Original Article

MORPHOLOICAL EFFECTS OF RIBAVIRIN ON ADULT OVARY OF ALBINO RAT

Hafiz Moeen-ud-Din, Muhammad Suhail and G.P.William

Objective: To evaluate the morphological effects of ribavirin on adult ovary of albino rats.
Material and Methods: This experimental study was conducted in the Department of Anatomy, Shaikh Zayed Postgraduate Medical Institute, Lahore in collaboration with Department of Zoology, Quaid-e-Azam Campus, University of Punjab. Doses of 20.200mg/kg of ribavirin were give to albino rats orally for five consecutive days at intervalsof 24 hrs. The ovaries were processed for histopathological analysis on day 14 after this last exposure. the gross parameters studies were body weight, paired ovarian wight, relative tissue weight index (RTWI). Data was analysed by ANOVA and Turkey's test for statistically significant difference.
Results: There was decrease in body wight of experimental groups compared with control at higher dose-levels i.e 100 and 200mg/kg. When paired ovarian weight were compared among groups the overall difference was significant. Post hoc analysis revealed that low dose group had significantly low ovarian weight as compared to control. when RTWI was compared among groups the overall difference was significant. Post hoc analysis revealed that low dose group i.e. 20mg/kg had significantly low RTWI as compared to control.
Conclusion: Ribavirin administration in adult female rat showed significant harmful effects on ovary, and these gonadotoxic effects of ribavirin may cause infertility in females during reproductive period.
Key words: Ribavirin, gonadotoxicity, antiviral drugs, guanosine analogue nucleoside

Introduction

Ribavirin is a guanosine analogue nucleoside with broad spectrum antiviral activity.[1] It acts by inhibiting inosine monophosphate dehydrogenase (IMPDH), which is the key step in de novo guanine synthesis responsible for viral replication.[2,3] Acute control of HCV RNA levels occurs through a brisk intrahepatic T-helper and T-suppressor cell response, a shift toward a T-helper (Th_1) cytokine profile and up-regulated natural killer cell activity.[2,3] Ribavirin is rapidly absorbed after oral administration (time to maximum concentration = 1.5 hours) followed by rapid distribution and prolonged elimination phases. Uptake from the proximal small bowel is active via concentrative N1 sodium-dependent nucleoside transporters.[4] Ribavirin has been used against flavi viruses such as yellow fever which is a life-threatening mosquito-borne flavi viral hemorrhagic fever characterized by severe hepatitis, renal failure, hemorrhage, and rapid terminal events with shock and multi-organ failure.[5] The recommended ribavirin dose is based on body weight, being generally 1000 mg/day if less than 75 kg and 1200 mg/day if more than 75 kg. The oral LD50 of ribavirin is 2 and 5.3g/kg in mice and rats respectively and intraperitoneal LD50 has been reported 0.9-1.3 and 2g/kg respectively.[6]

anaemia, neutropenia, thrombocytopenia, skin rashes, anorexia, pulmonary edema and depression.[6,7] Pre-natal exposure of ribavirin in pregnant women suggests birth defects with torticollis, hypospadiasis, polydactyly, natal teeth, glucose-6-phosphate dehydrogenase deficiency (G6PD), Ventricular septal defect and cyst of 4th ventricle of brain.[8]

Materials and Method

This experimental study was conducted in the Department of Anatomy, Shaikh Zayed Postgraduate Medical Institute, Lahore in collaboration with Department of Zoology, Quaid-e-Azam Campus, University of Punjab.

The sample Size was estimated by using 5% level of significance and 80% power of test with expected mean body weight increase of 40+ 5 gm, 35+ 5 gm, 30+ 5 gm and 29+ 4 gm in rats of control group, groups with 20 mg/kg, 100 mg/kg and 200 mg/kg respectively at day 14.9 Based on this a total of 40 female Abino rats Wistar Strain weighing between 175 and 230 grams were used after randomization by balloting method. All these animals were kept in cages for 14 days in the animal house of Zoology Department, University of Punjab Lahore for the purpose of acclimatization. A twelve hour light and dark cycle was maintained at room temperature between 22-250C. The food and water was provided

35+ 5 gm, 30+ 5 gm and 29+ 4 gm in rats of control group, groups with 20 mg/kg, 100 mg/kg and 200 mg/kg respectively at day 14.9 Based on this a total of 40 female Abino rats Wistar Strain weighing between 175 and 230 grams were used after randomization by balloting method. All these animals were kept in cages for 14 days in the animal house of Zoology Department, University of Punjab Lahore for the purpose of acclimatization. A twelve hour light and dark cycle was maintained at room temperature between 22-250C. The food and water was provided to these animals ad libitum. The food given was in the form of chick feed.

