VIA MEDICA

ORIGINAL ARTICLE

Folia Morphol.
Vol. 64, No. 2, pp. 65–71

ISSN 0015–5659
www.fm.viamedica.pl

The antiviral drug ribavirin reversibly affects the reproductive parameters in the male Wistar rat

K. Narayana[1], Urban J.A. D'Souza[2], Prashanthi Narayan[1], Ganesh Kumar[3]

[1]*Department of Anatomy, Centre for Basic Sciences, Kasturba Medical College, Bejai, Mangalore, Karnataka, India*
[2]*Department of Physiology, School of Medical Sciences, PPSP University Science Malaysia, Kubang Kerian, Kelantan, Malaysia*
[3]*Department of Physiology, International Center for Health Sciences, Madav Nagar, Manipal, Karnataka, India*

[Received 21 October 2004; Revised 6 February 2005; Accepted 11 February 2005]

The present study was planned to evaluate the toxic effects of ribavirin on the reproductive parameters in the male Wistar rat. Rats (11–13 weeks old) were treated with 5 injections (i.p.) of 20, 100 or 200 mg/kg/day ribavirin at intervals of 24 h. The testes were processed for histopathological analysis on days 14, 35, 70 and 105 after the last exposure. The parameters studied were body weight, the weights of the testis, epididymis, seminal vesicle and prostate, seminiferous tubular diameter (STD), epithelial height (SE), epithelial sloughing, incidence of stage XIV tubules, sperm abnormality and total serum level of testosterone. Data were analysed by ANOVA and the Bonferroni post hoc test for significances between different groups. There was a decrease in body weight and organ weights, excluding those of the testis and epididymis, against control at higher dose-levels. Ribavirin induced the formation of vacuoles, gaps and sloughing of the seminiferous epithelium. The STD, SE and the incidences of stage XIV tubules decreased on days 14 and 35. Ribavirin also induced the formation of sperm with microcephaly and cephalocaudal junction defects, with or without fibrils jetting out. All these morphological defects recovered to control limit by day 105. The serum level of testosterone was decreased at all dose-levels and time points, although recovery had started by day 105. In conclusion, ribavirin is gonadotoxic in male rats but the effects are reversible after a period of 105 days. However, the endocrine-disrupting properties of ribavirin persist beyond this period.

Key words: antiviral drugs, germ cells, testosterone, testis, cytotoxicity, gonadotoxicity

INTRODUCTION

Ribavirin (1-β-D-ribofuranosyl-1, 2, 4 triazole-3--carboxamide) is a unique antiviral drug that is clinically effective against unrelated viruses, including families of paramyxoviruses, flaviviruses, picornaviruses, orthomyxoviruses, arena viruses and reoviruses [4]. Although its mechanism of action is uncertain, it is known to inhibit the activity of inosine monophosphate dehydrogenase [9]. The toxicity profile of this drug, especially on the genetic material and reproductive system, has not been properly understood. Ribavirin has acted as a genotoxic agent in the mouse bone marrow [19] although other studies have not considered it as severe a mutagen as other antiviral

Address for correspondence: Dr. K. Narayana, Department of Anatomy, Centre for Basic Sciences, Kasturba Medical College, Bejai, Mangalore, 575004. Karnataka, India, tel: ++91 824 2211746 (o), fax: ++91 824 2428183, e-mail: narayana68@yahoo.com

drugs [18]. Reports are available, which reveal its toxicity on haematological parameters and, therefore, induced abnormal clinical conditions in both animals and humans [1]. Recently, the suppressing effects of ribavirin at dose-levels of 20–200 mg/kg have been demonstrated on the sperm-population in the epididymis of the rat in a dose-dependent and time-dependent pattern [15]. This result was due to the toxicity of the drug on germ cells, exerted in terms of inhibition of cell renewal and acceleration of cell death akin to apoptosis [6]. Evaluation of mutagenicity by a sperm morphology test in the rat at dose-levels of 20–200 mg/kg revealed an increased incidence of abnormal sperm, probably as a result of mutations, although these effects were transient [16]. Serious effects, such as microcephaly and midpiece defects in sperm were not evaluated in the above study. Hoffmann et al. [12] reported that ribavirin could not induce any dominant lethal mutations, hence concluding that ribavirin had no impact whatsoever on the fertility of the rat. In the light of the above findings, the present study has been undertaken to evaluate the effect of ribavirin on the reproductive parameters in the rat.

