Reversible Effects of Ribavirin on the Testicular Weight of Albino Rats

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Abstract

Introduction: Ribavirin because of its genotoxic quality causes adverse effects on the gonads in a temporary way that has been studied on the testicular tissues of various experimental animals.

Objectives: To study the changes in mean testes weight of albino rats after treatment with Ribavirin in different doses at different time points.

Methods: The research lab and the animal house of the Anatomy Department of Postgraduate Medical Institute, Lahore was the place of this experimental study where 72 adult male albino rats were divided into 4 groups: A, B, C and D; with 18 rats each. Intraperitoneal injections of distilled water were given to control group A and Ribavirin in the doses of 20mg, 100mg and 200mg/kg body weight, as a single dose for 5 days was administered to experimental groups B, C and D. Every group was further split up into 3 subgroups according to the sacrificial days 20th, 40th and 60th since after administration of the last dose of the drug. On each sacrificial day, 6 rats were randomly selected from a group and sacrificed. Their testes were dissected out and weighed after the removal of the epididymis. The mean testes weight was also calculated.

Results: In comparison with the control groups on the 20th and 40th sacrificial days all experimental groups showed a decrease in the values of mean testes weight (MTW) which was more in medium and high-dose treated groups due to the toxic effects of Ribavirin on rat's testes. On the 60th sacrificial day, only low-dose treated groups showed the values of MTW very close to that of controls due to the reversibility of changes induced by Ribavirin on rat's testes. On the same time point medium and high dose treated groups still had reduced values of MTW in comparison with controls due to the late recovery.

Conclusion: A patient on Ribavirin therapy must be informed by a physician regarding its toxic effects on his reproductive health and the reversibility of its gonadotoxicity after discontinuation of this drug.

Key words: Ribavirin, Testicular toxicity, Mean testes weight.

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Introduction

Hepatitis is a huge community health issue in the world which is linked with the loss of many lives due to liver ailments.[1] The Hepatitis C virus (HCV) was detected in 1989 and disseminated through contact with infected blood. 60 to 80 percent of long-standing infections bring about liver cirrhosis and hepatic cancer.[2] Ribavirin (RBV) orally and injectable Interferon alpha is an adjuvant therapy that has proved to be a powerful treatment for chronic Hepatitis C. RBV, an antiviral drug was produced in 1970 and its wide-ranging activity against viruses was spotlighted in 1972.[1]

Ribavirin is a broad-spectrum antiviral drug active against many RNA and DNA viruses. Ribavirin along with interferon-alpha is very effective to control hepatitis C virus infection. It is also used in the treatment of chro-

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nic hepatitis E virus infection, respiratory syncytial virus infection and various hemorrhagic fevers. This drug is being considered for the treatment of cancers on the basis of some studies. Ribavirin intracellularly changes into Mono-, Di- and Triphosphates and gets active. Ribavirin triphosphate concentration is found high inside the cells that stop the replication of viruses. Its mode of action is to decrease the guanosine triphosphate GTP level in the cell by inhibition of inosine monophosphate dehydrogenase IMDH resulting in stoppage of viral mRNA capping and sometimes may cause modification of the immune response of the host cell. Another mechanism of action is viral mutagenesis.[3]

Ribavirin is harmful to the developing baby if any one of the parents is taking ribavirin. That's why both parents should adopt useful contraceptive measures during ribavirin therapy and even six months after its last dose.[4] RBV is not suitable for either sex during six months prior to conception and its use is also prohibited during pregnancy due to its teratogenicity. The pregnancy registry of RBV was initiated in 2003 for documentation of its most probable harmful effects on embryos.[5]

In the light of the results of various studies done on Ribavirin, it is known for causing deleterious effects on the morphology and physiology of different tissues like bone marrow, liver, epididymis and testis of laboratory animals.[6,7,8,9]

Ribavirin exerts its cytotoxicity by causing cell death due to the stoppage of cell division.[6]

Due to its metabolites, Ribavirin was proved reversibly genotoxic in patients of Crimean-Congo hemorrhagic fever taking this drug in the doses advised by their physicians.[7]

Chromosomal aberrations were noted in patients with long-standing infection of hepatitis C, taking adjunctive medication containing Pegylated interferons and Ribavirin.[8]

The cytotoxicity of RBV has been noted in seminiferous tubules and sperms of testes in previous studies. The current research was undertaken to observe the effects of different doses of Ribavirin on the mean testes weight of rats at three sacrificial times and reversibility of these changes was noted at these time points after discontinuation of this drug.