After 14 days the animals were randomly divided into four groups. Group A was labelled as control, the other three groups were experimental, groups B, C & D. Each group comprised of 10 animals. All the rats were weighed and properly recorded in proforma. These rats were then marked with a permanent marker for identification and placed in their respective cages labelled with allocated tags. Ribavirin (Xolox, Ferozsons Ltd. Pakistan) 20mg/kg, 100mg/kg and 200mg/kg were used respectively in this study which was given to the rats through the nasogastric tube (NG tube). Control group A was having ten female rats and were not given any medication except for equivalent proportion of distilled water according to body weight by nasogastric tube for 5 consecutive days. Experimental groups B,C & D were given 20,100 & 200mg/kg of ribavirin dissolved in 1ml of distilled water were given by Nasogastric tube respectively for 5 consecutive days.

Ribavirin tablets were removed from the blister pack and were placed in a small mortis. These tablets were then crushed to powder form using a small wooden crusher. The powder form of medicine was then transferred to electronic weighing machine and then according to body weight of animal dose was calculated and then it was transferred to a beaker and 1ml of distilled water was added. Using a glass mixer the contents were mixed together. This was then given to each of the experimental rat with the help of insulin syringe by a NG tube.

By making an incision at the base of abdomen, it was opened. The anterior abdominal viscera were reflected and the ovaries were removed after identifying the uterine tubes for the detailed morphological study. Ovaries were then weighed and fixed in 10% formalin.

The following gross parameters were observed in this study.

A. Quantitative Parameter.

1. Weight of the Rat Normal or any abnormality.
2. Paired ovarian weight.
3. Relative tissue weight index.

Parameters Observations

1. Normal or any abnormality
2. Normal or any abnormality

It was calculated as following:-

$$RTWI = \frac{\text{Mean weight of ovary}}{\text{Mean body weight of animal}} \times 100$$

The obtained results were tabulated and compared using computer software Statistical Package for Social Sciences (SPSS) version 17.0. The arithmetic mean of observations and standard deviations values were calculated. The significance between three means for body weight, paired ovarian weight, RTWI was calculated by analysis of variance (ANOVA) and Tukey. P-Value <0.05 will be considered statistically significant.

Discussion

This study was conducted to evaluate the harmful effects of Ribavirin, an antiviral drug, on the morphology of the ovaries. The drug literature of Ribavirin did not mention any side effects related to the ovarian cycle and there is lack of research work on its effects on the morphology of female gonads. As younger population of Pakistan, especially ,Infections especially hepatitis C, so this research study was carried out to investigate the effects of Ribavirin on the morphology of the adult ovaries.

Ribavirin has known effects on adults such as anemia, neutropenia, thrombocytopenia, weight loss, pulmonary embolism, pulmonary edema, myocardial infarction, cerebral haemorhage, hypothyroidism etc.[6,12] It also has known effects on rat testis regarding abnormal morphology of sperms and decrease in the weights of seminal vesicle and prostate.[9]

Table-1: Mean body weight (g) for female albino rats in various groups at start of experiment.

Groups	Mean	SD	Minimum	Maximum
Group A	203.0	±13.37	180	220
Group B	197.0	±13.37	180	220
Group C	197.0	±13.91	175	221
Group D	210.0	±15.15	1183	230

AControl Group, BExperimental Group, CExperimental Group, DExperimental Group, SDStandard Deviation

Table-2: Comparison for mean body weight (g) for female rats among various groups at start of experiment.

