Effect of Ribavirin on the Testes of Adult Albino Rats (Light Microscopic Study)

Mohamed Abdel Hay Autifi, Elsayed Ahmed Abou Salem, Elsayed Abdel Rhman Abdel Hady And Amr Mohamed Younis

Medical Anatomy & Embryology Department – Faculty of Medicine – Al-Azhar University

Abstract: **Background:** Ribavirin is one of the approved antiviral drugs indicated for various viral infections. So it is used as a current therapy with interferon alpha for treatment of viral hepatitis C that affects 200 million patients worldwide. This treatment regimen is poorly tolerated because of its side effects and toxicity. The testis is a complex organ concerned with the production of sperms to fertilize the ova and secretion of androgen to maintain the secondary sexual characters and proper spermatogenesis. **Objective:** The present study was performed to assess ribavirin toxicity on the structure of testicular tissue of adult male rats after exposure to thedrugfor different periods as well as to evaluate the extent of improvement of testicular tissue structure after cessation of drug administration. **Results & Conclusion:** ribavirin administration produced toxicity and mutagenicity leading to marked serious histological changes of the testis including the spermatogenic cells, sperms, Sertoli cells and Leydig cells. These testicular changes persisted after cessation of ribavirin administration indicating the cumulative toxic effects of ribavirin that caused hypospermatogenesis, oligospermia and then hypospermia. Because it is used nowadays on a large scale allover the world especially in middle aged males (30- 50 years), these serious complications on testicular structure should be considered when physicians prescribe ribavirin in the protocol of viral hepatitis C management.

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Keywords: Effect; Ribavirin; Testes; Adult; Albino; Rat; (Light Microscopic Study

1. Introduction

Hepatitis is a major public health problem worldwide, responsible for considerable morbidity and mortality from liver disease ***(Lavanchy, 2011).***

Common causes of hepatitis include viral infection, side effects of certain prescribed drugs and over doses of the drugs ***(Jihan et al., 2013).*** Until 2011, the combination of pegylated interferon ***(PegIFNalph)*** and Ribavirin for 24 or 48 weeks was the approved treatment for chronic hepatitis C (European Association for the Study of the Liver***) (EASL, 2011)***.

Ribavirin is a non specific antiviral drug. It was synthesized in 1970. The broad spectrum antiviral activity was reported in 1972, the aerosolic form was approved for the treatment of respiratory syncytial virus in children ***(Trevors et al., 2010)***.

The oral ribavirin and interferon alpha (injections) combination therapy was approved by United States Regulatory Authorities in 1998 for the treatment of hepatitis C infection ***(Lau et al., 2002)***.

The chemical name of ribavirin is 1 beta D ribofurnosy l-1*H*-1, 2, 4-triazole-3-carboxamide. It is a purine (guanosine) nucleoside analog with modified base and d-ribose sugar, both are necessary for its antiviral activity. Ribavirin has three metabolites mono-, di- and triphosphates that are effective against various RNA and DNA viruses. Ribavirin-5' – triphosphate is the principal intracellular form ***(Acosta and Flexner, 2011).***

Ribavirin exerts its cytotoxicity in the testes after intra peritoneal administration by getting absorbed from peritoneal cavity and reaching to the germ cells. It acts a germ cell mutagen in rats ***(El Brashy et al., 2007)***.

Weiss et al. (1993) reported that ribavirin administration in cats resulted in pathological changes including hepatocellular vacuolization and centrilobular necrosis. Ribavirin is reversibly cytotoxic to germ cells and decreases the production of sperms ***(Rao et al., 2002)***.

A decrease in sperm count in a dose and time dependent pattern in the epididymis of rats receiving ribavirin, and its mutagenic agent to germ cells in a transient fashion inducing anomalies of head and tail of *sperms (**Narayana et al., 2002).***

In humans, ribavirin was found reversibly genotoxic due to its toxic metabolites in patients of Crimean Congao hemorrhagic fever treated with the therapeutic doses of the drug ***(Tatar et al., 2009)***.

2. Material and Methods

This study was carried out on 100 male adult albino Wistar rats (weight 200- 250 gm). They were

obtained from the laboratory animal unit in Nile center, El-mansoura. They were housed in cages in controlled laboratory environment with a constant 12hours light/ 12 hours dark cycle and at a temperature maintained at 25˚C, fed a standard balanced laboratory diet and had water.

The animals were divided into three main groups; Control group of 10 rats (G1), Experimental treated group of 30 rats and Experimental Recovery group of 60 rats.

The experimental treated group was divided into 3 subgroups (30 rats); G.2, G.3 and G.4 having 10 rats each. Each of the three subgroups were given intraprietoneal ribavirin in doses of 20, 100, 200 mg/kg/day respectively for 15 days.

Experimental Recovery group was divided into 6 subgroups; G5, G6, G7, G8, G9, and G10 having 10 rats each. G5, G6 and G7 were given intraprietoneal ribavirin in doses of 20, 100, 200 mg/kg/day respectively for 15 days then stopped and received an equivalent doses of intraperitoneal injection of normal saline-0.9% for another 15 days.

G8, G9 and G10 were given intraprietoneal ribavirin in doses of 20, 100, 200 mg/kg/day respectively for 15 days then stopped and received an equivalent doses of intraperitoneal injection of normal saline-0.9% for another 40 days.

All the doses for experimental animals was calculated according to Paget's tables in which the recommended daily dose of human can be transformed into its equivalent in rat regarding the body weight and the total body surface area ***(Paget and Barnes, 1964)***.

