ORIGINAL ARTICLE

Histological Effects of Ribavirin on Adult Ovary of Albino Rat

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ABSTRACT

The present study was planned to evaluate the histological effects of ribavirin on adult ovary of albino rat in doses of 20,100,200mg/kg orally for five consecutive days of estrous cycle at interval of 24 hrs. Estrous cycle was measured by vaginal smear method. The ovaries were processed for histopathological analysis on day 14 after the last exposure. The histological parameters were capsule, primordial follicle, secondary follicle, diameter of Graafian follicle and secondary oocyte. Data was analysed by ANOVA and Tukey's test for statistically significant difference. Capsule was of same nature in all groups. Primordial follicles, Secondary follicles were significantly atrophied in high dose groups i.e., 100 and 200mg/kg as compared to control. Post hoc analysis revealed significantly low diameter of Graafian follicle and diameter of secondary oocyte in high dose groups i.e., 100 and 200mg/kg 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.

Keywords: Ribavirin, ovary, albino rat

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 75kg and 1200mg/day if more than 75kg. 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 & 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].

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MATERIAL AND METHODS

This study was conducted in Anatomy Department, Shaikh Zayed Postgraduate Medical Institute, Lahore in collaboration with Zoology Department, Quaid-e-Azam Campus, University of Punjab Lahore (PUL).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 20mg/kg, 100 mg/kg and 200 mg/kg respectively at day 14.[9] Based on this a total of 40 female Albino rats Wistar Strain weighing between 175 and 230 grams were used after randomization by balloting method. All these animals were kept for 14 days in cages in the animal house of Zoology Department, (PUL) for the purpose of acclimatization. A twelve hour light and dark cycle was maintained at room tem between 22^0C & 25^0C.

Experimental design: Animals were randomly divided into four groups after 14 days. Group A was control, the other three groups B, C & D were experimental, groups. Each group has 10 animals. Weights of all the rates were 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 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 medicine 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 of estrous cycle. Estrous cycle was measured by vaginal smear method (Fig. 2)

Dosage calculation: Ribavirin tablets were removed from the blister pack and were placed in a small mortis.

Further methodology: At 14th day of experiment, when ribavirin was stopped, the weight of the animals of all the groups were recorded in proforma. These animals were then euthanized by intraperitoneal injection of sodium pentobarbital as anaesthetic in doses of 45mg/kg[9,10] and morphine as analgesic in doses of 0.3-0.5mg/kg[11]. By making an incision anterior abdominal was opened. The abdominal viscera were reflected and the ovaries were removed for the detailed histological study. Ovaries were weighed and fixed in 10% formalin. By using a rotary microtome Serial 5 micrometer tissue sections were cut and stained with Haematoxyline & Eosin (H&E)[12,13].

The obtained results were tabulated and compared using SPSS version 17.0. The significance between three mean diameters of Graafian follicles, secondary follicles was calculated by analysis of variance (ANOVA) and Tukey. The Qualitative variables (capsule, primordial follicle, secondary follicle) were evaluated by using Chi-square test. P-Value <0.05 will be considered statistically significant.

RESULTS

Histological study of the ovaries of both groups revealed the presence of ovarian follicles in various stages of development. There were well defined cortex and medulla. Primordial, primary, secondary and Graafian follicles were present in the cortex of the ovary.The capsule of the group A was made up of a thin layer of connective tissue immediately underlying the lining epithelium, which was simple squamous to cuboidal type.

In the experimental groups B, C & D, it was observed that the capsule was thin and normal looking. It was also observed that the primordial follicles in group A occupying the peripheral part of the cortex of ovaries. The primordial follicles in the groups B, C and D were relatively decreased in number and somewhat smaller in appearance.

In group wise comparisons it was noted that the atrophy in primordial follicle was significantly higher in experimental group B, C and D as compared to control group A, all with p-values <0.001, while there was no difference noted between the experimental group B and D. The difference of experimental group C with experimental group B and D both was insignificant with p-value 0.528 (Table1).