Materials & Methods

This randomized controlled experimental study was conducted at the Research lab and animal house of Postgraduate Medical Institute, Lahore, and approved by the review board of the University of Health Sciences, Lahore. 72 adult male albino rats weighing in the range of 180 - 200gms were obtained from the National Institute of Health, Islamabad. The rats were kept at 24±2°C and a 12hrs light and dark cycle was maintained. All animals were fed a normal diet and were given water ad libitum. After adaptation of a week rats were split up into 4 groups A, B, C and D with 18 rats in each group by using a computer-generated random numbers table. Ribavirin used was purchased from Getz Pharma Company, Karachi, Pakistan. Scientific balance (Sartorius precision balance®, Germany) was used to measure doses of Ribavirin. The intervention done was as follows:

Control group A: Intraperitoneal injection of 0.75ml/kg body weight (b.w) of distilled water was given to the rats once daily at 24 hrs. intervals for 5 days.

Subgroups according to schedule of sacrifice:

A1,20th day

A2, 40th day

A3, 60th day

Experimental group B: Intraperitoneal injection of Ribavirin 20mg/kg b.w dissolved in 0.75ml of distilled water was given to the rats once daily at 24 hrs. intervals for 5 days.

Subgroups according to schedule of sacrifice:

B1, 20th day

B2, 40th day

B3, 60th day

Experimental group C: Intraperitoneal injection of Ribavirin 100mg/kg b.w dissolved in 0.75ml of distilled water was given to the rats once daily at 24 hrs. intervals for 5 days.

Subgroups according to schedule of sacrifice:

C1, 20th day

C2, 40th day

C3, 60th day

Experimental group D: Intraperitoneal injection of Ribavirin 200mg/kg b.w dissolved in 0.75ml of distilled water was given to the rats once daily at 24 hrs. intervals for 5 days. .

Subgroups according to schedule of sacrifice:

D1, 20th day

D2, 40th day

D3, 60th day

3 sacrificial days 20th, 40th and 60th from the last dose were selected and 3 subgroups of every group were made in accordance with these sacrificial times forming 12 subgroups in total. From each study group, six randomly selected rats were sacrificed on each sacrificial day. Their testes were dissected out and weighed after the removal of the epididymis. The weight of each testis was recorded and the mean testes weight of all study groups was calculated. The data was entered in Table-1 at various sacrificial times and dose-related changes in mean testes weight were studied in comparison with the controls.

Statistical Package for Social Sciences (SPSS), version 23 was used to analyze the data. For quantitative variables Mean (± SD) was calculated. Analysis of variance (ANOVA) was applied to show the statistical difference in the mean of all groups. Post-hoc Tukey test was applied to evaluate the difference of means between the groups at 5% level of significance ($p < 0.05$).

Results

In comparison with the controls mean values of testes weight (MTW) showed a reduction in all experimental groups though it was more marked in medium and high-dose treated groups, on the 20th and 40th days from the last treatment (Fig 1). This decrease in values was more pronounced on the day 20th. By applying ANOVA comparison was made and an overall reduction in the values was found significant with p-value<0.001. A statistically significant difference between all study groups in all possible combinations was found when the Post Hoc test was applied. (Table 1). Between C2 and D2 groups significant difference was not found (Table 1). On the 60th day from the last dose of the drug in comparison with control groups, a reduction in the values of mean testes weight was noticed but it was not as noticeable as was observed on days 20th and 40th. The values of the low-dose group were very close to the controls in comparison with the medium and high-dose

Table 1: *Comparison of mean values of testes weight (MTW) in grams among study groups at different sacrifice times*

Taaimes	Groups	n	MTW	ANOVA	Post Hoc Test	
			Mean ±SD	p-value	Comparison groups	MTW p-value
20th day	A1 (Control group)	6	0.83 ±0.089	<0.001**	A1 - B1	<0.001**
	B1(RBV20mg/kg b.w)	6	0.64 ±0.04		A1 - C1	<0.001**
	C1(RBV100mg/kg b.w)	6	0.37 ±0.01		A1 - D1	<0.001**
	D1(RBV200mg/kg b.w)	6	0.28 ±0.01		B1 - C1	<0.001**
					B1 - D1	<0.001**
					C1 - D1	0.026*
40th day	A2 (Control)	6	0.89 ± 0.05	<0.001**	A2 - B2	<0.001**
	B2(RBV20mg/kg b.w)	6	0.72 ± 0.10		A2 - C2	<0.001**
	C2(RBV100mg/kg b.w)	6	0.62 ±0.008		A2 - D2	<0.001**
	D2(RBV200mg/kg b.w)	6	0.58 ± 0.01		B2 - C2	0.034*
					B2 - D2	0.002*
					C2- D2	0.576
60th day	A3 (Control)	6	0.84 ± 0.03	<0.001**	A3 - B3	0.56
	B3(RBV20mg/kg b.w)	6	0.80 ± 0.08		A3 - C3	0.004*
	C3(RBV100mg/kg b.w)	6	0.73 ± 0.02		A3 - D3	<0.001**
	D3(RBV200mg/kg b.w)	6	0.67 ± 0.03		B3 - C3	0.074
					B3 - D3	0.001*
					C3 - D3	0.164

*-p-value≤ 0.05 statistically significant change
**-p-value< 0.005 highly significant change
n= Number of rats in each group

groups (Fig 1). This reduction in the values was compared by applying ANOVA, which was significant with a p-value<0.001. Post Hoc test showed a statistically significant difference between A3 and C3, A3 and D3, and B3 and D3 groups. No significant difference between A3 and B3, B3 and C3, C3 and D3 groups was found (Table 1).