At the determined end date for each group, all rats were sacrificed from each group after giving deep anesthesia by ether inhalation. A mid line incision was done. The testis were excised and washed with normal saline and fixed in the fixative (10% formal saline containing 100 ml of formalin, 8.5 grams of sodium chloride and 900 ml of water) contained in glass bottles with glass stoppers which had been properly labeled before handling. Tissue processing was done. Histological sections of 3-5 µm thickness were taken and stained with routine Hematoxylin and Eosin stain. Slides were observed under the light microscope in low and high magnifications ***(Bancroft and Gamble, 2002).***

3. Results

[1] Results of control group 1(G1):

Light microscope examination:

Examination of H & E stained sections from testes of control group of adult albino rats revealed that testicular parenchyma was formed of a large number of seminiferous tubules (S.Ts.) resting on thin layer of basement membrane (B.M.). There was a large number of mature spermatozoa in lumena of S.Ts. and normal series of spermatogenic and Sertoli cells in their walls. Narrow interstitium between S.Ts. was present.

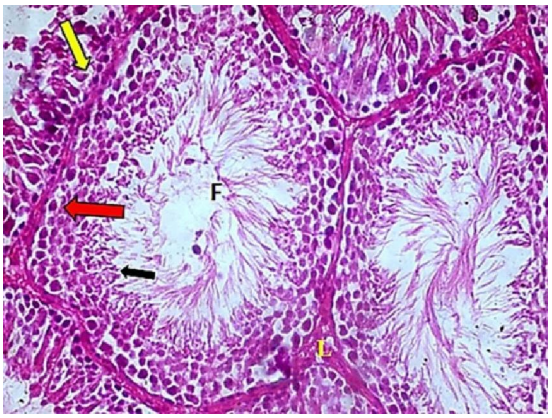

Fig. (1) A photomicrograph of adult albino rat's testis of control group **(G1)** showing multiple seminiferous tubules, each one is resting on a thin straight basement membrane (**yellow arrow**) and interstitial Leydig cells (**L**) in between. The wall of each S.T. was formed of series of spermatogenic cells (**red arrows**) and elongated spermatids **(black arrows).** The lumena of S.Ts. were filled with large number of sperm flagella (**F**). [H & E x 400].

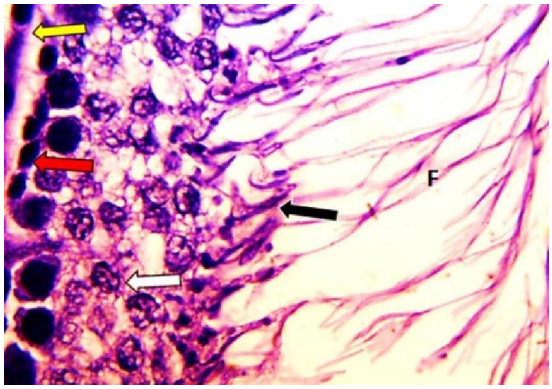

Fig (2) A photomicrograph of adult albino rat's testis of (G1) showing the basement membrane (yellow arrow) forming the wall of S.T., a series of spermatogenic cells (red arrow) resting on the basement membrane. Large number of spermatocytes (white arrows) and elongated spermatids (black arrow). The lumen of S.T. was filled with many sperm flagella (F). [H & E x 1000].

The interstitial connective tissue (I.C.T.) showed groups of interstitial cells of Leydig between S.Ts. (Fig. 1). The S.Ts. were lined by a stratified germinal epithelium and had patent lumina containing clumps of spermatozoa. Spermatocytes were stacked in several layers occupying the space between B.M. and lumen of S.Ts. Filamentous bundles of sperms were seen at the adluminal side of S.Ts. Spermatogonia appeared as small rounded cells with rounded nuclei resting on thin B.M.

Primary spermatocytes appeared as large cells with large nuclei and were arranged into one or two layers. (Fig. 2).

[2] Results of the experimental group 2(G2):

Histological examination of testicular tissue of adult albino rats after ribavirin administration (20 mg /kg /day) (G2) showed mild widening of interstitial spaces. The lumena of most S.Ts. revealed mild hypospermia. The walls of S.Ts. were tortuous and revealed many variation in thickness with mild hypospermia in some S.Ts. (Fig. 3), mild vacuolation of spermatogenic cells, spermatocytes and spermatids. (Fig. 4).

[3] Results of the experimental group 3(G3):

Histological examination of testicular tissue of adult albino rats after ribavirin administration (100 mg /kg /day) (G3) showed moderate widening of interstitial spaces, the lumen of most S.Ts. revealed moderate hypospermia. The walls of S.Ts. were tortuous and revealed many variation in thickness with moderate hypospermia in some S.Ts**.** (Fig. 5), moderate vacuolation of spermatogenic cells, spermatocytes and spermatids. (Fig. 6).

[4] Results of the experimental group 4(G4):

Histological examination of testicular tissue of adult albino rats after ribavirin administration (200mg /kg /day) (G4) showed marked widening and congestion in interstitial spaces. The lumena of most S.Ts. revealed marked hypospermia. The walls of S.Ts. were tortuous and revealed many variations in thickness. There was marked atrophy in some S.Ts. (Fig. 7). There are also marked separation and vacuolation of spermatogenic cells, spermatocytes and spermatids with marked hypospermia. (Fig. 8).