Table 1: Group wise comparison for appearance of primordial folicle in female albino rats among various groups after administration of ribavirin

Group	Mean difference	Std. error	P-value
Group A			
Group B	21.02	1	<0.001*
Group C	16.91	1	<0.001*
Group D	21.02	1	<0.001*
Group B			
Group C	0.40	1	0.528++
Group D	0.00	1	1.000

DF Degree of Freedom, *Significant difference (P<0.01)
++ Non significant difference (P>0.05)

The number and size of the secondary follicles decreased in experimental groups B, C and D as compared to the control group A. When secondary oocyte diameters were compared among groups the overall difference was significant with p-value <0.001 (Table2). Post hoc analysis revealed that the experimental groups B, C and D had significantly low secondary oocyte diameter as compared to group A and all differences were significant with p-value <0.001. Experimental group B had significantly high secondary oocyte diameter as compare to experimental group C and experimental group D, both with p-values <0.001. Similarly the experimental group C had significantly higher secondary oocyte diameter as compare to experimental group D with p-value <0.001 (Table3).

Table 2: Comparison for mean diameter of secondary oocyte in female albino rats among various groups after administration of ribavirin

	Sum of squares	DF	Mean square	F
Between groups	37.750	3	12.583	116.759
Within groups	3.880	36	0.108	
Total	41.629	39		

P value <0.001* Based on ANOVA, DF Degree of Freedom, *Significant difference (P<0.01) Ff-test (Ratio of variances)

Table 3: Group wise comparison for mean diameter of secondary oocyte in female albino rats amongvarious groups

Group	Mean difference	Std. error	P-value
Group A			
Group B	0.745(*)	0.147	<0.001*
Group C	1.805(*)	0.147	<0.001*
Group D	2.535(*)	0.147	<0.001*
Group B			
Group C	1.060(*)	0.147	<0.001*
Group D	1.790(*)	0.147	<0.001*
Group C			
Group D	0.730(*)	0.147	<0.001*

Based on TUKEY'S Test

When diameters of Graafian follicles were compared among groups the overall difference was significant with p-value <0.001 (Table4)

Table 4: Comparison for mean diameter of Graafian follicle in female albino rats among various groups after administration of ribavirin

	Sum of squares	DF	Mean square	F
Between Groups	260.869	3	86.956	349.183
Within Groups	8.965	36	0.249	
Total	269.834	39		

P value <0.001* Based on ANOVA
DF Degree of Freedom, *Significant difference (P<0.01)

Table 5: Group wise comparison for mean diameter of Graafian follicle in female albino rats among various groups

Group	Mean difference	Std. error	P-value
Group A			
Group B	2.20	0.223	<0.001*
Group C	3.85	0.223	<0.001*
Group D	7.00	0.223	<0.001*
Group B			
Group C	1.65	0.223	<0.001*
Group D	4.80	0.223	<0.001*

Fig 1: Photomicrograph - panoramic view of ovary of female albino rat of control group A showing Epithelium (EP), Capsule (C) , Follicular Antrum (FA), Ovum (O) Cumulus Oophorus (CO), ZonaGranulosa (ZG), Theca Interna (TI), Theca Externa (TE), Corpus Luteum (CL), Primary follicle (PF) (H&E, 5X)

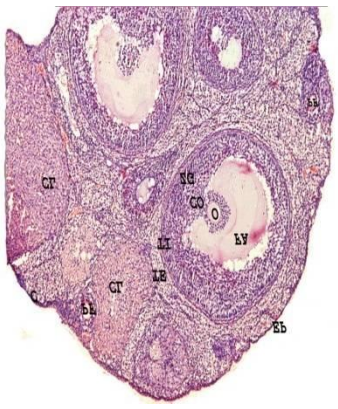
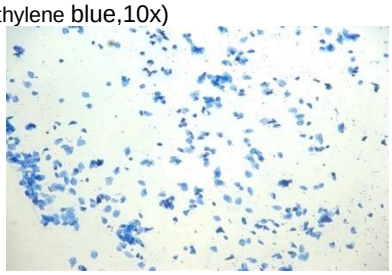


Fig. 2: Photomicrograph of a proestrous vaginal smear of female albino rat showing nucleated epithelial cells (ep) of experimental group b (methylene blue,10x)



DISCUSSION

This study was carried out to evaluate the harmful effects of Ribavirin, an antiviral drug, on the histology of the ovaries. The drug literature of Ribavirin did not mention any side effects related to the ovarian cycle and less research work was done on its effects on the female gonads' morphology. As younger population of Pakistan, especially females of reproductive age group have been affected by viral infections especially hepatitis C, so this research study was carried out to investigate the effects of Ribavirin on the histology 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[6,14]. It also has known effects on rat testis especially affecting the normal morphology of sperms and decrease in the weights of seminal vesicle and prostate[15].