MATERIAL AND METHODS

Animals

Male Wistar rats (11–13 weeks old) were housed in polypropylene cages with paddy husk bedding under controlled temperature and humidity. They were fed on laboratory chow and tap water *ad libitum*. All experiments were conducted in accordance with the institutional ethical committee guidelines and those of the Government of India.

Treatment and tissue processing

The rats were segregated into 16 groups of 5 rats each. Of these, 4 groups served as controls, receiving only water. Ribavirin (Virazole, ICN Pharmaceuticals, Inc., California, Lot No. 94 J 02) was administered (i.p.) at dose-levels of 20, 100 or 200 mg/kg in such a way that 4 groups received 20 mg/kg, another 4 groups received 100 mg/kg, and the remaining 4 groups received 200 mg/kg, for 5 consecutive days at intervals of 24 h. The dose selection and the route of exposure were based on earlier studies [15, 16, 19], and 20 mg/kg was an antiviral dose, whereas the other doses used were higher to test the adverse effects at higher dose-levels. On days 14, 35, 70 and 105 following the last exposure 4 sample points were selected, based on a previous study [15]. On each of the days assigned for sacrificing the rats one control group and one group from each of the three dose-levels was randomly selected, each group consisting of 5 animals. The animals were anesthetised (Pentabarbitone sodium, 45 mg/kg, Sigma Chemicals), and the thoracic wall was excised to expose the heart. The blood was collected from the heart for the evaluation of testosterone in the serum (*vide infra*). The testis was fixed in Bouin's fluid [20] (on all sampling points), and the epididymis, seminal vesicle and prostate (at day 35 after exposure only) were fixed in 10% buffered formalin, and then processed for paraffin embedding [5]. The body weights of the animals were measured up to day 14 and the organ weights were measured on day 35, with the exception of the testis, which was measured on all sampling points.

Sperm morphology test

The epididymes of the control and treated rats (from the 35-day sample only) were minced in 1 ml of phosphate buffered saline (pH 7.2) on day 35 after the last exposure. The suspension was then filtered through 80 μ nylon mesh and the filtrate was stained with 1% eosin Y and smears were prepared on clean glass slides [16]. In all 1,000 sperm per animal were examined and sperm with microcephaly and those with cephalocaudal bending with or without fibrils jetting out were counted and expressed as percentage incidence.

Staining and histopathological analysis

Sections of 5 micron in thickness (Jung-Biocut, Leica, 2035, Germany) were stained with haematoxylin and eosin and also by PAS-haematoxylin [5]. Testes were analysed for qualitative changes by observing the vacuoles, gaps, epithelial sloughing and abnormal cells in the epithelium. The seminiferous tubule diameter (STD) and epithelial height (SE) were measured using an ocular micrometer, calibrated with the stage micrometer. The stage VII tubules only were selected to avoid any stage-dependent variations in morphometrical values. In each testis 10 transversely cut seminiferous tubules were selected for measuring STD and SE. In each tubule both maximum and minimum diameters were measured and then their average taken. In the same tubule the SE was measured from the basement membrane to the surface of the epithelium at 2 locations and their average taken. Finally, the representative values of STD or SE for each animal were obtained by taking the averages of 10 measurements each. In each testis

100 tubular sections were screened continuously and tubules that were encountered with epithelial sloughing and stage XIV of the seminiferous cycle were subsequently recorded to express their percentage incidence [11]. In each testis 10 inter-tubular spaces were selected and the number of Leydig cells was counted. An average of 10 counts served as the number of Leydig cells/inter-tubular space for that animal.

Estimation of testosterone level

Total serum level of testosterone was estimated by Automated Chemiluminescence System (ACS 180; Chiron Diagnostics Corporation, East Walpole, USA) in accordance with the manufacturer's instructions.