[5] Results of the experimental group 5(G5):

Histological examination of testicular tissue of adult albino rats after recovery of ribavirin administration (20 mg /kg /day) for 15 days (G5) showed many S.Ts. with moderate restoration of regularity in thickness of basement membrane, restoration of spermatogenic cells and spermatocytes in lumena of S.Ts. and decrease in interstitial Leydig cells between adjacent S.Ts. (Figs.9 & 10).

[6] Results of the experimental group 6 (G6):

Histological examination of testicular tissue of adult albino rats after recovery of ribavirin administration (100mg /kg /day) for 15 days (G6) showed that many S.Ts. with moderate restoration of regularity in thickness of basement membrane, mild loss of spermatogenic cells and spermatocytes and mild hyposprmia in lumena of some S.Ts. Mild increase in interstitial Leydig cells between adjacent S.Ts. (Figs. 11 & 12).

[7] Results of the experimental group 7(G7):

Histological examination of testicular tissue of adult albino rats after recovery of ribavirin administration (200 mg /kg /day) for 15 days (G7) showed many S.Ts. with moderate irregularity in thickness of basement membrane, mild loss of spermatogenic cells and spermatocytes and moderate hyposprmia in lumena of some S.Ts. and moderate increase in interstitial Leydig cells between adjacent S.Ts. (Figs. 13 & 14).

[8] Results of the experimental group 8(G8):

Histological examination of testicular tissue of adult albino rats after recovery of ribavirin administration (20 mg /kg /day) for 40 days (G8) showed many S.Ts. with restoration of regularity in thickness of basement membrane**,** restoration of spermatogenic cells and spermatocytes and resoration of number of interstitial Leydig cells between adjacent S.Ts. (Figs. 15 & 16).

[9] Results of the experimental recovery group 9(G9):

Histological examination of testicular tissue of adult albino rats after recovery of ribavirin administration (100 mg /kg /day) for 40 days showed many S.Ts. with mild irregularity in thickness of basement membrane**,** mild loss of spermatogenic cells and spermatocytes, and mild hypospermia in lumen of S.Ts. Mild increase in interstitial Leydig cells between adjacent S.s. (Figs. 17 & 18).

[10] Results of the experimental group 10(G10):

Histological examination of testicular tissue of adult albino rats after recovery of ribavirin administration (200 mg /kg /day) for 40 days (G10) showed many S.Ts. with restoration of thickness of basement membrane, partial loss of spermatogenic cells and spermatocytes and partial hypospermia in lumena of few S.Ts. restoration of interstitial Leydig cells between adjacent S.Ts. **(**Figs. 19 & 20).

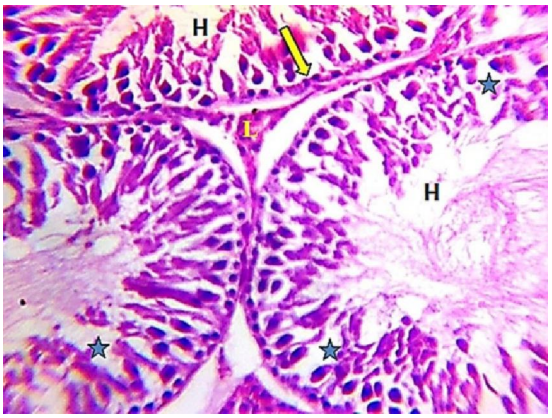

Fig. (3) A photomicrograph of adult albino rat's testis (G2) showing many S.Ts. with mild irregularity in thickness of basement membrane (yellow arrow), mild decrease of spermatogenic cells and spermatocytes (star) and mild hyposprmia (H) in lumena of S.Ts. Mild increase in interstitial Leydig cells (L) between adjacent S.Ts. was noticed (H & E x 400).

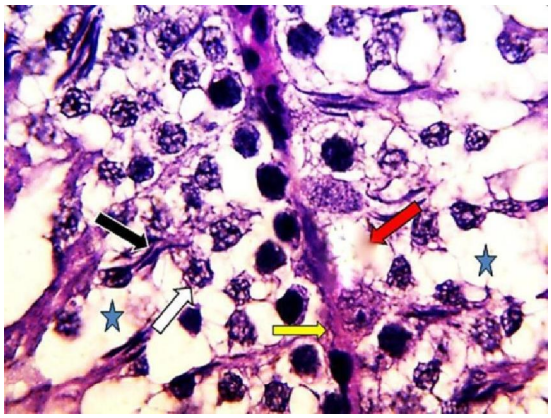

Fig. (4) A photomicrograph of adult albino rat's testis (G2) showing adjacent S.Ts. with mild irregularity in thickness of basement membrane (yellow arrow), mild decrease of spermatogenic cells (red arrow), spermatocytes (white arrow), spermatids (black arrow) and mild vacuolation in lumena of S.Ts (stars). (H & E x 1000).

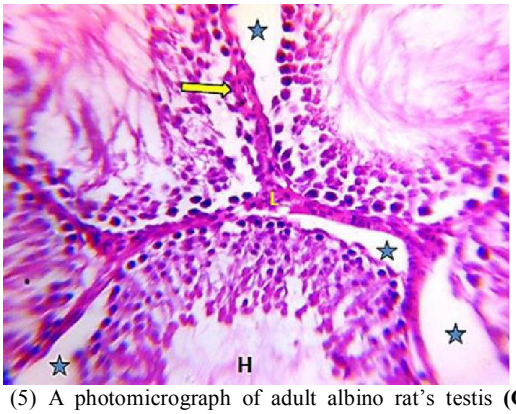

Fig. (5) A photomicrograph of adult albino rat's testis **(G3)** showing many S.Ts. with moderate irregularity in thickness of basement membrane **(yellow arrow)**, modrate separation and loss of spermatogenic cells and spermatocytes **(stars)** and moderate hyposprmia **(H)** in lumena of S.Ts. Moderate increase in interstitial Leydig cells **(L)** between adjacent S.Ts. (H & E x 400).

