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Histological Study on the Effects of Sofosbuvir and/or Ribavirin on Normal Rats

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Abstract

The present study was conducted to demonstrate the histological effects of ribavirin and/or sofosbuvir on liver and kidney of rats. Rats were treated with ribavirin (0.0227 mg/kg body weight), sofosbuvir (0.01 mg/kg body weight) and mixture of ribavirin and sofosbuvir for 12 weeks. Liver and kidney were stained with H/E stain. Liver tissue also stained with AgNORs stain. The results showed that no histological alteration or abnormal counts were observed after treatment with ribavirin and / or sofosbuvir. Also the count of AgNORs is non-significantly differs between the normal non-treated rats and those treated with ribavirin and/or sofosbuvir. The present study concluded that, treatment with ribavirin and / or sofosbuvir or both has no side effects on normal rats.

Keywords: HCV- sofosbuvir- ribavirin- liver- kidney.

Introduction

Hepatitis C virus (HCV) infection exhibits a very important medicinal services load, with 184 million individuals influenced around the world **(Mohd Hanafiah *et al*.2013).** An expected 185 million individuals are living with hepatitis C virus infection worldwide and up to 500,000 deaths are caused by the infection every year **(Mohd Hanafiah *et al* 2013; Lozano *et al*. 2012)**. In spite of the fact that there is no protective immunization against HCV, current treatments give high cure rates, which are required for development of more powerful therapies **(Schmidt *et al.* 2014).** This has brought guarantee that HCV can be wiped out or more adequately averted by treating patients with hepatitis C, a supposed treatment as prevention methodology **(Martin *et al.* 2013).** However, there are serious challenges, including access to HCV testing, care and treatment services **(Yehia *et al.* 2014; Lemoine *et al.* 2013).** Moreover, the danger of re-infection after effective treatment, which is common among injection drug users (IDU), should also be taken into consideration **(Grebely *et al*. 2012).** In Egypt, hepatitis C virus (HCV) infection is a main reason of chronic liver disease and the most well-known sign for

liver transplantation **(National Institutes of Health, 1997; Detre *et al*. 1996).** Interferon alfa (IFN-α) was the main treatment accessible for patients with chronic hepatitis C for a long time. Nonetheless, following 48 weeks of treatment, serum HCV RNA levels are very low in only 15 to 20 percent of patients (**Hoofnagle and Di Bisceglie, 1997; Tine *et al*. 1991; Poynard *et al*. 1996; Poynard *et al*. 1996; Lin *et al*. 1995; Carithers and Emerson. 1997).** Pilot investigations of patients who have relapsed and of previously untreated patients suggest that a combination of interferon and ribavirin is more successful treatment than using interferon alone **(Schvarcz *et al*. 1995; Schalm *et al*. 1997).** For already untreated cases, using interferon and ribavirin together for six months was more effective than interferon alone **(Reichard *et al*. 1998).**

Ribavirin (Virazole, 1_b-D-ribofuranozyl-1, 2, 4-triazole-3-carboxamide) is a C-nucleoside analogue known for its powerful affect against a wide range of DNA and RNA viruses **(Smith and Wade, 1986).** Moreover, it exhibits a medium affinity for adenosine A1 receptors **(Franchetti *et al*., 1995),** which have been distinguished in numerous areas of the brain, including the basal ganglia **(Rivkees et al. 1995; Ochiishi *et al*., 1999)** which is responsible of the regulation of motor activity.

Sofosbuvir commercially had known as SOVALDI, a strong, once every day, orally administered nucleotide analog inhibitor of the hepatitis C virus (HCV) NS5B polymerase. Sofosbuvir powerfully suppress genotype 1–6 HCV RNA replicons in vitro, has a high genetic barrier to resistance, and has shown a strong virologic reaction (SVR) rates when used with ribavirin to patients with chronic genotype 2 and 3 HCV infection and with pegylated interferon ribavirin to cases with chronic genotype 1, 2, 3, 4, or 6 HCV infections. The sofosbuvir/ribavirin or sofosbuvir/pegylated-interferon/ ribavirin therapy gives significantly shorter treatment periods. The present study aimed to evaluate the effects of sofosbuvir and ribavirin and ribavirin/ sofosbuvir mixture on liver and kidney histology of normal rates

Materials and Methods

A total of 32 female albino rats (weighing about 180 ± 10 g) were used for the experimental study. Animals were purchased from Abu Rawash (Cairo, Egypt). Rats were housed in the animal house in Zoology Department, Faculty of Science, Damietta University, New Damietta, Egypt. They were housed in plastic cages under controlled temperature. They were fed on standard pellet diet. Experiments were started after the animals were allowed to adapt for 2 weeks. After adaptation, rats were divided into 4 groups of each of 8 rats.

Control Group:

Fed on basal diet and served as a control group.

