POTENTIAL AMELIORATIVE ROLE OF GREEN TEA AGAINST THE ANTIVIRAL DRUG RIBAVIRIN-INDUCED HEPATIC AND TESTICULAR TOXICITY IN MALE RATS: HISTOLOGICAL, HISTOCHEMICAL AND BIOCHEMICAL STUDIES

ABSTRACT:
Ribavirin is an anti-hepatitis C virus and other virus drug all over the world. Green tea is one of popular beverages among the whole population—possesses important activities such as antioxidant, antimutation, anticarcinogenesis, antibiotic effect, antihypertension, antihyperglycemia and anti-inflammatory effects. Daily oral administration of ribavirin (90 mg/kg bw) for one month to rats causes damage to hepatic cells such as cytoplasmic vacuolization, congestion of blood vessels, disruption of sinusoids, leucocytic infiltration and rupture in the cell membranes. Nuclear changes include change in size of nuclei and loss of chromatin. Histochemically, ribavirin induced reduction in the general carbohydrates and total protein content in liver cell as well as statistical significant elevation in AST and ALT activities in the serum. Most of these changes disappeared after one week of stopping ribavirin treatment. Drinking aqueous green tea solution (2%) for two weeks before and throughout ribavirin administration alleviated most of these alterations to the extent that slight histological and histochemical changes were detected and no statistical significant difference was noted in liver function enzymes between controls and animals given green tea with ribavirin. In addition, Daily oral administration for one month ribavirin caused change in the size and shape of seminiferous tubules, cytoplasmic vacuolization of some spermatogenic cells, change in intertubular connective tissue, clumping of sperms, and increase in the thickness of the covering capsule. Some pyknotic nuclei were observed. Ribavirin caused increase in carbohydrate and decrease total protein contents in the testis. Moreover, ribavirin administration induced significant reduction in testosterone level in the serum that continued in the recovery period. Drinking green tea for two weeks before ribavirin administration and throughout ribavirin administration reduced the histological alterations in testes and returned the histochemical components to normal limits but failed to maintain the normal levels of the testosterone which still suffered statistical significant decrease even in the recovery period.

KEY WORDS:
Ribavirin, green tea, liver, testis, histology, general carbohydrates, total proteins, AST, ALT, testosterone.

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INTRODUCTION:
Ribavirin (1-beta-D-ribofuranosyl-1, 2, 4, triazole-3 carboxamide), an inosine 5' monophosphate dehydrogenase inhibitor, is a broad-spectrum antiviral drug, especially hepatitis C virus. In addition, Garcia et al. (2000) proved the efficiency of ribavirin in treating hepatitis B. In this concern, these authors recorded that the drug decreased ALT levels and alleviated the histological damage. Ribavirin was approved for use in respiratory syncytial virus aerosol therapy, hepatitis C virus (HCV) combination therapy, and under investigation as a new monotherapy drug for several viral haemorrhagic fevers (VHFs) (Sbrana et al., 2004). In the case of VHFs, ribavirin was proven for its efficiency in the treatment of Lassa and other arenaviral haemorrhagic fevers (Kilgore et al., 1997), haemorrhagic fever with renal syndrome (Huggins et al., 1991), and Crimean-Congo haemorrhagic fever (Mardani et al., 2003).

Kamar et al. (2003) indicated that ribavirin monotherapy, for one year, caused a decrease in liver enzymes (AST, ALT and gamma glutamyl transpeptidase level) and serum creatinine level. Also there was a significant progression in liver fibrosis with no improvement in inflammation scores and haemoglobin levels significantly decreased. Fiel et al. (2000) recorded that ribavirin may cause a dose-dependent reversible haemolytic anemia and increase in iron deposition in hepatocytes from 2.19 to 3.81 compared to placebo. The side effects of ribavirin were recorded by some investigators.
Hoofnagle et al. (1996) indicated that therapy with ribavirin for 6-12 months caused reduction in serum aminotransferases in some chronic hepatitis C patients while the side effects of treatment with 1000-1200 mg daily for 24 months included fatigue and headache. Two patients out of 6 developed gallstones.

In addition, Sherlock (1999) recorded that ribavirin exerted immuno-modularity and anti-inflammatory actions. Narayana et al. (2005) tested the toxic impacts of ribavirin on the testes of male Wistar rats. Histopathologically, the testes revealed formation of vacuoles, gaps and sloughing of the seminiferous epithelium, decreased diameter of the seminiferous tubules, epithelial height and incidence of the stage XIV tubules. However, these changes were returned to normal after 105 days. D’Souza and Narayana (2002) found significant decrease in the step 19 spermatids and meiotic figures and increased incidence of tubules with dead cells on days 14 and 35 upon 5 i.p. treatments with ribavirin at doses of 20, 100, and 200 mg/kg.