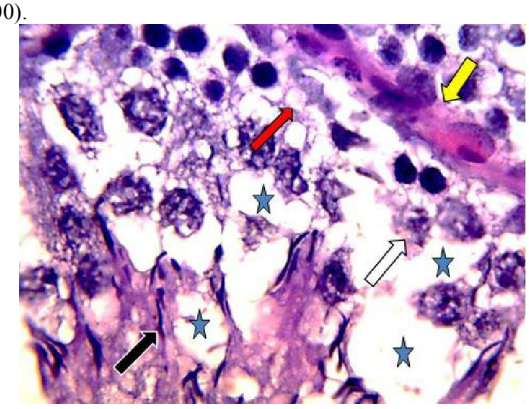

Fig. (6) A photomicrograph of adult albino rat's testis **(G3)** showing adjacent S.Ts. with moderat irregularity in thickness of basement membrane **(yellow arrow)**, moderate loss of spermatogenic cells **(red arrow)**, spermatocytes **(white arrow)**, spermatids **(black arrow)** and moderate vacuolation in lumen of S.Ts **(stars)**. (H & E x 1000).

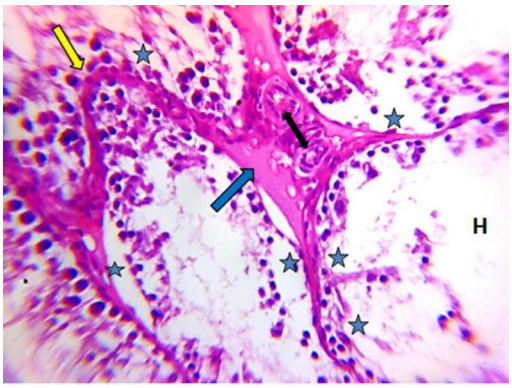

Fig. (7) A photomicrograph of adult albino rat's testis **(G4)** showing many S.Ts. with marked irregularity in thickness of basement membrane **(yellow arrow)**, marked separation and loss of spermatogenic cells and spermatocytes **(stars)** and marked hyposprmia **(H)** in lumena of S.Ts. Marked congestion and increase in interstitial Leydig cells **(double sided arrow)** and widening of interstitial space between adjacent S.Ts**. (blue arrow)** were noticed (H & E x 400).

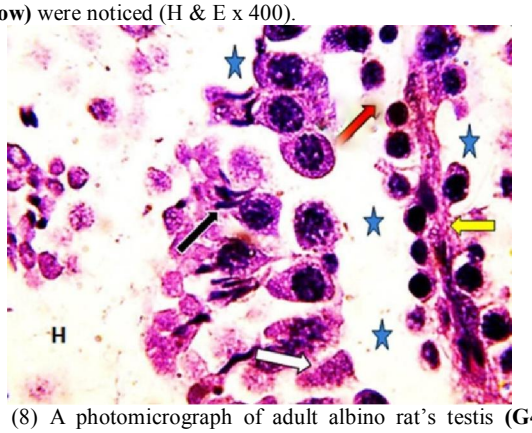

Fig. (8) A photomicrograph of adult albino rat's testis **(G4)** showing adjacent S.Ts. with marked irregularity in thickness of basement membrane **(yellow arrow)**, marked loss of spermatogenic cells **(red arrow)**, spermatocytes **(white arrow)**, spermatids **(black arrow)** and moderate spaces inside the lumen of S.Ts **(star)** and marked hyposprmia **(H)**. (H & E x 1000).

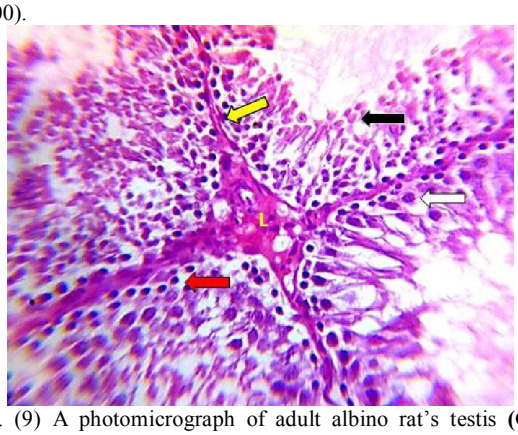

Fig. (9) A photomicrograph of adult albino rat's testis **(G5)** showing many S.Ts. with moderate restoration of regularity in thickness of basement membrane **(yellow arrow)**, restoration of spermatogenic cells **(red arrow)** and spermatocytes **(white arrow)** andspermatids **(black arrow)** in lumena of S.Ts. There is decrease in interstitial Leydig cells **(L)** between adjacent S.Ts. (H & E. x 400).