Statistical analysis

The data were expressed as means ± SD or SEM for each group (5 animals per group) and subjected to statistical analysis by one-way Analysis of Variance for differences between groups. Multiple comparisons were performed by the Bonferonni post hoc test. The level of significance was set at $p < 0.05$.

RESULTS

There were no qualitative changes in the control testes and they showed normal cell associations in the seminiferous tubules (Fig. 1A). Occasionally, the gaps (Fig. 1B) or vacuoles (Fig. 1C) were seen in the seminiferous epithelium of treated testes on days 35 and 70. Testes from the treated rats showed epithelial sloughing (Fig. 1D) and dark basophilic cells on days 14 and 35. Ribavirin caused neither the formation of multinucleated cells nor tubular atrophy at any dose-level or time point. Body weight had decreased by day 6 at 100 mg/kg ($p < 0.05$) and on day 14 at two higher dose-levels ($p < 0.05$–0.01; Fig. 2). On the other hand, there was no significant effect on the weights of the testis or the epididymis at any of the dose-levels or sampling points tested (data not shown). The weights of the seminal vesicles and prostate decreased ($p < 0.01$–0.001) at two higher dose-levels in a dose-dependent pattern (Table 1).

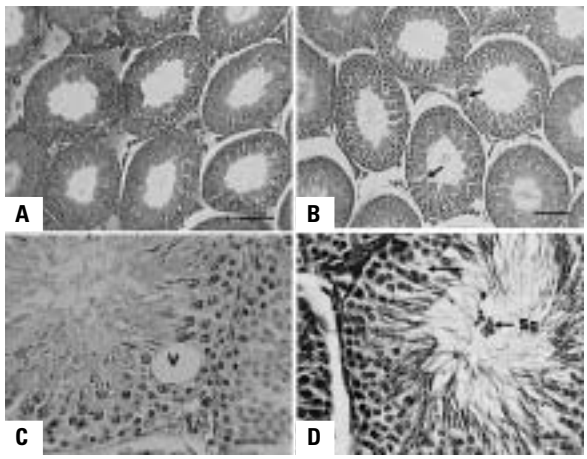

Figure 1. Effect of ribavirin on the rat testis. **A.** A testicular section from a control rat showing normal cell association. **B.** A section of a testis from a rat treated with 20 mg/kg on day 35 showing gaps (arrows) in the epithelium, H and E; A and B — scale bar = 93 μm; **C.** A section of a testis from 100 mg/kg on day 35 showing a large vacuole (V) in the epithelium; **D.** A section of a testis from a rat treated with 200 mg/kg on day 35 showing sloughed germ cells in the lumen (Sg) and the gaps in the epithelium (arrow); PAS-haematoxylin; C and D — scale bar = 23 μm.

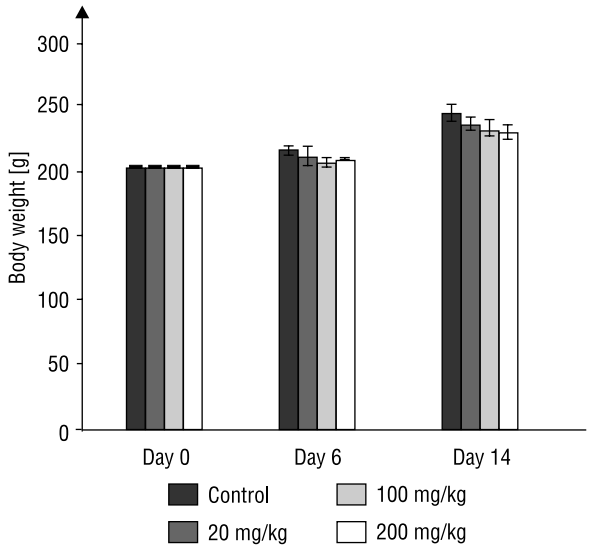

Figure 2. Effect of ribavirin on the body weight of animals treated with ribavirin and sacrificed on day 14. Data are represented as means ± SD from 5 animals per group. Inter-group differences were significant on days 6 ($p < 0.05$) and 14 ($p < 0.01$). After multiple comparisons there were no differences between control and treated or between treated animals on day 0. On day 6 control vs. 100 mg/kg ($p < 0.05$) and on day 14 control vs. 100 mg/kg ($p < 0.05$) and control vs. 200 mg/kg ($p < 0.01$) were significant.