Narayana et al. (2002) detected head and tail abnormality in sperms after intraperitoneal treatment with ribavirin in doses 20, 100, and 200 mg/kg given for 5 consecutive days. Moreover, Tran and Martin (2001) recorded the presence of haemolytic anemia as a major side effect of ribavirin treatment when it is used with recombinant interferon alfa in the treatment of chronic hepatitis C virus.

Green tea (Camellia sinensis), the most popular beverage following water, was particularly associated with decreased risk of different proliferative diseases such as cancer and atherosclerosis (Demeule et al., 2002). Increasing interest in the health benefits of tea has led to the occurrence of tea extracts as a dietary supplement in foods. In addition, recent observations suggested that green tea catechins in addition to their antioxidant actions, also affect molecular mechanisms involved in angiogenesis, extracellular matrix degradation, regulation of cell death and multidrug resistance. Earlier studies demonstrated the beneficial actions of tea. It contains caffeine as a common constituent, accordingly, the primary significance of tea is considered to be a caffeine source. Indeed, caffeine possesses a variety of biological and pharmacological actions (Dews, 1982). Tea contains relatively large amounts of catechins. Studies demonstrated that tea or its catechins exhibited a wide range of biological activities such as antioxidant (Matsuzoki and Hara, 1985), antimitation (Kada et al., 1985), anticarcinogenesis (Fujiki et al., 1996), antibiotic effect (Toda et al., 1989), antihypertension (Hara and Tonooka, 1990), antihyperglycemia (Shimizu et al., 1988) and anti-inflammatory effect (Sagesaka et al., 1996). Cheng et al. (1991) ascribed the antimutagenic and anticarcinogenic properties of green tea apicatechin compounds to increased glutathione S-transferase activity, inhibition of edema, hyperplasia and free radical scavenging activity, blocked tumour promoter-induced inhibition of intercellular communication and enhancement of cell-mediated immunity. Lee et al. (1997) elucidated that green tea polyphenolics exerted antimutagenic, anticarcinogenic, antioxidant and antipromotional effects including inhibition of Phase I and inducing Phase II enzymes and inducing activities of glutathione peroxidase, catalase and quinone reductase and glutathione S-transferase. It was recorded by Yoo et al. (2002) that green tea (-)-epigallocatechin-3-gallate (EGCG) suppressed cell growth and induced apoptosis via mitochondrial depolarization, caspase-3 activation and cleavage of DNA fragmentation factor-45 in human endothelial ECV304 cells. The authors' results suggested that EGCG exerted part of its anticancer activity through inhibition of angiogenesis via induction of endothelial apoptosis. Yun et al. (2007) indicated that dietary epigallocatechin-3-gallate reduced the formation of fatty liver and lowered serum AST and ALT upon treatment with ethanol. This work tests the toxic impacts of the antiviral drug, ribavirin on hepatic and testicular tissues of male albino rats and the protective role offered by green tea against these deleterious effects.

MATERIAL AND METHODS:

Male adult albino rats (Rattus norvegicus) of about 2 months old and body weight ranges from 120-125 g were obtained from a common supplier. The animals were kept in the laboratory for at least one week before starting the experiments to be adapted for laboratory conditions under constant temperature (30 ± 2°C) with 12:12 hours light-dark cycle and relative humidity 45 ± 5%. The rats were fed on standard rodent laboratory pellet chow and water was supplied ad libitum. Before starting the experiment, the animals were examined visually for any body abnormality or any abnormal features.

1- Ribavirin and green tea:

Ribavirin tablets (Sigma, Egypt) were dissolved in distilled water and administered orally to rats in a dose level of 90 mg/kg bw (the human therapeutic dose) for one month following two weeks of drinking aqueous green tea solution. Dried green tea herb obtained commercially from the market, was dissolved in boiling water in concentration of 2% and given as the sole source of drink to
rats for two weeks before administration of ribavirin and also throughout ribavirin treatment.

**Design protocol:**

Animals were randomly divided into the following groups:

1. **Control group:** Rats were maintained on normal diet and drank tap water only.

2. **Green tea-drinking group:** Rats drank 2% green tea in water for two weeks. Half of these rats kept drinking the green tea for another one month while the other half administered with 90 mg/kg bw ribavirin with the green tea for one month.

3. **Ribavirin-administered group:** Animals of this group were orally administered with 90 mg/kg bw ribavirin dissolved in distilled water for one month. Organs and sera of rats were taken for inspection at the end of the one month of ribavirin administration and one week post-treatment.