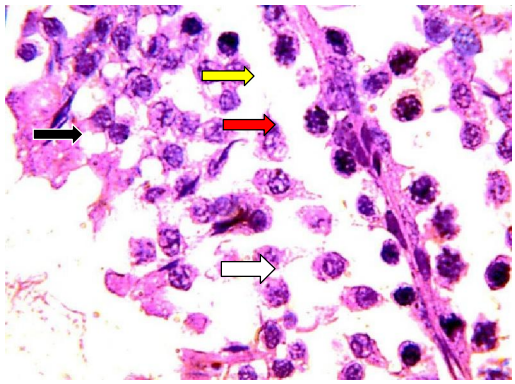

Fig. (10) A photomicrograph of adult albino rat's testis **(G5)** showing adjacent S.Ts. with moderate restoration of regularity in thickness of basement membrane **(yellow arrow)**, restoration of spermatogenic cells **(red arrow)** and spermatocytes **(white arrow)** andspermatids **(black arrow)** in lumena of S.Ts. (H & E. x 1000).

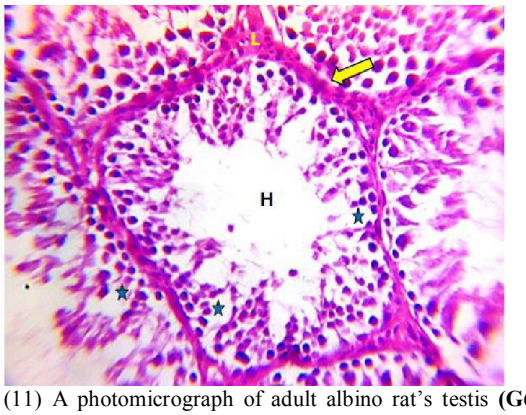

Fig. (11) A photomicrograph of adult albino rat's testis **(G6)** showing many S.Ts. with moderate restoration of regularity in thickness of basement membrane **(yellow arrow)**, mild loss of spermatogenic cells and spermatocytes **(stars)** and mild hyposprmia **(H)** in lumena of some S.Ts. Mild increase in interstitial Leydig cells **(L)** between adjacent S.Ts. (H & E x 400).

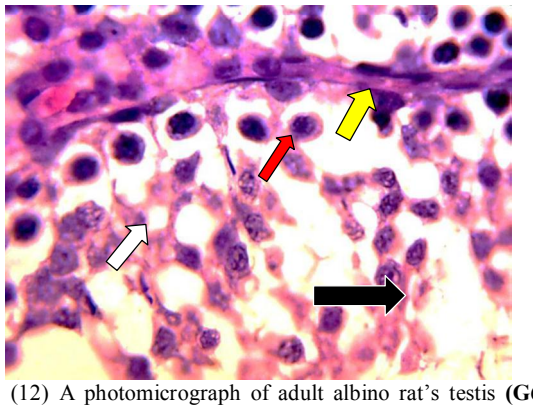

Fig. (12) A photomicrograph of adult albino rat's testis **(G6)** showing many S.Ts. with moderate restoration of regularity in thickness of basement membrane **(yellow arrow)**, mild loss spermatogenic cells **(red arrow)** and spermatocytes **(white arrow)** andspermatids **(black arrow)** in lumena of S.Ts. (H & E. x 1000).

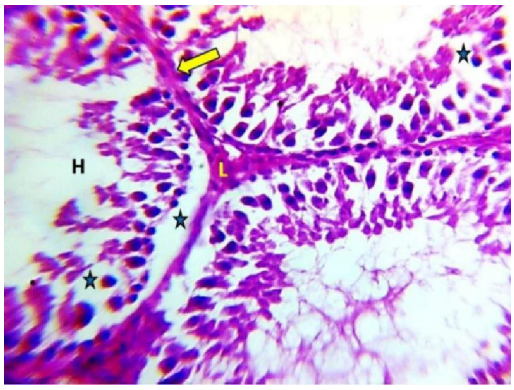

Fig. (13) A photomicrograph of adult albino rat's testis **(G7)** showing many S.Ts. with moderate irregularity in thickness of basement membrane **(yellow arrow)**, mild loss of spermatogenic cells and spermatocytes **(stars)** and moderate hyposprmia **(H)** in lumena of some S.Ts. Moderate increase in interstitial Leydig cells **(L)** between adjacent S.Ts. (H & E x 400).

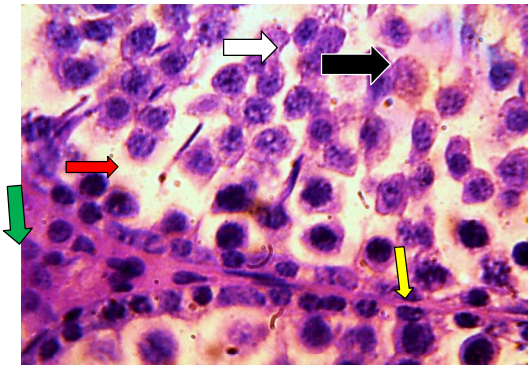

Fig. (14) A photomicrograph of adult albino rat's testis **(G7)** showing many S.Ts. with moderate irregularity in thickness of basement membrane **(yellow arrow)**, mild loss of spermatogenic cells **(red arrow)**, spermatocytes **(white arrow)** and spermatids **(Black arrow)** and resoration of interstitial Leydig cells **(green arrow)** between adjacent S.Ts. (H & E x 1000).

