.66 ± 2.73</td>
<td>19.16 ± 2.48</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>7</td>
<td>20.83 ± 1.47</td>
<td>18.83 ± 2.92</td>
</tr>
</tbody>
</table>

Data represented as mean ± SD from six animals. Significant values are; normal control Vs. treated, ***p < 0.001. No significant differences were found between 50 mg Vs. 100 mg groups.

### Table 4: Effect of phenytoin sodium on incidence of abnormal sperms in fifteen week sampling time

<table>
<thead>
<tr>
<th>Drug/ Dose</th>
<th>Sample time (weeks)</th>
<th>Normal sperm</th>
<th>Abnormal Sperms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Amorphous</td>
</tr>
<tr>
<td>Normal Control</td>
<td>15</td>
<td>4.83 ± 2.48</td>
<td>5.16 ± 0.75</td>
</tr>
<tr>
<td>Gum acacia</td>
<td>15</td>
<td>5.66 ± 3.55</td>
<td>4.16 ± 0.75</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>15</td>
<td>6.16 ± 2.71</td>
<td>5.16 ± 0.98</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>15</td>
<td>5.66 ± 2.25</td>
<td>5.16 ± 0.98</td>
</tr>
</tbody>
</table>

Data represented as mean ± SD from six animals. No significant differences were found between normal control Vs. treated groups.
2nd week, the incidence of sloughing was elevated by both the doses and showed progressive increase till the 7th week. However, complete recovery was observed by the 15th week. The cytoarchitecture of the epididymis did not show any disturbance.

**Effect on gross architecture of testes**

Weight of the testes was not altered. At higher dose the seminiferous tubular diameter showed a linear decrease as the sampling weeks increased and highest drop in the diameter was seen during the 7th week. However at the lower dose the tubular diameter showed a significant increase in diameter during the 2nd week and showed gradual decrease in the diameter in the following sampling weeks. The least tubular diameter was seen during the 5th sampling week. Recovery period was same for both the doses and returned to normal control levels by the 15th week (Table 5).

At the 2nd week there was significant decrease in the epithelial height regardless of the dose. Similar trend was also seen during the other sampling weeks and recovery to normal height was seen only by the 10th week. Even though dose dependent decrease was seen in epithelial height during the sampling time, values were not significant. The peak decline of epithelial height was observed during the 5th week for both the doses. The time response graph shows that phenytoin sodium affects the epithelial height of the seminiferous tubules in a time dependent manner (Table 6).

**DISCUSSION**

Sperm count is one of the most sensitive tests for spermatogenesis, since, it gives the cumulative result of all stages in sperm production, and it is highly correlated with fertility[16]. Our results show that phenytoin sodium is cytotoxic to the sperm since it decreased the sperm count significantly.

<table>
<thead>
<tr>
<th>Dose</th>
<th>2 weeks</th>
<th>4 weeks</th>
<th>5 weeks</th>
<th>7 weeks</th>
<th>10 weeks</th>
<th>15 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>280.2 ± 6.26</td>
<td>284.3 ± 7.4</td>
<td>286.4 ± 5.6</td>
<td>288.4 ± 5.73</td>
<td>301.4 ± 7.35</td>
<td>315.7 ± 4.34</td>
</tr>
<tr>
<td>Gum acacia control</td>
<td>288.9 ± 5.72</td>
<td>286.7 ± 7.04</td>
<td>286.3 ± 5.07</td>
<td>285.1 ± 6.93</td>
<td>302.6 ± 6.27</td>
<td>313.7 ± 5.75</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>302.2 ± 6.26*</td>
<td>273 ± 4.18</td>
<td>266.4 ± 5.73***</td>
<td>275 ± 3.09**</td>
<td>315.5 ± 7.03*</td>
<td>320.2 ± 3.01</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>286.2 ± 1.22aa</td>
<td>266.3 ± 6.6**</td>
<td>245.2 ± 4.28***aaa</td>
<td>240.3 ± 3.02*****aaa</td>
<td>300.6 ± 6.35a</td>
<td>316.7 ± 5.05</td>
</tr>
</tbody>
</table>