Table 1. Effect of ribavirin on organ weights in the rat on day 35 after the last exposure. Data are represented as means ± SD from 5 animals per group. Inter-group differences were significant ($p < 0.05$–0.01)

Groups	Seminal vesicle [g]	Prostate [g]
Control	0.949 ± 0.05	0.836 ± 0.04
20 mg/kg	0.931 ± 0.03	0.837 ± 0.01
100 mg/kg	0.838 ± 0.02*	0.612 ± 0.01**
200 mg/kg	0.701 ± 0.03**	0.591 ± 0.02**

*$p < 0.01$, **$p < 0.001$ control versus treated

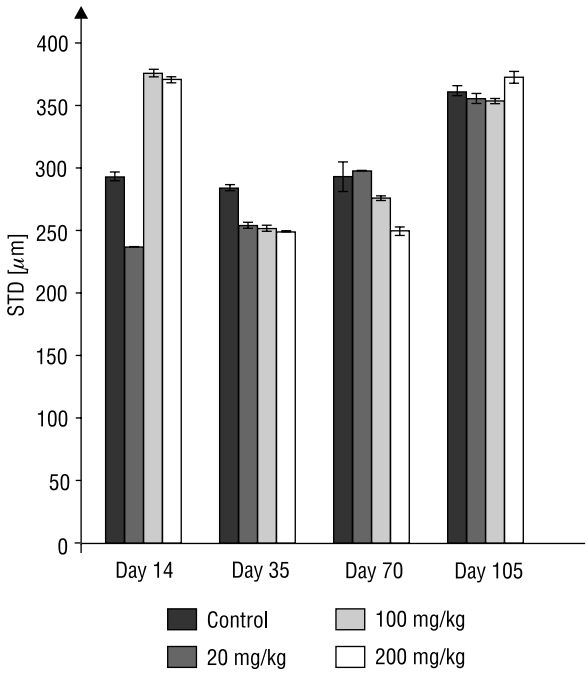

Figure 3. Effect of ribavirin on STD [μm] in the rat testis. Data are represented as means ± SEM from 5 animals per group. The effect was significant against respective controls on days 14 and 35 ($p < 0.01$) and not at other two sampling times. Inter-group differences were significant from day 14 to day 35 ($p < 0.01$) and until day 70 ($p < 0.05$). However, there were no differences between the groups on day 105.

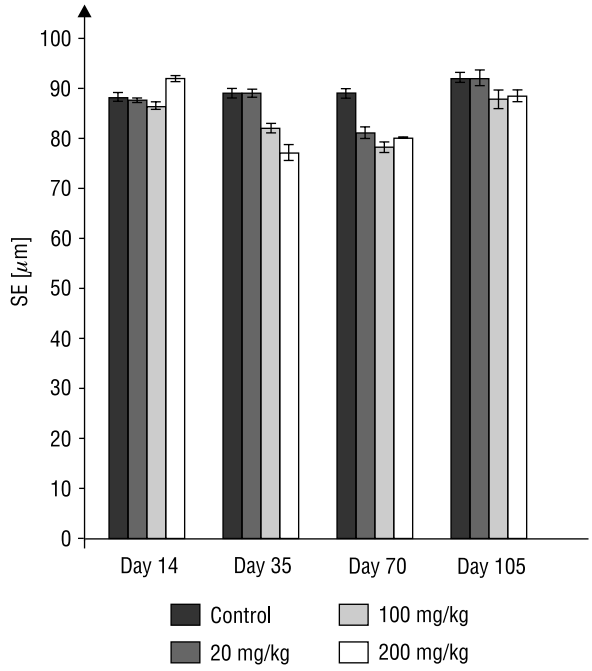

Figure 4. Effect of ribavirin on SE [μm] of seminiferous tubules in the rat testis. Data are represented as means ± SEM from 5 animals per group. The SE was decreased at 100 ($p < 0.05$) and 200 mg/kg ($p < 0.01$) on day 35 and on day 70 ($p < 0.01$) against respective controls. Inter-group differences were significant on day 35 ($p < 0.05$) and day 70 ($p < 0.01$), while at the other two sampling times there were no significant differences.