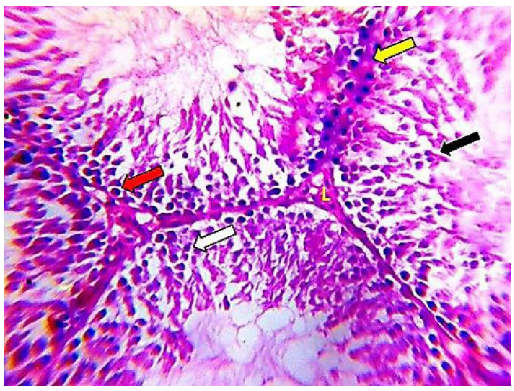

Fig. (15) A photomicrograph of adult albino rat's testis **(G8)** showing many S.Ts. with restoration of regularity in thickness of basement membrane **(yellow arrow)** restoration of spermatogenic cells **(red arrow)**, spermatocytes **(white arrow)** and spermatids **(black arrow)** and resoration of interstitial Leydig cells **(L)** between adjacent S.Ts. (H & E x 400).

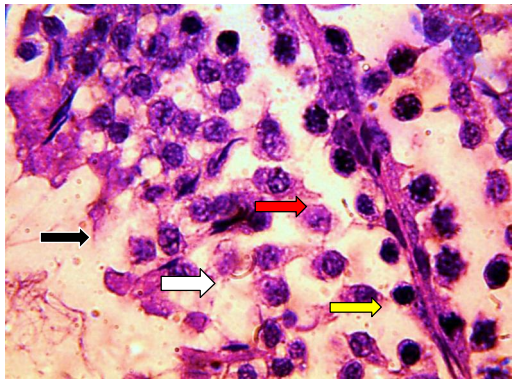

Fig. (16) A photomicrograph of adult albino rat's testis **(G8)** showing many S.Ts. with restoration of regularity in thickness of basement membrane **(yellow arrow)**, restoration of spermatogenic cells **(red arrow)**, spermatocytes **(white arrow)** and spermatids **(black arrow)** (H & E x 1000).

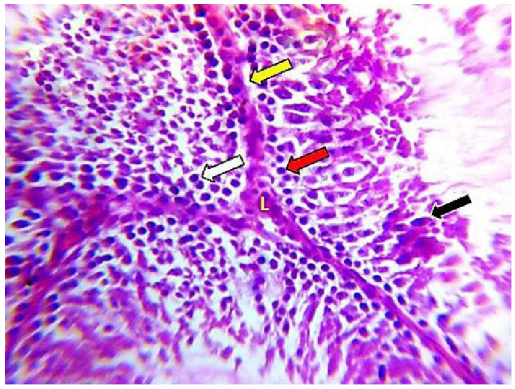

Fig. (17) A photomicrograph of adult albino rat's testis **(G9)** showing many S.Ts. with mild irregularity in thickness of basement membrane **(yellow arrow)**, mild loss of spermatogenic cells **(red arrow)** and spermatocytes **(white arrow)** and spermatids **(black arrow)** in lumena of S.Ts. Mild increase in number of interstitial Leydig cells **(L)** between adjacent S.Ts. (H & E x 400).

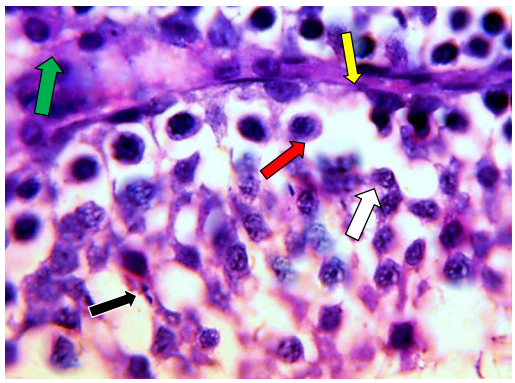

Fig. (18) A photomicrograph of adult albino rat's testis **(G9)** showing S.Ts. with mild irregularity in thickness of basement membrane **(yellow arrow)**, mild loss of spermatogenic cells **(red arrow)** and spermatocytes **(white arrow)** and spermatids **(black arrow)** in lumena of S.Ts. Mild increase in number of interstitial Leydig cells **(green arrow)** between adjacent S.Ts. (H & E x 1000).

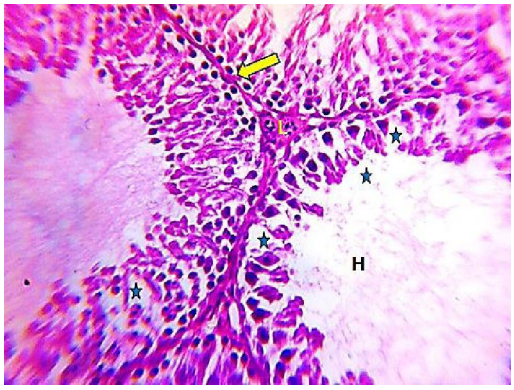

Fig. (19) A photomicrograph of adult albino rat's testis **(G10)** showing many S.Ts. with restoration of thickness of basement membrane **(yellow arrow)**, partial loss of spermatogenic cells and spermatocytes **(stars)** and partial hypospermia **(H)** in lumena of few S.Ts. restoration of number of interstitial Leydig cells **(L)** between adjacent S.Ts. (H & E x 400).

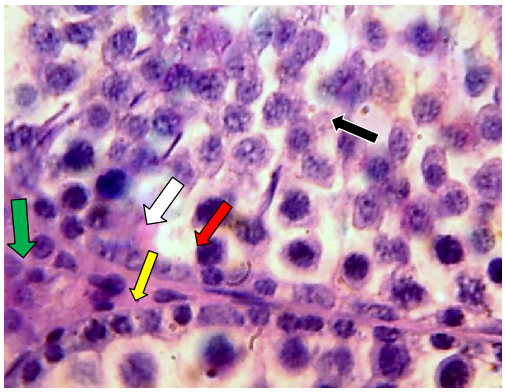

Fig. (20) A photomicrograph of adult albino rat's testis (G10) showing S.Ts. with restoration of thickness of basement membrane (yellow arrow), partial loss of spermatogenic cells (red arrow), spermatocytes (white arrow) and spermatids (black arrow) in lumena of S.Ts. Restoration of number of interstitial Leydig cells (green arrow) between adjacent S.Ts. (H & E x 1000).