	Sum of Squares	DF	Mean Square	F	P-value
Between Groups	28.675	03	79.558	0.408	0.748++
Within Groups	7026.100	36	195.169		
Total	7264.775	39			

Based on ANOVA. DFDegree of Freedom, Ff-test (Ratio of variances) ,++Non significant difference (P>0.05)

Table-3: Group wise comparison for mean body weight (g) of female albino rats among various groups at end of experiment after ribavirin administration

Groups	Group	Mean Difference	STD. Error	P-value
	Group B	31.7	6.31	<0.001*
Group A	Group C	39.0	6.31	<0.001*
	Group D	48.2	6.31	<0.001*
Group B	Group C	7.3	6.31	0.657++
	Group D	16.5	6.31	0.059++
Group c	Group D	9.2	6.31	0.472++

Table-4: Comparison for mean paired ovarian weight (g) of female albino rats among various groups after administration of ribavirin

	Sum of Squares	DF	Mean Square	F	P-value
Between Groups	0.006	03	0.002	6.665	0.001
Within Groups	0.011	36	0.0003		
Total	0.017	39			

*Based on ANOVA DFDegree of Freedom Ff-test (Ratio of variances) **Highly significant difference (P<0.01)*

Table-5: Group wise comparison for mean paired ovarian weight (g) of female albino rats among various groups after ribavirin administration.

Groups	Group	Mean Difference	STD. Error	P-value
	Group B	0.014	0.008	<0.001*
Group A	Group C	0.014	0.008	<0.301++
	Group D	0.017	0.008	<155++
Group B	Group C	-0.021	0.008	0.053++
	Group D	-0.018	0.008	0.121++
Group c	Group D	90.3	0.008	0.981++

*Based on TUKEY'S Test AControl Group BExperimental Group CExperimental Group DExperimental Group **Highly significant difference (P<0.01) ++Non significant difference (P>0.05)*

Table-6: Comparison for relative tissue weight index for ovaries of female albino rats among various groups after administration of ribavirin.

	Sum of Squares	DF	Mean Square	F	P-value
Between Groups	0.00951	03	0.000317	9.195	<0.001**
Within Groups	0.001241	36	0.00034		
Total	0.002192	39			

*Based on ANOVA DFDegree of Freedom Ff-test (Ratio of variances) **Highly significant difference (P<0.01)*

Table-7: Group wise comparison for relative tissue weight index for ovaries of female albino rats among various groups after administration of ribavirin.

Groups	Group	Mean Difference	STD. Error	P-value
	Group B	0.00840	0.0026	0.015*
Group A	Group C	-0.00307	0.0026	0.650++
	Group D	-0.00394	0.0026	0.447++
Group B	Group C	-0.01147	0.0026	0.001*
	Group D	-0.01235	0.0026	<0.001*
Group c	Group D	-0.00088	0.0026	0.987++

*Based on TUKEY'S Test, AControl Group BExperimental Group, CExperimental Group, DExperimental Group, *Significant difference (P<0.05), ++Non significant difference (P>0.05)*

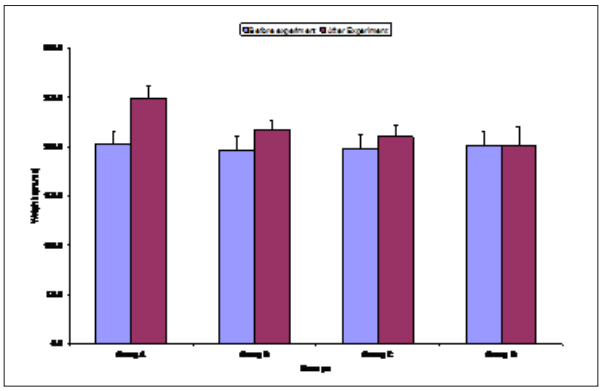
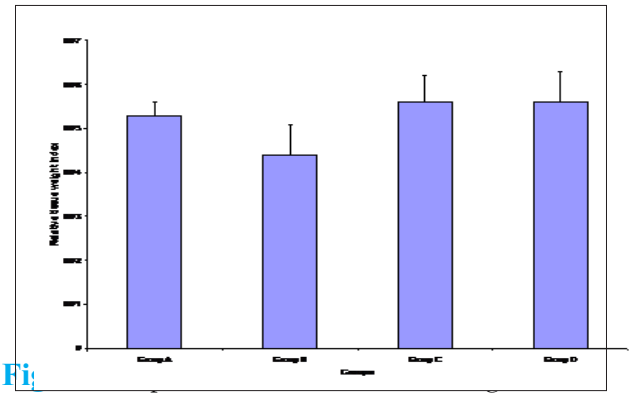


Fig-1: Comparison of mean weight difference of female albino rats-control & experimental groups after ribavirin administration.