The STD decreased in the 20 mg/kg group on days 14 and 35 ($p < 0.01$) and the two higher doses increased it on day 14 but had decreased it by day 35 (Fig. 3). The two higher doses decreased the SE on day 35 in a dose-dependent pattern ($p < 0.05$–0.01) and it was further decreased on day 70 at 100 mg/kg, although without any effect on day 105 (Fig. 4). The incidences of stage XIV tubules significantly decreased at all three dose-levels on days 14 and 35 ($p < 0.05$–0.01; Table 2), and only the two higher doses imparted similar effects on day 70. This effect was exerted in a dose-dependent pattern on days 14 and 35, with greater toxicity at the latter sample time. The incidences of tubules with epithelial sloughing increased in a dose-dependent pattern and the maximum damage was observed on day 70. This morphological defect showed a complete recovery by day 105 (Table 2). Ribavirin induced the formation of sperm with microcephaly and cephalocaudal junction defects at two higher dose-levels (Table 3). Some sperm also showed the fibrils jetting out of the bending at the cephalocaudal junctions (Fig. 5).

The Leydig cells were fewer in number in the treated testes those in the control. Their decrease in number was dose-dependent at all sample points and there was no recovery to the control limit even on day 105 (Fig. 6). Ribavirin decreased the serum level of testosterone in a dose-dependent pattern at all dose-levels and sampling points ($p < 0.05$–0.01). The lowest serum level of testosterone was observed on day 70 in the 200 mg/kg dose group (Fig. 7). The hormone level had shown some recovery by day 105 when compared to previous sampling points, but maintained significant differences with the control.

DISCUSSION

We have observed decreased food and water intake during and after the treatment, especially at higher dose-levels, leading to a significant decrease in the body weight of the animals. The appearance of vacuoles in the seminiferous epithelium indicates that the drug caused damage to the Sertoli cell structure in terms of dilatation of the endoplasmic reticulum [7], although a few investigators are of the opinion that the vacuoles indicate a non-specific injury, occurring during unrelated conditions of testicular damage [3]. The gaps in the seminiferous epithelium appeared as a result of the removal of germ cells

Table 2. Effects of ribavirin on rat testis. Data are represented as means ± SEM from 5 animals per group. The incidences of stage XIV tubules decreased significantly at the two higher dose levels on days 14 and 70 ($p < 0.01$), and at all dose-levels on day 35 ($p < 0.05$–0.01) against respective controls. Inter-group differences were significant on days 14, 35 ($p < 0.01$) and 70 ($p < 0.05$). A significant increase in sloughing was observed in the first three time points ($p < 0.05$–0.01). Inter-group differences were significant up to day 70 ($p < 0.01$)

Parameter	Drug/dose	Day 14	Day 35	Day 70	Day 105
Stage XIV tubules (%)	Control	4.80 ± 0.37	3.60 ± 0.40	3.80 ± 0.37	3.40 ± 0.51
	20 mg/kg	3.80 ± 0.37	1.80 ± 0.37	3.80 ± 0.30	3.00 ± 0.71
	100 mg/kg	2.00 ± 0.32	1.00 ± 0.32	1.60 ± 0.40	2.60 ± 0.51
	200 mg/kg	1.20 ± 0.37	0.60 ± 0.25	1.61 ± 0.51	2.80 ± 0.58
Tubules with sloughing (%)	Control	0	0.20 ± 0.20	0.20 ± 0.20	0.60 ± 0.24
	20 mg/kg	0.40 ± 0.24	3.20 ± 0.37	2.60 ± 0.51	0.60 ± 0.24
	100mg/kg	4.40 ± 0.51	7.00 ± 0.55	10.00 ± 0.71	0.40 ± 0.24
	200 mg/kg	5.20 ± 0.58	7.40 ± 0.51	10.80 ± 0.66	0.80 ± 0.37

Table 3. The effect of ribavirin on sperm abnormalities in the rat on day 35 after the last exposure. Data are represented as means ± SD from 5 animals per group. Inter-group differences are significant ($p < 0.05$–0.01)