4. Discussion

Ribavirin is a synthetic purine nucleoside analogue, which is structurally similar to guanosine and inosine. It appears to inhibit viral protein synthesis through its interference with the function of mRNA. Ribavirin is phosphorylated into ribavirin triphosphate (RTP), which is the active form of the drug with antiviral activity against a broad group of DNA and RNA viruses. It is further catabolized in the liver to triazolecarboxamide, which has no antiviral activity ***(El-Brashy et al., 2007).***

The testis is a complex organ concerned with the production of sperms to fertilize the ova, and secretion of androgens to maintain the secondary sexual characters and proper spermatogenesis ***(Narayana et al., 2002).***

The objective of this work was to investigate the histological changes due to the effect of ribavirin

administration for different periods on the testicular structure of adult male

albino rats in the experimental treated subgroups (G2, G3 & G4) by using light microscope. Then assessment of the effect of drug withdrawal after cessation of drug intake for different durations in the follow up subgroups (G5, G6, G7, G8, G9 & G10) by using light microscope.

The testis of rats administered ribavirin 20mg /kg/day intraperitoneal (IP) for 2 weeks (G2) showed mild affection. Most of the S.Ts. revealed mild vacuolation of spermatogenic cells, Sertoli cells and Leydig cells. Also, S.Ts. showed a thin straight B.M. These observations coincide with those of ***Richman et al. (2002)*** who mentioned that ribavirin was added to the list of chemicals known to cause male reproductive toxicity on the basis of developmental toxicity studies.

In the present study, the degeneration in the testis of rats administered ribavirin 100mg /kg/day IP for 2 weeks **(G3)** showed more affection. Most of the S.Ts. revealed moderate vacuolation of spermatogenic cells. Also, S.Ts. showed irregular B.M. These observations coincide with those of ***Shin et al. (1999)*** who showed that the cell death after ribavirin administration was significant and the number of tubules with dead cells increased at higher dose levels.

In the present work, the testis of adult rats administered ribavirin 200mg /kg/day IP for 2 weeks **(G4)** showed disorganization of S.Ts. and their stratified germinal epithelium. Few sperms, were detected in the lumena of the S.Ts. These results are in accordance with ***Ohta et al., (1996)*** who reported that the earliest changes in the testis of adult rats after ribavirin adminstration occurred by loss of spermatocytes and early spermatids.

The present results also coincide with that of ***Corritori and Brown (2001)*** who evaluated the effects of testicular toxicity of ribavirin and its reversibility in mice. There was significant testicular damage after six months at the human equivalent dose of 400mg/day. So ribavirin is potentially very toxic and represents a challenge to the effective management of patients receiving combination therapy with interferon alpha plus ribavirin.

Also in the (G4) of the present study, marked loss of spermatogenic cells was noticed leaving wide intercellular spaces. These findings are in agreement with ***Urban et al. (2002)*** who reported serious destruction of spermatogenic cells following chronic administration of ribavirin and also with ***Khan and Sinha (1996)*** who mentioned that spermatogenic cells received mutagenic molecules produced in two ways; via those produced in situ as well as those elaborated from hepatic metabolism. The two together, increase the mutagen load in the testes.

This was in agreement with ***Urban et al. (2002)*** who mentioned that high dose administration of ribavirin has marked affection on spermatogenic and Leydig cells.

Moderate hypospermia were detected in G3 and no sperms were detected in G4. These observations coincide with ***Dalmay et al. (2000)*** who mentioned that ribavirin is a teratogen and causes problems with sperm development.

Rao et al. (2003) stated that ribavirin induced germ cell apoptosis. Apoptosis can be induced by various pathological conditions such as heat stress, exposure to ionizing radiation, hormonal depletion or toxic substances such as ribavirin.

Also, abnormal spermatogenesis in our finding is in agreement with ***Narayana et al. (2002)*** who explained this abnormal spermatogenesis and abnormal sperms as that ribavirin was mutagenic to germ cells and produced abnormal shapes of sperms and reduced sperm counts.

In contrary to our explanation, ***Narayana et al. (2002)*** explained the mechanism of these changes as follows: ribavirin inhibits the activity of inosine monophosphate dehydrogenase (IMPDH) that catalyses the oxidation of inosine 5 monophosphate to xanthosine 5 monophosphate which is one of the key enzymes of de novo guanine nucleotide biosynthesis. As IMPDH inhibitors selectively reduce the guanylate concentration, the incomplete guanosine triphosphate (GTP) level possibly down-regulates the G-protein function, a process that hinders the cell growth or induces the apoptosis; so ribavirin was found to elevate the incidence of dead cells in the bone marrow smear and in the testis.

Also, our findings of hypospermia in groups receiving high doses of ribavirin were in agreement with ***Dobrzynska and Gafewski (1999)*** who mentioned that ribavirin induced significant reduction in sperms in the testis of Wistar rats. There was a dose dependent decrease in the sperms in ribavirin treated animals. This study confirmed that ribavirin affects the sperm production and therefore caution should be taken while administering ribavirin to the patients during the reproductive age.