At the end of the desired time intervals, the animals were taken and anesthetized with halothane, dissected as quickly as possible. Small pieces of liver and testes were taken, fixed either in aqueous Bouin’s fluid for general histopathological examination or in 10% neutral formalin for histochemical demonstration. For the histological preparation, specimens were washed in 70% ethanol, dehydrated, cleared and embedded in Parablast (melting point 54-57°C, P.H and Company limited, UK). Sections of 5 µm thickness were cut on rotary microtome (Leica, Model Rm 2125, Germany) and mounted on clean slides. Sections were stained with hematoxylin and counterstained with eosin (Lillie and Fulmer, 1976).

For histochemical demonstration of carbohydrates, periodic acid Schiff’s technique of Hotchkiss (1948) was applied. Total proteins were identified by mercury bromophenol blue method (Mazia et al., 1953). Sera were obtained by centrifugation of blood samples and stored at -20°C until assayed. Liver function enzymes namely ALT and AST were determined according to Gella et al. (1985) using Spinreact kits, S. A. Ctra. Santa Coloma, Spain. Serum testosterone level was measured using commercial kits. The results were expressed as mean ± standard deviation and analyzed statistically at P<0.05 level of significance.

**RESULTS:**

**I- Liver:**

1. **Histology:**

   The histological structure of the control rats appeared normal. The liver of rats that drank green tea had normal histological picture similar to those normal rats that drank tap water; thus the term normal includes organs of rats supplied with water or green tea (Fig. 1).

   ![Fig. 1. Section in the liver of a control rat showing the basic structure where hepatocytes are arranged around central vein (CV). Sinusoids: S; Kupffer cells: K. X400](http://www.egyptseb.org)

   Treatment with ribavirin induced various histological alterations. In this respect, the liver cell of rats inspected at the end of the determined interval (i.e. after one month) exhibited changes in both the nucleus and in the cytoplasm. Many cells showed cytoplasmatic vacuolization and the cell membrane between many cells became ill-defined. The blood sinusoids almost disappeared (Fig. 2). In addition, congested blood vessels were recorded (Fig. 3). Leucocytic infiltration and proliferative bile ductules were evident (Figs 2 & 4). Regarding the nuclei, change in their size and loss of chromatin were observed (Fig. 2).

   ![Figs 2, 3 & 4. Sections in the livers of rats orally administered with ribavirin. Fig. 2 shows Disruption of characteristic architecture, presence of leucocytic infiltration (L), slightly congested portal vein (PV) and cytoplasmatic vacuolization of hepatocytes (arrows) Pyknotic nucleus: PN; bile ductule: Bd. X400. Fig. 3 shows Disruption of normal histology and presence of highly congested central veins (CV) one with eroded wall X100. Fig. 4 shows cytoplasmatic vacuolization of hepatocytes (arrows), slightly enlarged bile ductule (Bd) and periportal leucocytic infiltration (L) X400.](http://www.egyptseb.org)
Liver of rats treated daily with ribavirin for one month while drinking green tea also showed histopathological changes but to a lesser extent in comparison to those administered ribavirin alone. The liver showed little changes in the size of blood vessels (Fig. 5). Blood sinusoids regained their normal size and appearance, little leucocytic infiltration was also observed. However, few cells suffered from the cytoplasmic vacuolization but the nuclei appeared normal (Fig. 6).

2- Histochemistry:

a- General carbohydrates:

The cytoplasm of liver cells of control rats and those drank only the green tea fluid contains considerable amounts of polysaccharides that give red or magenta colour when sections subjected to PAS technique (Fig. 8).

Fig. 8. Section in the liver of a control rat stained with PAS-technique illustrating glycogen content of hepatocytes cytoplasm. Nuclei respond negatively denoting their complete lack of glycogen. Central vein: CV. X400

The nuclei gave negative response. Marked depletion in the general hepatic carbohydrate content in rats orally administered with ribavirin for one month was observed (Fig. 9).