Groups	Microcephaly	c-c bending without fibrils	c-c bending with fibrils
Control	0.60 ± 0.89	2.60 ± 1.82	3.20 ± 1.30
20 mg/kg	0.80 ± 0.84	7.60 ± 2.41*	6.40 ± 2.79*
100 mg/kg	10.60 ± 2.70**	14.20 ± 1.92**	6.20 ± 2.04*
200 mg/kg	12.00 ± 2.45**	16.40 ± 3.20**	22.18 ± 5.89**

*$p < 0.05$, **$p < 0.01$ against control

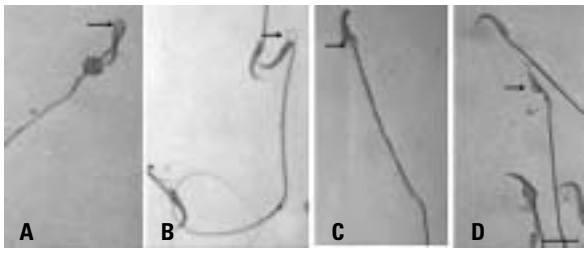

Figure 5. Effect of ribavirin on sperm morphology in the rat. **A.** A sperm from 100 mg/kg group with cephalocaudal bending without fibrils jetting out; **B.** A sperm from 200 mg/kg group with cephalocaudal bending with fibrils jetting out (arrows); **C.** and **D.** Microcephalous sperm (arrows) from 20 mg/kg and 100 mg/kg groups respectively. Note that normal sperm are shown in B and D, eosin Y, scale bar = 50 μm.

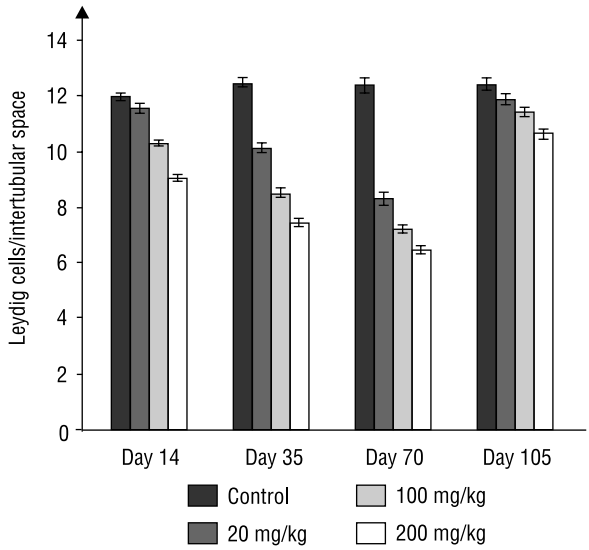

Figure 6. The effect of ribavirin on Leydig cell number/inter-tubular space in the rat. Data are expressed as means ± SEM from 5 animals/group; $p < 0.05$–0.01, control versus treated and inter-group differences.

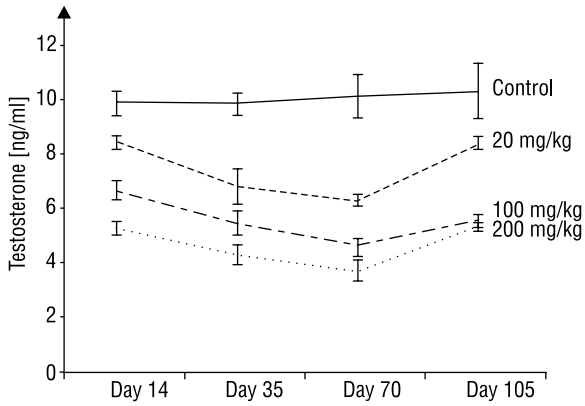

Figure 7. Effect of ribavirin on serum testosterone level in the rat. Data are expressed as means ± SEM from 5 animals/group. Hormone level decreased in a dose-dependent manner ($p < 0.05$–0.01) with significant inter-group differences ($p < 0.05$–0.01) except between 100 and 200 mg/kg on days 70 and 105.

by sloughing. The sloughing of epithelial cells in this case may be due to damage to intercellular bridges formed by the microtubules between the Sertoli cells and the germ cells, although a detailed study is needed to confirm these as effects of ribavirin.