Fi

among control and experimental groups after administration of ribavirin.

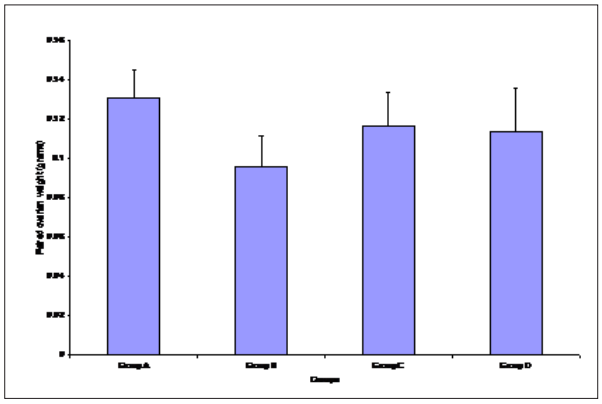


Fig-2: Comparison of mean paired ovarian weight of female albino rats-control & experimental groups.

In the present research work the body weight of the female albino rats among groups before starting the experiment was statistically non significant (p-value 0.748, **Table 2**). When mean body weights of albino rats were compared after Ribavirin administration in doses of 20mg/kg in experimental group B, 100mg/kg in experimental group C & 200mg/kg in experimental group D showed overall difference among these groups which was statistically

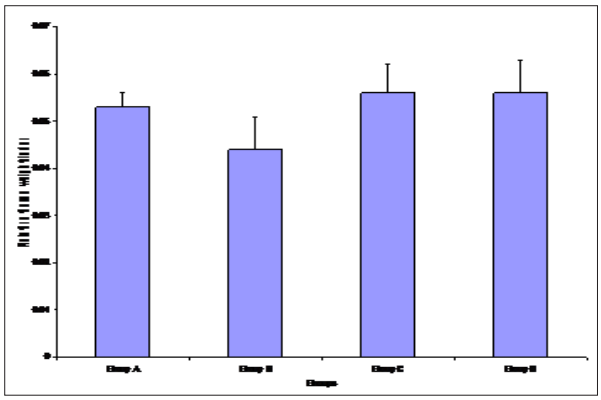


Fig-4: Comparison of relative tissue weight index among control and experimental groups after administration of ribavirin.

significant (p- <0.001, **Table 3**). Post hoc analysis revealed that the experimental groups B,

C and D all had significantly low weights as compared to control group A (p-values <0.001). Experimental groups C and D had low weights as compared to experimental group B but statistically insignificant (p-values 0.657 and 0.059) respectively. The difference between experimental groups C and D was also statistically insignificant (p-value 0.472, **Table 4**).

The results of body weight after Ribavirin administration in the present study coincides with the research and study performed by Naryana. K et al who also observed decrease in body weight after ribavirin in the experimental animals.[9]

In the present research work there was a decrease in the mean paired ovarian weights after ribavirin administration in the experimental groups B, C and D as compared to the control group A. This may be due to overall decrease in the weights of the rats in these experimental groups. To the best of researcher's knowledge no previous data is available to co-relate with our results. When weights were compared among groups the overall difference was statistically significant (p-value 0.001,**Table 4**). Post hoc analysis revealed that after 20mg/kg Ribavirin administration in the experimental group B showed statistically significant low ovarian weight as compared to control group A (p-values <0.001). Experimental groups C and D had statistically insignificant low mean paired ovarian weights as compared to control group A (p-values 0.301 and 0.155) respectively. The experimental group C had high ovarian weight as compared to experimental group B but insignificant (p-value 0.053). The difference of experimental groups B and C from experimental group D was also insignificant (p-value 0.121 and 0.981, **Table 5**) respectively. These results coincide when compared with the research and study performed by Naryana. K et al in which after Ribavirin administration there was decrease in the weights of organs such as seminal vesicles and prostate but no change in the weights of testis were found in experimental animals.[9]

The relative tissue weight index (RTWI) in this study was calculated to see the changes in weights of paired ovaries as compared to the body weights of the animals. Reduction in ovarian weight was accompanied by a relative decrease in the total body weight of the albino rats in the experimental groups after Ribavirin administration when compared with the control group A.