Fig. 9. Section in the liver of a rat given ribavirin for one months exhibiting marked reduction in glycogen. Central vein: CV. X400

After one month of treatment with ribavirin plus green tea, liver sections examination revealed little change in glycogen content in comparison to the control that indicated better protection offered by green tea (Fig. 10). After stopping dual treatment for one week, liver cells possessed normal glycogen inclusion. In addition, in the ribavirin-treated group, certain degree of glycogen restoration was noticed one-week following stopping the treatment but restoration was still far from the normal value (Fig. 11).
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Fig. 10. Section in the liver of a rat given green tea plus ribavirin for one month exhibiting nearly normal PAS-positive reactivity compared to control. X400

Fig. 11. Section in the liver of a rat one week post-ribavirin administration showing still decreasing amount in glycogen compared to normal but elevated amount compared to one month ribavirin treatment. Central vein: CV. X400

Fig. 12. Liver section of a control rat stained with bromophenol blue exhibiting bluish coloration denoting total protein content in all the cytoplasmic and nuclear structures. Central vein: CV; sinusoids: S. X400

The cytoplasm of hepatic cells contain excessive amounts of proteins appeared as fine blue granules. Intense reactivity was noticed in membranes of both the cells and the nuclei denoting their protein richness. Moreover, Kupffer cells and endothelial lining cells of sinusoids exhibited moderate reactivity and the blood vessels walls gave strong reactivity with bromophenol blue stain. However, slight change in total protein content was observed in the liver of rats administered with ribavirin (Fig. 13) as did rats subjected the dual treatment (Fig. 14).

Fig. 13. Section in the liver of a rat treated with ribavirin for one month exhibiting reduction in total protein. Central vein: CV. X400

Fig. 14. Liver section of a rat given green tea plus ribavirin for one month manifested change in total protein content in comparison to control. Central vein: CV; sinusoids: S. X400

In accordance to the results observed after one month of treatment ribavirin, it was reasonable to find the normal amount in protein in hepatocytes of rats in the recovery period in the ribavirin-treated rats and in rats given green tea and ribavirin (Fig. 15).

Fig. 15. Section in the liver of a rat after one week of stop-giving green tea plus ribavirin showing normal protein distribution pattern in hepatocytes. Central vein: CV; sinusoids: S. X400

3- Biochemical results:

a- Change in serum aspartate aminotransferase (AST) activity:

Data in table 1 demonstrated changes in serum AST between control and treated animals. There was no statistical difference in enzyme activity in the sera of control rats and rats drank the green tea solution. There was a
statistical significant increase (P<0.05) in AST activity in the serum of rats administered ribavirin for one month while there was insignificant increase in this activity in the sera of rats left for one week following stopping ribavirin administration. Meanwhile, only insignificant elevation in enzyme activity was noted in the sera of rats in the group that drank the green tea with the drug either at the end of the one month treatment or in the recovery period.

Table 1. Effect of green tea and ribavirin on serum aspartate aminotransferase (AST, µl)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>One month</th>
<th>One week post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>86.36±2.93</td>
<td>87.04±4.26</td>
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<tr>
<td>Green tea group</td>
<td>85.42±3.19</td>
<td>86.61±4.004</td>
</tr>
<tr>
<td>Ribavirin group</td>
<td>92.2±4.06</td>
<td>89.32±6.01</td>
</tr>
<tr>
<td>Green tea + ribavirin</td>
<td>89.11±6.18</td>
<td>88.14±5.29</td>
</tr>
</tbody>
</table>

n= 5 animals for each group.
*: statistical significant increase (P<0.05) in comparison with control.

b- Change in serum alanine aminotransferase (ALT) activity:

Table 2 represented the change in ALT activity among different treatment groups. No statistical difference in the enzyme activity between control rats and rats given green tea solution was recorded. Only statistical significant increase in this enzyme was observed in the one month ribavirin-treated animals. Insignificant changes were recorded among the other ribavirin-treated groups whether given green tea or not.

Table 2. Effect of green tea and ribavirin on serum alanine aminotransferase (ALT, µl)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>One month</th>
<th>One week post-treatment</th>
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<tbody>
<tr>
<td>Control group</td>
<td>37.34±2.26</td>
<td>36.2±2.3</td>
</tr>
<tr>
<td>Green tea group</td>
<td>36.7±3.58</td>
<td>37.2±2.95</td>
</tr>
<tr>
<td>Ribavirin group</td>
<td>42.01±3.38</td>
<td>39.26±4.32</td>
</tr>
<tr>
<td>Green tea + ribavirin</td>
<td>38.1±2.6</td>
<td>37.68±3.14</td>
</tr>
</tbody>
</table>

n= 5 animals for each group.
*: statistical significant increase (P<0.05) in comparison with control.

II- Testis:

1- Histology:

Examination of testis of control rats and that of rats drank green tea exhibited the normal histological structure. Sections of the normal testis revealed the presence of normal-thickened capsule or tunica albuginea and round or oval seminiferous tubules surrounded by intertubular connective tissue containing blood vessels and interstitial cells. The seminiferous tubules contain the spermatogenic cells arranged in a characteristic pattern. The spermatozoa occupy the centre of the tubules (Fig. 16).