The lack of significant effect on testis weight indicates that severe damage, such as the atrophy of seminiferous tubules or backflow of exfoliated cells into the tubules as an aftermath of blockade of the efferent ductules [14], was not caused by ribavirin. The drug did not induce the atrophy of seminiferous tubules at any of the dose-levels or time points studied. As an indicator of gonadotoxicity, ribavirin affected the STD and SE; the increase in STD on day 14 could be due to the induction of secretion of tubular fluid, although such an effect of this drug has not so far been recorded. The decrease in STD and SE occurred in positive correlation with the sloughing of epithelial cells, indicating the shrinkage of seminiferous tubules as a result of cell loss.

Cytotoxicity was seen in the form of a decreased incidence of stage XIV tubules. This effect was due to the prevention of progression of previous stages of the seminiferous epithelial cycle, especially stages IX–XII, similar to the effects of 1, 3-dinitrobenzene in the rat [11]. The present findings are, however, in consensus with previous reports on cytotoxicity in rats, in which ribavirin has been found to decrease the sperm count [15] and step 19 spermatids and to increase cell death [6] and abnormal sperm [16]. This study adds some other sperm abnormalities induced by the drug which have not been quantified in a previous study [16]. The tail defects and cephalocaudal junction defects have shown strong positive correlations with infertility [8]. This suggests that ribavirin might affect the ability of treated males to fertilise the female rats. Furthermore, the sperm abnormalities also indicate gross testicular injury, although various other factors also seem to be responsible for their formation [16]. The fact that the fertilizing abilities of both male and female rats pre-exposed to ribavirin are remains an unresolved puzzle [1], it may be argued that ribavirin influences these through structural damage to the testis.

Ribavirin seems to have a moderate but prolonged effect on testosterone levels in the rat. The lowered level of the hormone was due to a decreased number of Leydig cells. Ribavirin showed some cytotoxic effects on Leydig cells, which resulted in a decrease in their number. We have not evaluated the effect on leutinising hormone level in the serum, which must have only been increased as a result of a negative feed-back mechanism. Nevertheless, it could be said that ribavirin functions like an endocrine-disruptor in the rat. Although a very low level of testosterone in the testis is sufficient to maintain normal spermatogenesis [21], an immediate response to its decrease may be a decrease in parameters such as the number of advanced spermatids [2]. The decline in weights of the seminal vesicle and prostate must be due to a decreased testosterone level, since they are androgen-dependent accessory sex organs [13, 17].

In an interesting study on the *in vitro* interaction of ribavirin and Leydig cells the former was able to restore the testosterone level, which was originally decreased by the mumps virus [10]. This paves the way for the hypothesis that ribavirin may not have any effect on the structure or function of Leydig cells. In fact, the toxicity of ribavirin might be considerably less *in vitro* for the simple reason that ribavirin requires active metabolism to generate severely toxic metabolites, namely ribavirin 5′ triphosphate and ribavirin 5′ monophosphate, to impart its toxicity [4, 6, 9]. Hence, lack of metabolism was responsible for the absence of any direct effect on Leydig cells *in vitro* [10], therefore not affecting their function. The restoration of testosterone level in the mumps virus-Leydig cells-ribavirin complex was therefore not due to the direct action of ribavirin but to the elimination of the virus from the system by the drug. In this study, however, the Leydig cells were affected by the drug up to day 105, hence forming a basis for a sustained decrease of testosterone level in the serum.

Curiously, all the parameters tested here showed recovery by day 105, except the testosterone level

and the number of Leydig cells. Since ribavirin is used as an aerosol, and occupational exposure may be an important means of inadvertent exposure, it is noteworthy that ribavirin has adverse effects on the testis and other reproductive parameters. It should also be noted that the drug does not induce atrophy of the seminiferous tubules, but damages the testis in a transient fashion and, furthermore, that the endocrine-disrupting properties of this drug are prolonged and disappear at a comparatively slower rate in the rat testis.