When RTWI were compared among groups the overall difference was statistically significant (p-value <0.001, **Table 6**). Post hoc analysis revealed that after 20mg/kg Ribavirin administration in experimental group B showed significantly low RTWI as compared to control group A (p-value 0.015) and experimental group C with 100mg/kg Ribavirin and experimental group D with 200mg/kg Ribavirin showed statistically insignificant results with high RTWI (p-values 0.650 and 0.447) respectively when compared with control group A. Experimental group B when compared to experimental groups C and D showed statistically significant results (p-value <0.001). Experimental groups C and D showed insignificant results when compared with each other (**Table 7**). These results showed that experimental group B when given Ribavirin in doses of 20mg/kg showed decrease in RTWI as compared to other experimental groups C and D. Ribavirin in high doses cause pulmonary edema[12] and also damages blood vessel endothelium[13,14] and similar effects like interstitial edema may be responsible for slight reduction in RTWI in female albino rats which were exposed to high dose i.e 100mg/kg and 200mg/kg of Ribavirin in experimental groups C and D respectively. In these groups interstitial edema in ovaries were also observed.

Conclusion

This study showed that Ribavirin when given for shorter duration of time has a negative impact on the morphology of rat ovaries. The results of present research has shown deleterious effects on weight of ovaries in doses of 20,100 and 200mg/kg of ribavirin. This may increase the risk of infertility in those females who are taking this anti viral drug during their reproductive age group. Further research work is also required regarding its effects on the hormonal assays in females of reproductive age group and to correlate this with the adverse effects on ovarian structure.

Department of Anatomy,
Allama Iqbal Medical College, Lahore
www.esculapio.pk

References

1. Feld J, Hoofnagle J. Mechanism of action of interferon and ribavirin in treatment of hepatitis C. Nature 2005; (436): 96772.
2. Robert G. Gish. Treating HCV with ribavirin analogues and ribavirin-like molecules. Journal of Antimicrobial chemotherapy advance access 2005 17; (1):1-6.
3. Robert G. Gish. Treating HCV with ribavirin analogues and ribavirin-like molecules. Journal of Antimicrobial Chemotherapy 2011 6; 57(1):8-13.
4. Glue P. The Clinical Pharmacology of Ribavirin: Semi Liver Dis 1999. 19 Suppl 1:17-24 Cit:90.
5. Usefulness of monitoring ribvirin plasma concentrations to improve treatment response in patients with chronic hepatitis C. Journal of Antimicrobial Chemotherapy 2011; 6(66):1174-1180.20739.

6(66):1174-1180.
6. Monath TP. Treatment of Yellow Fever: Antiviral Res. 2008 Apr;78 (1): 116-24.
7. Canonico PG, Kasteelo MD et al. Effects of Ribavirin on red blood cells. Toxicol Appl pharmacol. 1984 Jun 30; 74(2); 155-62.
8. Toxicology Data Network HSDB [online].2011 July 6 [cited 2011-09-14]: Available from:http://www. Toxnet.Org/
9. Narayana.K, Urban J.A. D' Souza, Narayan.P, Kumar.G The Antivral drug reversibly affects the reproductive parameters in the male wistar rat 2005 Feb 11;64(2):65-71.
10. AVMA guidelines on Euthenasia.
11. Lee-Parritz,D. Analgesia For Rodent Experimental Surgery ISRAEL Journal Of Veter Medi 2007;62(3-4).
12.Drugs.com [online] 2013 Feb 16 avilable from: URL: http://www. Drugs. Com
13.Michaekis.M, Michaelis.R, Suhan.T et al Ribavirin inhibits angiogenesis by tetrahydrobiopterin depletion The FASEB Journal 2007; 21(1):81-87
14.Hatakeyama K, Herada T, Kagraniyama H. IMP dehydrogenase inhibitors reduce intracellular tetrahydrobiopterin levels through reduction of intracellular of GTP level. Indications of the regulation of GTP cyclohydrolase 1 activity by restriction of GTP availability in the cells. J. Biol.Chem. 1992; 267:20734-20739.