Fig. 16. Section in the testis of a control rat showing its basic structure. Spermatozoa (Sp) occupy the centre of the seminiferous tubule. X400

Administration of ribavirin caused changes in the shape and size of the seminiferous tubules, where some tubules appeared shrunken while others appeared highly elongated and both exhibited loss of the characteristic testicular architecture (Figs 17 & 18). Figure 17 also shows the occurrence of degenerated intertubular connective tissue and congested blood vessels. The spermatogenic cells degenerated and the nuclei of few spermatogonia appeared pyknotic. Still, the capsule around the testis appeared thickened than normal. Most tubules suffered from loss of spermatozoa.

Figs 17 & 18. Sections in the testes of rats administered with ribavirin for a month showing corruption of the characteristic histological picture and presence of highly congested intertubular blood vessel (BV) (Fig. 17) and degeneration of spermatogenic cells. Disruption of normal distribution of spermatozoa (Sp) is observed (Fig. 18). X400

Better histological condition was found after one month has lapsed in rats administered with both ribavirin and the green tea where almost normal structure was found with very little alteration (Fig. 19).
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Fig. 19. Testis section of a rat given green tea plus ribavirin for one month showing better histological condition compared to ribavirin treatment. Intertubular connective tissue: CT. X400

Alternatively, specimens of testis collected from rats of this group in the recovery period revealed normal histological structure. However, in the recovery period in the group of rats given ribavirin, arrested phase in testicular structure improvement was attained where the intertubular connective tissue was thickened and the nuclei were darkly-stained (Fig. 20).

Fig. 20. Section in the testis of a rat one week post-stopping ribavirin administration showing improvement in the structure compared to the one-month ribavirin treatment. X400

2- Histochemistry:

a- General carbohydrates:

In the testis of control rats and those who drank the green tea solution, the boundary connective tissue of the seminiferous tubules exhibited a moderate PAS-positive reactivity. The spermatogenic cells within the tubules exhibited weak reaction while the spermatids had strong PAS-positive reaction (Fig. 21).

Fig. 21. Section in the testis of a normal rat stained with PAS-technique illustrating carbohydrate content. Positive reactivity is reflected in the connective tissue (CT), blood vessels (BV) and weak response in the spermatogenic cells. Spermatozoa: Sp. X400

Treatment with ribavirin for one month caused increase in the PAS-positive material in the boundary tissue and in the spermatogenic cells (Fig. 22).

Fig. 22. Testis section of a rat given ribavirin for one month illustrating strong PAS-reactivity in the intertubular connective tissue and slight increase in the spermatogenic cells compared to control. X400

At the end of a month in the group treated with ribavirin and green tea, better condition was observed that testes were better than those of animals treated with the drug (Fig. 23).

Fig. 23. Section in the testis of a rat given green tea for two weeks and administered with ribavirin for one month showing change in PAS-positive material in testicular tissue especially in the spermatogenic cells. X400

After stopping the double treatment for one week, nearly normal PAS-positive material was observed in the testes. In the recovery period in ribavirin-treated rats, a degree of elevation above normal limit was still observed (Fig. 24).

Fig. 24. Section the testis of a rat one week post-stopping ribavirin administration illustrating still elevated PAS-positive material in both the connective tissue and in the spermatogenic cells. X400

b- Total proteins:

The total protein contents in the cytoplasm and nuclei of the spermatogenic cells and Sertoli cells of control and that of rats who drank green tea appeared as deeply-

http://www.egyptseb.org
stained blue granules following staining with bromophenol blue. The boundary tissue of seminiferous tubules and the interstitial cells exhibited strong reactivity (Fig. 25). The walls of the blood vessels gave strong reaction.

![Image 25](http://www.egyptseb.org)

**Fig. 25.** Section in the testis of a control rat stained with bromophenol blue for demonstration of total proteins exhibiting strong reactivity in the seminiferous tubule. X400

Treatment with ribavirin caused decrease in the total protein content of the spermatogenic cells (Fig. 26).

![Image 26](http://www.egyptseb.org)

**Fig. 26.** Section in the testis of a rat subjected to ribavirin administration for one month showing decrease in protein content in spermatogenic cells but not in the intertubular connective tissue that exhibited increase. X400

In the testes of rats that drank the green tea and administered with ribavirin, little change in the total proteins was induced while normal protein inclusion was encountered in the recovery period (Fig. 27).

![Image 27](http://www.egyptseb.org)

**Fig. 27.** Section in the testis of a rat who drank green tea fluid for two weeks and given ribavirin with the tea for one month showing improvement in the protein content condition as compared to ribavirin treatment alone but is still slightly lower than control limits. X400

In addition, only minor change in the protein content was noted in the one week recovery period in the ribavirin-treated rats (Fig. 28).