REFERENCES

1. Arky R (1996) Physicians' desk reference. In: Sifton DW (ed.) Medical economics company, Montvale NJ, pp. 1264–1267.
2. Chakraboty S, Pakrashi A (1991) Antifertility effect of chronically administered Malviscus conzattii flower extract on fertility of male rats. Contraception, 43: 273–285.
3. Chapin RE, Dutton SL, Ross MD, Sumrell BM, Lamb JC (1984) The effects of ethylene glycol monomethyl ether on testicular histology in rats. J Androl, 369–380.
4. Crotty S, Cameron C, Andino R (2002) Ribavirin's antiviral mechanism of action: lethal mutagenesis? J Mol Med, 80: 86–95.
5. Culling CFA, Allison RT, Barr WT (1985) Cellular pathology technique. 4th Ed. Butterworths, London, pp. 27–50.
6. D'Souza UJA, Narayana K (2002) Mechanism of cytotoxicity of ribavirin in the rat bone marrow and testis. Indian J Physiol Pharmacol, 46: 468–474.
7. De Krester DM, Kerr JB (1994) The cytology of the testis. In: Knobil NE, Neil JD (eds.) The physiology of reproduction. 2nd Ed. Raven Press Ltd., New York, pp. 1177–1280.
8. Ellis MK, Richardson AG, Foster JR, Smith FM, Widdowson PS, Farnworth MJ, Moore RB, Pitts MR, Wikramaratne GAS (1998) The reproductive toxicity of molinate and metabolites to the male rat: effects on testosterone and sperm morphology. Toxicol Appl Pharmacol, 151: 22–32.
9. Franchetti P, Grifantini M (1999) Nucleoside and non-nucleoside IMP dehydrogenase inhibitors as antitumor and anticancer agents. Curr Med Chem, 6: 599–614.
10. Goffic RL, Mouchel T, Ruffault A, Pattard JJ, Jegou B, Samson M (2003) Mumps virus decreases testosterone production and gamma interferon-induced protein 10 secretion by human Leydig cells. J Virol, 77: 3297–3300.
11. Hess RA, Linder RE, Strader LF, Perreault SD (1988) Acute effects and long term sequelae of 1,3-dinitrobenzene on male reproduction in the rat II. Quantitative and qualitative histopathology of the testis. J Androl, 9: 327–342.
12. Hoffman SH, Wade MJ, Staffa JA, Mc Gregor DB, Holmstrom M, Dayan AD (1987) Dominant lethal study of ribavirin in male rats. Mutation Res, 188: 29–34.
13. Jana D, Maitti R, Ghosh D (2003) Effect of *Stephania hernandifolia* leaf extract on testicular activity in rats. Asian J Androl, 5: 125–129.
14. Nakai M, Hess RA, Moore B, Gutroff RF, Strader LF, Linder RE (1992) Acute and long term effects of a single dose of the fungicide carbendazim (Methyl 2 benzimidazole carbamate) on the male reproductive system in the rat. J Androl, 13: 507–518.
15. Narayana K, D'Souza UJA, Rao KPS (2002) Effect of ribavirin on epididymal sperm count in rat. Indian J Physiol Pharmacol, 46: 97–101.
16. Narayana K, D'Souza UJA, Rao KPS (2002) Ribavirin-induced sperm shape abnormalities in Wistar rat. Mutation Res, 513: 193–196.
17. Niu YJ, Ma TX, Zhang J, Xu Y, Han R-F, Sun G (2003) Androgen and prostatic stroma. Asian J Androl, 5: 19–26.
18. Phillips MD, Nascimbeni B, Tice RR, Shelby MD (1991) Induction of micronuclei in mouse bone marrow cells: an evaluation of nucleoside analogues used in the treatment of AIDS. Environ Mol Mutagen, 18: 168–183.
19. Rao KPS, Rahiman MA (1989) Cytogenetic effects of ribavirin on mouse bone marrow. Mutation Res, 224: 213–218.
20. Rocha DCM, Debeljuk L, Franca LR (1999) Exposure to constant light during testis development increases daily sperm production in adult Wistar rats. Tissue Cell, 31: 372–379.
21. Sharpe RM (1994) Regulation of spermatogenesis. In: Knobil E, Neil JD (eds.) The physiology of reproduction. 2nd Ed. Raven Press Ltd., New York, pp. 1363–1434.