In addition, ***Howel and Shalet (2001)*** mentioned that the treatment with cytotoxic chemotherapy or radiotherapy is associated with asignificant gonadal damage in men and women.

Although ***Tan et al. (2001)*** reported that the fertility of ribavirin treated animals has not been fully investigated, several additional toxicology studies showed that ribavirin caused testicular lesions (tubular atrophy) in adult rats at oral dose levels as low as 6mg/kg/day.

In the present study, many spaces between germinal epithelial cells, vacuoles and thickening of

B.M. were observed in G3 and G4. ***Maekawa et al. (1996)*** concluded that these vacuoles appeared to arise from local dilatation of the intercellular spaces between opposing inter-sertoli cell junctions and shrinkage of germ cells of the S.Ts. These results are in agreement with ***Sinha and Swerdloff (1993)*** who explained that the vacuoles inside the wall of the S.T. were due to phagocytosis of pyknotic bodies and exfoliated germ cells by Sertoli cells. Many reports have demonstrated that such vacuolations of germinal epithelial cells are mainly due to mitochondrial affection.

In the present study, the experimental recovery group G5 **(**that were left for 2 weeks without ribavirin administration after 15 days of daily administration of 20 mg ribavirin**)** showed many S.Ts. with moderate restoration of regularity in thickness of basement membrane, restoration of spermatogenic cells and spermatocytes in lumena of S.Ts. as well as decrease in interstitial Leydig cells between adjacent S.Ts.

These observations coincide with the opinion of ***Narayana et al. (2002)*** who mentioned that ribavirin was mutagenic to rat germ cells and affected the testicular tissue in a transient fashion when given for short periods.

In the present study, the experimental recovery group G6 **(**that were left for 2 weeks without ribavirin administration after 15 days of daily administration of 100 mg ribavirin**)** showed many S.Ts. with moderate restoration of regularity in thickness of basement membrane, mild loss of spermatogenic cells, spermatocytes and mild hyposprmia in lumena of some S.Ts. and mild increase in interstitial Leydig cells between adjacent S.Ts.

These observations are in agreement with ***Schering (2005)*** who stated that ribavirin administration between 20-150 mg /kg /day resulted in significant S.T. atrophy, decreased sperm concentrations, and increased number of sperms with abnormal morphology. Partial recovery was apparent at 15-45 days following dose cessation.

In the present study the experimental recovery group G7 **(**that were left for 15 days without ribavirin administration after 2 weeks of daily administration of 200 mg ribavirin **)**, there were many S.Ts. with moderate irregularity in thickness of basement membrane, mild loss of spermatogenic cells and spermatocytes and moderate hyposprmia in lumena of some S.Ts. and moderate increase in interstitial Leydig cells between adjacent S.Ts. This could be explained according to ***Kenneth et al. (1991)*** who mentioned that the Leydig cells may be expected to receive a higher level of testosterone than centrally located cells.

The experimental recovery subgroups G5, G6 and G7 revealed persistence of affection of testicular tissue in the treated animals after 15 days from stoppage of ribavirin. S.Ts. showed a decrease in the number of germ cell layers and multiple exfoliated germ cells in their lumena. Also vacuolation of spermatogenic cells, sertoli cells and leydig cells was observed.

The experimental recovery group G8 showed restoration of regularity of thickness of basement membrane, spermatogenic cells and Leydig cells. These observations coincide with the opinion of ***Narayana et al. (2002)*** who mentioned that ribavirin was mutagenic to rat germ cells and affected the testicular tissue in a transient fashion when given for short periods.

Marked loss of spermatogenic cells was observed in G9 and G10 while wide intercellular spaces between germ cells and Sertoli cells were observed. These observations coincide with ***Maekawa et al. (1996)*** who mentioned that vacuoles appeared to arise from destruction of spermatogenic cells and Sertoli cell occur due to shrinkage of germ cells and Sertoli cell of the affected S.Ts.

These observations coincide with ***Urban et al. (2002)*** who reported serious destruction of Sertoli and Leydig cells following chronic administration of ribavirin, and with ***Anderson et al. (2004)*** who mentioned that prolonged effect of ribavirin leads to irreversible testicular damage.

From the previous finding, it is important to know that the testicular function of males taking ribavirin presented azospermia and severe disturbances in the germinal cell line on testicular biospsy. This confirms the remote toxicity of alkylating agents of drugs ***(Aubier et al., 1989).***

Similarly, prolonged effect of ribavirin and its metabolites may explain the persistence of testicular affection in the recovery groups of G5-G10 in the present work (except G8).

In conclusion, ribavirin is currently a significant component of the current standard of care for the treatment of chronic hepatitis C. However, it is potentially very toxic and represents a challenge to the effective management of patients using combination therapy with interferon alpha plus ribavirin.

The present work showed that ribavirin administration produced toxicity, mutagenicity leading to marked, and adverse histological changes of the testis at the level of light studies (including the spermatogenic cells, sperms, Sertoli cells and Leydig cells).

These changes persisted after cessation of ribavirin administration indicating the cumulative toxic effects of ribavirin that causes hypospermia that may lead to male infertility. Because it is used nowadays on a large scale especially in middle aged males (30- 50 years), this possible side effect should

be considered when physicians perscribe ribavirin in the protocol of viral hepatitis C management allover the world.

The planned recovery period in this work was not able to determine the reversibility of pathological changes of the testis under effect of ribavirin in a wide range.

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