Each dose from a particular time represents mean ± SD from six animals. Significant values are, normal control Vs. treated *p < 0.05, **p < 0.01, ***p < 0.001; 50 mg/kg Vs. 100 mg/kg, ap < 0.05, aap < 0.01
in a linear manner from 2nd to 7th week sampling time, regardless of the dose. In adult mice, duration of spermatogenic cycle is 34 to 35 days\cite{10,17} and these sample times correspond to spermatids and spermatocytes in origin respectively. However, the duration of spermatogenic cycle in rats is 52 to 60 days and our findings point out that the germ cells affected are approximately the spermatids, spermatocytes and spermatogonia. The sperm count returned close to normal values by the end of the 10th week. Phenytoin sodium appears to be more toxic to the spermatocytes as the sperm count was least by the end of the 5th week.

Phenytoin sodium decreased the percentage of sperm motility in a time dependent manner. Similar results were observed by earlier workers\cite{17,31,32}. The decrease in motility was observed right from the 2nd week sample time and complete recovery was observed by the end of 10th week. The sperm motility largely depends on the microtubular apparatus of the sperm tail\cite{33}. In the current study it was also observed that a considerable number of abnormal sperms were with a defect in their tail. It is possible to confirm that sperm motility might have been hindered mainly because of the presence of a large number of abnormal sperms between the 2nd and 7th week as well as the interference of these drugs on the sperm membrane.

Phenytoin sodium increased the number of abnormal sperms in a time dependent manner. Sperm abnormalities were observed in both doses. The number of dysmorphological sperm was significantly high during the 4th to 7th week sampling time. It is essential that, to label any drug as mutagen, it should induce double the sperm abnormality compared to the control level\cite{11}. Phenytoin sodium treatment resulted in more than double the percentage of abnormal sperms and hence they could be considered as mutagens. Their mutagenic effect was more pronounced during the 4th to 7th week sampling time. As mentioned earlier, motility of sperm was also least during these sampling weeks, which indicates that sperm motility and morphology are related to each other. The higher dose of phenytoin sodium induced highest percentage of abnormal sperms at the end of 7th week. This indicates that spermatogonia are more vulnerable to phenytoin sodium. However, it is not known as to how this drug affect the fertility of rats and, moreover, it is not known how much percentage of abnormal sperms are required for infertility to occur. Earlier reports however suggest that the tail and head deformities are indicators of infertility\cite{34,35}. Moreover, it is not possible to say whether these antiepileptics induce any chromosomal aberrations or mutations in germ cells as this assay identifies only point mutations. Future studies could therefore address these specific effects of phenytoin sodium.

A change in the weight of testes is an indicator of reproductive toxicity. In the current study, weight of the testes in the treated group did not differ significantly from that of the control group. It may be because of the lower dose in addition to the shorter duration of treatment. It must be considered that changes in the other important end points related to reproductive function may not be accompanied by a change in organ weight. A significant increase or decrease in testis weight can indicate an adverse effect, but it can be due to processes other than seminiferous tubular damage, such as edema, inflammation, cellular infiltration, Leydig cell hyperplasia or fluid accumulation due to blocked efferent ducts\cite{36,37}. Therefore, it is insufficient to evaluate organ weight alone to assess reproductive toxicity of an agent, as other end-points may be more sensitive indicators.

The present study confirms that phenytoin sodium decreases the diameter of the seminiferous tubules. On histological evaluation it had induced the formation of vacuoles and sloughing during the 2nd to 7th week sampling time. In some of the tubules structural deformity of the Sertoli cells were also observed, more so with the higher dose. The occurrence of vacuoles was more frequent during the 5th and 7th week sampling time. In the case of phenytoin sodium the tubular diameter showed