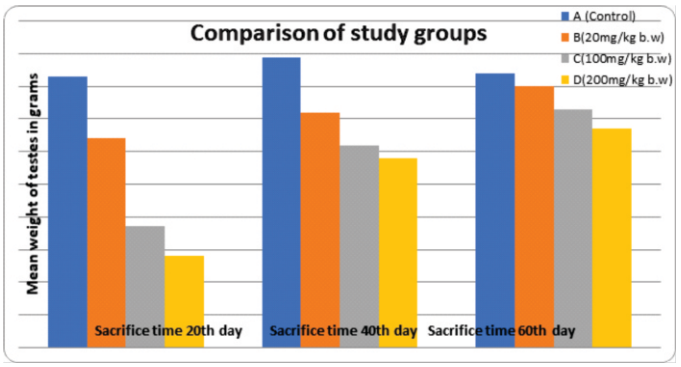

Figure 1: *Bar Graph Showing a Comparison of Mean Testes Weight (MTW) Among Study Groups at different Times of Sacrifice.*

Discussion

Regarding the effects of ribavirin on the mean weight of testes on the 20th and 40th days, it was noted that there was a significant decrease in mean testes weight in all experimental groups (p- value<0.001) (Table 1) (Fig 1). Reduction in values is suggestive of the presence of degenerative changes inside the seminiferous tubules of the testes. The reduction in values was more marked in high-dose groups due to more severity of the toxic effects. Because of the diminishing effects of Ribavirin reduction in MTW became less marked on the 60th days of sacrifice due to the beginning of regenerative changes as signs of recovery in the low dose group only. While high-dose groups still showed a more marked reduction in values probably due to late recovery. These findings are in accordance with a study by Narayana et al., (2005) that investigated the harmful effects of Ribavirin on body weight, testes weight, epididymis weight and many other reproductive parameters of albino rats. He observed that ribavirin is toxic to the reproductive parameters of rats in a transient way. This drug caused the vacuolization and sloughing of seminiferous epithelium that resulted in the reduction of body weight and organ weights in rats especially in high-dose groups in comparison with controls.[9]

Rao et al., (2005) studied the cytotoxicity of Ribavirin in mice testes and found that Ribavirin induced a reduction in testes weight due to decreased spermatogenesis and structural damage of germ cell chromosomes. Abnormal sperms formation due to point mutations was also noted in this study.[10]

Almasry et al., (2017) observed the effects of Ribavirin on the thickness of peritubular sheaths around seminiferous tubules of testes and testicular function in rats. He noticed that after 4 weeks of Ribavirin treatment, there was a significant reduction in the body weight of rats and a significant decrease in testes weight. He found that significantly increased thickness of peritubular sheath and sloughing of germinal epithelium caused shrinkage of tubules due to cell loss. After 4 weeks of Ribavirin administration decrease in Testosterone level was also found. Diminution in the values of body weight is due to side effects of this drug and testicular weight lessening is probably due to testicular damage and apoptosis in experimental groups.[11]

El-Kholy et al., (2019) noted that Sofosbuvir and Ribavirin administration caused harmful effects on the reproductive system and fertility of adult male rats as shown by a decrease in the level of serum testosterone. It had degenerative effects on the histology of the testes such as an increase in collagen deposits, an increased number of caspase-3 positive cells and DNA fragmentation. That's why the cytotoxic effects of these drugs must be kept in mind by physicians when advising them to patients. In order to find out the reversibility of their toxic effects, studies can be done and some agent that is protective against their gonadotoxicity must be explored.[12]

The present study highlighted the reversible cytotoxic effects of Ribavirin on the mean testes weight of albino rats that are dose-dependent and time-dependent.

Conclusion

Ribavirin administered to rats produced a reduction in testicular weight because of its harmful effects on testicular tissue but these effects were found reversible after discontinuation of the said drug. The low-dose group was the only one that exhibited more recovery as compared to the high-dose, which revealed less healing finally. Its probable toxicity on the fertility of a patient must be kept in mind while prescribing this medicine. The patient who is taking this medicine should be guided regarding the reversibility of its reproductive toxicity and the usage of effective contraceptives while taking ribavirin therapy.

Conflict of Interest *None*

Funding source *None*

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Author's Contribution

AB: Conceptualization of Project

FB: Data Collection

GPW: Revision

MF: Literature Search

FF: Wwriting of the Manuscript