In the present research the histological study of the group A ovaries revealed the ovulatory cycle in different stages of folliculogenesis, progressing from the primordial follicle to Graafian follicle. In addition the differentiation of both granulosa cells and theca interna cells, formation of antrum, cumulus oophorus and corpus luteum were also observed and depicted the normal histological findings and coincide with the findings reported by AminaBoubekri 2007, in her study of rat histology[16] (Fig. 1). The follicles were embedded in connective tissue stroma. The stroma of the cortex formed the cellular portion containing the above mentioned various stages of folliculogenesis, whereas the medulla occupied the central portion of the ovary. The thickness of connective tissue capsule was almost of same as in experimental groups (B, C and D) after Ribavirin administration as compared to the group A. follicles with a single layer of cells attached to the oocyte are the primordial follicles and primary follicles, as described by Tober Pedersen and Hannah Peters in their study for classification of oocytes and follicles[17,18]. The number of primordial follicles after Ribavirin administration in doses of 20, 100 & 200mg/kg in the experimental groups B,C & D were reduced respectively as compared to the control group A. These findings suggest that Ribavirin plays a negative role in the development of primordial follicle especially the growing oocyte.In group wise comparisons it was noted that the atrophy in primordial follicle was significantly higher in experimental group B, C and D after Ribavirin administration as compared to control group A (p-values <0.001) respectively (Table 1).

Due to limited research work of ribavirin on ovaries these findings cannot be compared with other studies. As Ribavirin is an antiviral drug, so some studies showing regarding effects of antiretroviral drugs on ovaries. However, these antiretroviral drugs use in HIV patients. These antiretroviral drugs cause

infertility by damaging the mitochondria in cells of sperm and egg[19].

When secondary oocyte diameters were compared among groups the overall difference was statistically significant (p-value <0.001, Table 2). Post hoc analysis revealed that the experimental groups B, C and D had significantly low secondary oocyte diameter as compared to control group A and all differences were statistically significant (p-value <0.001). Experimental group B which was given 20mg/kg of Ribavirin had significantly high secondary oocyte diameter as compared to experimental groups C and D, (p-values <0.001). Similarly the experimental group C which was given 100mg/kg of Ribavirin had significantly higher secondary oocyte diameter as compared to experimental group D which was given 200mg/kg of Ribavirin (p-value <0.001, Table 3) suggesting that an increase in the dose of Ribavirin affects the growth of the secondary oocyte adversely.

The Graafian follicle in the experimental groups showed decrease in size and diameter. When diameters were compared among groups the overall difference was significant (p-value <0.001, Table 4). Post hoc analysis revealed that the experimental group B, C and D had significantly low follicle diameter as compared to control group A and all differences were statistically significant (p-value <0.001). Experimental group B had significantly high follicle diameter as compared to experimental group C and D (p-values <0.001). Similarly the experimental group C had significantly higher follicle diameter as compared to experimental group D (p-value <0.001, Table 5).

The ovarian follicle growth, ovulation and formation of corpus luteum are complex processes that involve obvious changes in granulosa cell function. The changes are in a sequence and are characterized by specific, highly regulated responses to gonadotrophins, steroids and growth factors[20].

Preantral follicles were identified as oocytes with two or more layers of granulosa cells but no visible space between granulosa cells. The incipient follicles, which are the beginning of antral formation, were observed by the presence of visible space between granulosa cells. Small antral follicles were identified by the presence of a cavity with two or more compartments, while the Graafian follicles had one large continuous antral cavity[21].

CONCLUSION

This study showed that Ribavirin when given for short period of time has a negative impact on the morphology and histology of rat ovaries. Primordial follicles in the ovaries start to mature in cyclical basis at puberty and growing follicles contain very sensitive cells. The numbers of follicles are fixed and no more increase occur after birth. The results of present research have shown deleterious effects on growing follicles in the rat 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.

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