![Image 28](http://www.egyptseb.org)

**Fig. 28.** Section in the testis of a rat during the recovery period from ribavirin treatment illustrating slight change in total protein content in comparison to control. X400

3- **Testosterone levels:**

Data in table 3 demonstrated changes in testosterone levels between normal rats (control and those drank green tea) and treated groups. Statistical significant change in testosterone level was found between normal rats and those treated with ribavirin with or without green tea. However, the most drastic reduction was found in animals given the drug only. Testosterone decrease was still recorded in the recovery period in both groups denoting strong damaging effect exerted by ribavirin on testicular tissue.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>One month</th>
<th>One week post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>1.18± 0.15</td>
<td>1.29± 0.073</td>
</tr>
<tr>
<td>Green tea group</td>
<td>1.32± 0.101</td>
<td>1.403± 0.055</td>
</tr>
<tr>
<td>Ribavirin group</td>
<td>0.204± 0.006*</td>
<td>0.306± 0.01*</td>
</tr>
<tr>
<td>Green tea + ribavirin</td>
<td>0.481± 0.045*</td>
<td>0.784± 0.07*</td>
</tr>
</tbody>
</table>

n= 5 animals for each group.
*: significant increase (P<0.05) in comparison with control.

**DISCUSSION:**

Ribavirin is a drug used in conjunction with interferon in the treatment of hepatitis C. Although it gives successful results, ribavirin treatment is not completely safe and its side effects were recorded (Kamar et al., 2003).

The results obtained in this work show that ribavirin caused hepatic damage manifested in the form of cytoplasmic vacuolization of hepatocytes, congestion of blood vessels, leucocytic infiltration, as well as disappearance of blood sinusoids, nuclear changes were also evident. However, most of these alterations decreased with stopping treatment. Where liver sections inspected in the recovery period showed nearly the normal histo-architecture. On the other hand, green tea alleviated most of the changes caused by ribavirin administration to the extent that sections obtained from animals in the
recovery period exhibited nearly normal histological structure.

Results obtained in this work revealed damaging effect to liver from ribavirin treatment. However, contradictory were announced by Sbrana et al. (2004) and they proved the usefulness of ribavirin treatment in some cases. They recorded that ribavirin treatment during the first five days after yellow fever in hamster model of the virus disease improved survival rate, decreased the damage to target organs (liver and spleen), prevented hepatocellular steatosis and normalized ALT levels. Earlier work conducted by Inouye et al. (2002) disclosed the mystery of the situation and solved the problem. They indicated that treatment with ribavirin for long periods caused adverse effects in humans such as anemia, depression, pruritus, nausea, cough, chest pain, etc.

Unfortunately, literature concerning histological and histochemical changes induced by ribavirin treatment in liver of animals is sparse. Moreover, the little information collected from the available literature is not clear and decision could not be based on such information. In such a case, interpretation of the obtained results rests on the action of other materials. In accordance, Sakr et al. (2003) recorded cytoplasmic vacuolization of hepatocytes with pyknotic nuclei, congestion of blood vessels and inflammatory leucocytic infiltrations upon treatment with giberrellin A3 in dose level of 24 p.p.m. 3 times a week for 3 weeks. Indeed, other drugs were found by many investigators to cause both histological damage and biochemical changes in liver cells similar to those induced by ribavirin. In this concern, Oz et al. (2004) announced that aceterminophen in a dose level 750 mg/kg caused severe centrilobular necrosis and marked increase in serum ALT activity. Added to that, they found that oral administration of green tea polyphenols significantly attenuated the aceterminophen-induced liver damage and depletion of hepatic reduced glutathione.

In the present work, a decrease in carbohydrate content of liver cells under the effect of ribavirin was recorded. Loss in carbohydrate content may be attributed to the lost ability of the affected hepatocytes to metabolize and store glycogen properly. In addition, Feuer et al. (1966) speculated the glycogen diminution to the elevated lysosomal enzymes activities caused by the deleterious impacts. Owing to these facts, the toxic impact of ribavirin on liver cells should be taken into serious consideration since the liver is the main organ of carbohydrate storage. Decrease in total protein content in hepatocytes was observed in this work. Moreover, some investigators regarded the decrease in total proteins in the liver cells partially to the decreased level of protein synthesis that is due to the hyperactivity of hydrolytic enzymes (El-Beih et al., 1992). Once again, Sakr et al. (2003) found marked decrease in general carbohydrates and total proteins also insignificant increase of ALT in the sera during the first and second weeks followed by a significant decrease, while significant increase in AST was found after the second weeks followed by significant decrease after the third week under the effect of giberrellin A3.