<table>
<thead>
<tr>
<th>Dose</th>
<th>Sampling Time</th>
<th>2 weeks</th>
<th>4 weeks</th>
<th>5 weeks</th>
<th>7 weeks</th>
<th>10 weeks</th>
<th>15 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>89.89 ± 3.87</td>
<td>90.31 ± 3.41</td>
<td>90.78 ± 3.10</td>
<td>89 ± 3.16</td>
<td>90.15 ± 3.36</td>
<td>90.89 ± 3.35</td>
<td></td>
</tr>
<tr>
<td>Gum acacia control</td>
<td>89.15 ± 3.27</td>
<td>91.05 ± 3.18</td>
<td>90.78 ± 3.33</td>
<td>88.10 ± 3.50</td>
<td>89.15 ± 3.03</td>
<td>91.1 ± 3.33</td>
<td></td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>80.6 ± 3.23**</td>
<td>75.35 ± 3.25***</td>
<td>68.25 ± 3.41***</td>
<td>72.9 ± 3.53***</td>
<td>85.65 ± 3.40</td>
<td>89 ± 3.34</td>
<td></td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>77.05 ± 4.14***</td>
<td>70.5 ± 3.68***</td>
<td>65.25 ± 3.617***</td>
<td>71.85 ± 3.48***</td>
<td>86 ± 3.22</td>
<td>89 ± 3.36</td>
<td></td>
</tr>
</tbody>
</table>

Each dose from a particular time represents mean ± SD from six animals. Significant values are, normal control Vs. treated, **p < 0.01, ***p < 0.001. No significant differences were found between 50 mg/kg Vs. 100 mg/kg groups.
a slight increase by the end of the 2nd week in rats treated with the lower dose whereas the tubular diameter did not show any significant change with the higher dose. The increase in tubular diameter with the lower dose of phenytoin may be due to the induction of secretion of seminiferous tubular fluid as seen after the ligation of efferent ductules in the rat[38].

Intratesticular testosterone is thought to play a very important role in spermatogenesis; however, it is very rarely measured. According to Bauer et al[39], and Kuhn-Velten et al[40], valproate and phenytoin act directly on the testis to inhibit testosterone synthesis by the Leydig cells. It is now established that lowering of intratesticular testosterone concentration results in the apoptotic death of some germ cells (e.g., pachytene spermatocytes) in association with nuclear DNA fragmentation in the dying cells[41,42]. Previous studies of spermatogenesis in the rat have shown that the experimental reduction of intratesticular testosterone to low enough levels results in germ cell loss[43] and that the re-administration of testosterone restores spermatogenesis[7,8]. Androgen receptor expression in the seminiferous epithelium is restricted to the somatic Sertoli cells[43]. Therefore, in response to changes in intratesticular testosterone concentration, the Sertoli cell presumably communicates a signal to the attached and developing germ cells, which lack the androgen receptor, resulting either in the loss or restoration of germ cells[44].

Regulation of the reproductive axis begins at the level of the hypothalamus where neurosecretory cells synthesize and release gonadotropin-releasing hormone (GnRH) in a pulsatile fashion into the hypothalamic-hypophysial-portal circulation. In both men and women, gonadal failure results in increased LH, from loss of the negative feedback of estrogen at the hypothalamus and pituitary in women and from decreases in both androgen and estrogen feedback in men. In response to the decreased levels of the sex steroids and the loss of inhibin, FSH levels are also elevated following gonadal damage. Luteinising Hormone (LH), Follicle Stimulating Hormone (FSH), and testosterone are commonly analyzed. The sensitivity of this approach is limited, however, since serious disturbances in spermatogenesis are often observed with normal FSH[45]. The male reproductive system can be affected adversely by disruption of the normal endocrine balance. In adults, effects that interfere with normal concentrations or action of LH and/or FSH can decrease or abolish spermatogenesis, affect secondary sex organ (e.g., epididymis) and accessory sex gland (e.g., prostate, seminal vesicle) function and impair sexual behavior[46]. Significant alterations in circulating levels of testosterone, LH or FSH may indicate pituitary or gonadal injury and may be related to alterations in spermatogenesis, sperm maturation, mating ability or fertility. Furthermore, such hormonal effects can help understanding of the site or mechanism of toxicant action, especially for short-term exposures.

CONCLUSION

Findings from this study point out the gonadotoxic and cytotoxic potential of phenytoin sodium. Hence the work should pave way for the rational use of this drug unambiguously to control the adverse effects without compromising its efficacy.

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