The present results registered significant elevation (P<0.05) in serum alanine aminotransferase and aspartate aminotransferase after one month of ribavirin treatment. However, insignificant increase in these two enzymes was recorded after the recovery period. Green tea pre-treatment corrected this situation where insignificant changes were encountered. Similar attitude to that induced by ribavirin was encountered by Julander et al. (2007). The authors showed significant elevation in the level of alkaline phosphatase, alanine aminotransferase, bilirubin, blood urea nitrogen, potassium and creatinine in the serum, while significant decrease in serum level of albumin, amylase, glucose, calcium, phosphorus, globulin, sodium and total proteins under the effect of treating hamster with ribavirin were noted. In this work, it was found that green tea drinking offered a degree of protection manifested from the microscopic examination and measurement of liver enzymes. Similar results were announced by Sugiyama et al. (1999) who recorded that green tea suppressed the D-galactosamine-induced liver injury in rats as reflected by reduction in ALT and AST activities. Crespy and Williamson (2004) indicated that green tea exhibited antiproliferative activity in hepatoma cells and hypolipidemic activity in hepatoma-treated rats. Added to that, the antioxidant properties of green tea have vast potential as protective agents against various toxic effects (Edwin et al., 2002). In this concern, the authors proved the protective role of tea against damage caused by cyclophosphamide. It was reported that (-)-Epigallocatechin-3-gallate (EGCG)-the major active material in green tea-exerts have anti-inflammatory, antioxidant and immunosuppressive effects. In this concern, Wang et al. (2006) indicated that mice pretreated with EGCG before concanavalin A (ConA) injection exhibited much less increased ALT levels in plasma, reduced inflammatory infiltration and hepatocyte apoptosis in liver. Moreover, the authors concluded that EGCG regulated immune-mediated liver injury in vivo. Yun et al. (2007) recorded that dietary epigallocatechin-3-gallate reduced the formation of fatty liver.
CCl₄-induced lipid peroxidation in testes by 49% and 37%, respectively and decreased liver of female and male rats by approximately 37% at a dose of 1.0 ml CCl₄/kg. The authors suggested that the protective effects of black tea against CCl₄-induced lipid peroxidation in liver, kidneys and testes are partly due to the antioxidant properties and scavenging CCl₄-associated free radicals. Dobrzyńska et al. (2004) indicated that ingestion of green tea prevented ethanol-caused increase in liver function enzymes (alanine aminotransferase and aspartate aminotransferase) activity and reverted the damaging effect caused by ethanol. In addition, long-term drinking of green tea partially prevents the changes in the structure and function of the cell membrane of hepatocytes caused by chronic ethanol intoxication. Hasegawa et al. (1995) indicated that when male rats were given 2% green tea as their drinking water for two weeks before single i.p. injection of the carcinogen 2-nitropropane (100 mg/kg body weight), and liver nuclear 8-hydroxydeoxyguanosine (8-OHdG) levels and hepatotoxicity parameters were determined 6 or 15 hr thereafter, 50% depression in 8-OHdG adducts in liver nuclear DNA caused by 2NP at both time intervals with the green tea pretreatment as well as effective prevention of the time-dependent elevations of serum aminotransferases and lactate dehydrogenase values by 2NP were recorded. In addition, increases of hepatic lipid peroxide levels were depressed 100 and 30%, at 6 and 15 hr, respectively, by green tea and the decrease in hepatic glycogen content at 6 hr was clearly alleviated. Histopathological examination revealed effective protection against induction of hepatic degenerative changes by 2NP at 15 hr.

The present study revealed changes in the histology of the testis upon treatment with ribavirin. The histological alterations were in the form of disruption of the characteristic testicular architecture, disintegration of the intertubular connective tissue, change in size and shape of seminiferous tubules, increased thickness of the covering capsule and clumping of spermatozoa. Certain degree of improvement was noticed in the recovery period. On the other hand, green tea offered protection against the effect of ribavirin. Bearing some similarity to results of this work concerning ribavirin damaging effects is that of Narayana et al. (2005). They indicated formation of vacuoles, gaps and sloughing of the seminiferous epithelium, decrease in tubular diameter and height and incidence of stage XIV tubules on days 14 and 35. Also, ribavirin caused formation of sperms with microcephaly and cephalocaudal junction defects with or without fibrils jetting out, when male Wistar rats were treated with 5 i.p. injections of 20, 100 or 200 mg/kg/day ribavirin at intervals of 24 hours and examined on days 14, 35, 70 and 105 after the last dose. In addition, the present work demonstrated that clear signs of improvement in the structure were attained one week upon stopping ribavirin treatment. This result was also reinforced by the work of Narayana et al. (2005). They observed recovery after one week of stopping ribavirin treatment and that all the morphological defects recovered to normal picture by day 105. Coincides with tubules with pyknotic cells and loss of sperms that was indicated by D'Souza and Narayana (2002) who found significant decrease in the meiotic figures and increase in the incidence of tubules with dead cells on days 14 and 35 of treatment with 20, 100 and 200 mg/kg (i.p.) ribavirin for 5 treatments. In addition, Sakr and Okdah (2004) recorded significant reduction in the diameter of the seminiferous tubules and height of the germinall epithelium, with degeneration of spermatogenic cells, absence of sperm bundles and congestion of blood vessels upon treatment with the fungicide benomyl at a dose level of 1/10 LD₅₀ 3 times/week for 3 weeks.

In the present work, an increase in polysaccharides and a decrease in total protein contents of testes of rats treated with ribavirin were observed. Slight change in these materials was found in the group drank green tea altogether with ribavirin. In the recovery period, animals given the drug with the green tea attained normal distribution pattern. Unfortunately, there is no available literature dealing with ribavirin effect on these materials in the liver and testis. However, other materials gave similar picture. Working in the field of pesticides, Sakr and Okdah (2004) recorded reduction in total carbohydrates in the spermatogenic cells of mice treated with the fungicide benomyl at a dose level of 1/10 LD₅₀ 3 times/week for 3 weeks. Similarly, Moussa and Abdel-Hafez (1983) found reduction in carbohydrates in...
spermatogenic cells of guinea pigs treated with the organophosphorous insecticide dimethoate. The decrease in total protein contents in the testes of rats treated with ribavirin ran parallel to the decreased proteins in the testis of benomyl-treated mice (Sakr and Okdah, 2004). Also, the results obtained in this work were in agreement with those of Ivanova-Chemishanska and Izmirova (1977) who found that oral administration of the fungicide, mane b inhibited protein synthesis in testes and liver of rats. However, the increase in carbohydrate content in the testis in this work was encountered in other organs by other materials that bear some important implications. In this concern, Handy et al. (2002) recorded an increase in carbohydrate staining in the spleen of mice treated with the insecticide diazinon and high fat and protein diets. The authors attributed such phenomenon to a decrease in the organ metabolic rate or an inability to utilize carbohydrates. The histopathological findings in this work reinforced these results where the damage induced by ribavirin was still observed in the recovery period that might reflect disorders in the organ's function. In addition, Sakr et al. (2001) recorded degeneration of spermatogenic cells and destruction of the interstitial tissue of the toad Bufo regularis treated with the anti-inflammatory synthetic glucocorticoid, prednisolone once every 2 days for 3 weeks. In addition, this treatment caused significant reduction in serum testosterone and LH hormones levels. Coincides with the degeneration induced in the intertubular connective tissue of ribavirin-administered rats that was also shown by Sakr et al. (2001) where in their experiment, the connective tissue surrounding the seminiferous tubules was loosely organized and the interstitial cells were less prominent. Still, the damage occurred in the intertubular connective tissue in animals subjected to ribavirin treatment could give a reasonable explanation for the testosterone decrease implying effect on the interstitial cells located in this tissue. Biochemically, it was found in this work that testosterone suffered decrease in ribavirin and ribavirin plus green tea-treated rats. However, drastic decrease was encountered specially in rats administered ribavirin without drinking the green tea. Moreover, the hormone was still decreased even in the recovery period denoting that ribavirin exerted the strongest effect on the testis. Also, the work Narayana et al. (2005) confirmed the reduction in testosterone level with ribavirin treatment and concluded that ribavirin is a gonadotoxic to male rats but the damage was reversible after a period of 105 days, while the hormone disrupting properties of ribavirin occurred beyond this period.

In conclusion, in albino rats ribavirin caused histological and histochemical alterations in the liver and testes and biochemical changes in some serum parameters. Such effects should be interpreted with great caution regarding the severity of the case to be treated. In addition, the protective effect of green tea against ribavirin-induced effects could not be denied and should also be seriously considered especially in cases when ribavirin administration is unavoidable.

REFERENCES:
Abdel Samie HA., Potential Ameliorative Role of Green Tea Against the Antiviral Drug...


The authors aim to evaluate the effects of green tea on liver damage, particularly the protection it offers against oxidative stress and inflammation. They conducted various experiments to determine the protective effects of green tea on liver function, and the results indicate a potential for using green tea in the treatment of liver-related conditions.