Ribavirin Group:

Given dose (0.0227 mg/kg body weight) one time/ day administered by stomach gavage for 12 weeks. **Sofosbuvir Group**: given dose (0.01 mg/kg body weight) one time/ day administered by stomach gavage for 12 weeks.

Sofosbuvir-ribavirin group:

Given a combination of both sofosbuvir-ribavirin administered by stomach gavage according to previous doses one time/ day for 12 weeks. Liver and kidney tissues, from all groups, were collected directly after scarifying the animal, washed by saline solution and fixed in 10% formalin. Paraffin blocks were formed and sections were cut at 5 µm. for histopathological studies,

-Haematoxylin /Eosin stain:

The method was done according to **Drury and Wallington (1967).**

-AgNORs Stain:

The method was done according to **Crocker and Nar (1987).** Briefly, dewaxed sections were incubated with silver staining mixture (1 volume of gelatin at 2 g/dl in lg/dl formic acid and volumes 50 g/dl aqueous silver nitrate solution). The

staining was done at room in dark for about 40 min. from each section (liver or kidney) the nuclear organizer regions of 50 cells were calculated and the average of each group was calculated.

Results:

H/E stain:

1-Liver

Fig (1) showed that the liver of the control rats is composed of numerous hepatic lobules with indistinct outlines. Each lobule possesses a central vein and consists of hepatic strands of cells, these strands or cords anastomose with each other and enclose minute blood sinusoid. The hepatocytes are arranged in sponge-like plates one cell thick and appeared polygonal with centrally located nuclei. The lymphocytes are seen distributed throughout the hepatic tissue. The sinusoidal endothelium is formed of hardly distinct undifferentiated lining cells and the phagocytic Kupffer cells. The latter are distinctly large with oval nuclei. After treatment with ribavirin and/ or sofosbuvir no damage or alteration in liver tissue were observed (Fig 2,3 and 4).

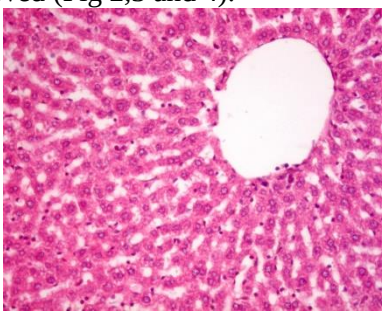

Fig (1): Photomicrograph of rat liver of the control group showing the normal architecture of the liver (H/E. X200)

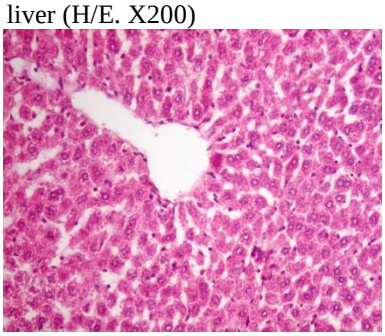

Fig (2): Photomicrograph of rat liver of the ribavirin group showing normal architecture of the liver (H/E. X200)

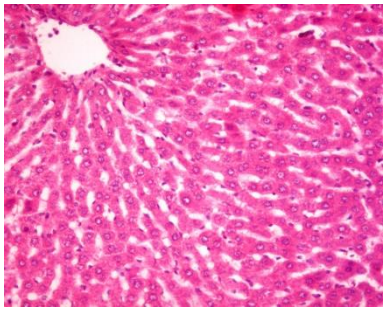

Fig (3): Photomicrograph of rat liver of the sofosbuvir group showing normal architecture of the liver (H/E. X200)

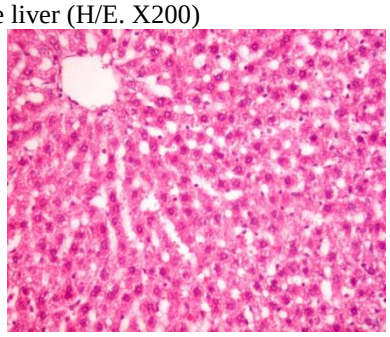

Fig (4): Photomicrograph of rat liver of the ribavirin/sofosbuvir mixture group showing normal architecture of the liver (H/E. X200)

2-Kidney:

Fig (5) showed the control of the kidney. In this figure, kidney is a compound tubular gland enclosed by a firm connective tissue capsule. The kidney is differentiated into; an outer cortex and an inner medulla. The uriniferous tubules are the structural and functional units of the kidney. The uriniferous tubule consists of Malpighian corpuscles, proximal convoluted tubule, Henele's loop and distal convoluted tubule. The cortex consists of Malpighian corpuscles and both proximal and distal convoluted tubules while the medulla consists mainly of the Henele's loop. The collecting tubules, however, are located in both cortical and medullar regions. In the cortex, numerous renal corpuscles are arranged in parallel rows at right angle to the capsule. Each renal corpuscle is roughly spherical in shape and consists of the Bowman's capsule enclosing the glomerulus, a tuft of blood capillaries. The proximal and distal convoluted tubules are lined with typical thick cubic epithelium. They have relatively regular distinct lumina. At light microscopic level, the control kidney is composed of the cortex and medulla. The cortex is distinguished by renal corpuscles, surrounding by an outer

envelope of simple squamous epithelium forming the Bowman's capsule and leaving a Bowman's space. The glomerular capillary loop is thin and delicate. The proximal and distal convoluted tubules showed normal lining epithelium and tubular lumina. In rats treated with ribavirin and /or sofosbuvir, the histology of the kidney is normal and no changes were observed **(fig 6, 7 and 8 respectively).**

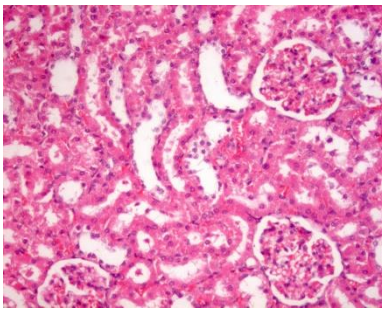

Fig (5): Photomicrograph of rat kidney of the control group showing the normal architecture of the liver (H/E. X200)

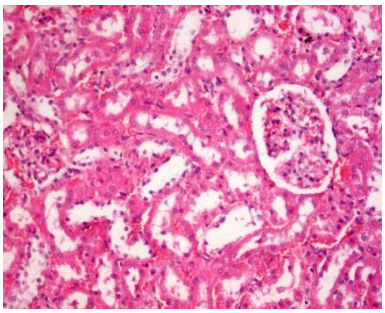

Fig (6): Photomicrograph of rat kidney of the ribavirin group showing the normal architecture of the liver (H/E. X200)

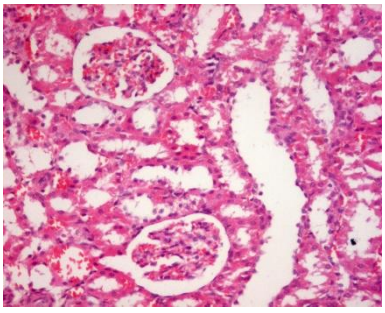

Fig (7): Photomicrograph of rat kidney of the sofosbuvir group showing the normal architecture of the liver (H/E. X200)

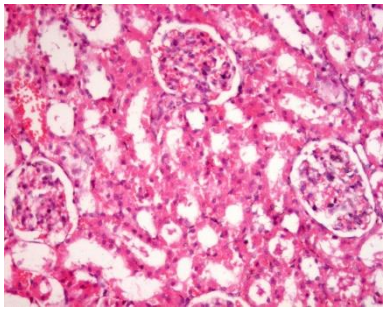

Fig (8): Photomicrograph of rat kidney of the ribavirin/sofosbuvir mixture group showing the normal architecture of the liver (H/E. X200)

AgNORS:

AgNORs counting of the control liver was 2.11. In ribavirin the count was 2.31. In addition m the count in sofosbuvir, the count was 2.18 but in mixed the count was 1.91. No significant changes in AgNORs count were observed between groups (figure 9).

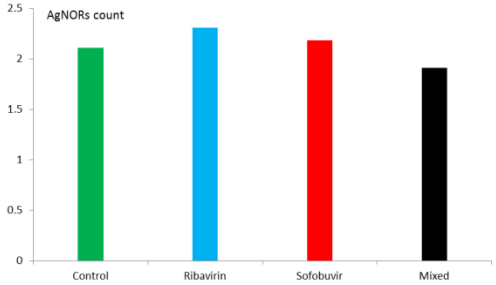

Fig (9): Count of AgNORs in liver of different experimental groups.

Discussion:

Around 25 % of HCV cases are self-constrained resulting in automatic clearance after acute infection, while the rest 75 % of cases advance into chronic disease **(Hajarizadeh et al 2013).** Up to 33% of patients with chronic hepatitis C progress liver cirrhosis or potentially hepatocellular carcinoma (HCC) **(Hajarizadeh et al 2013; Thomas et al 2013; Ly KN et al 2012).** Current treatments of HCV give high cure rates, which are required for development of more powerful therapies **(Schmidt *et al.* 2014).** The present study showed that there is no histological damage of liver or kidney due to the treatment with ribavirin and / or sofosbuvir. Many studies evaluated the impact of ribavirin and sofosbuvir on the improvement of inflammation and fibrosis that drive histological benefit and this may

reduce the rate of fibrosis progression in HCV patients **(Mchutchison et al., 1998; Poynard et al., 2002).** None of these previous studies examined the histologic effect of direct antiviral drugs such as sofosbuvir and ribavirin. From the present study, the applied treatments with ribavirin (0.0227 mg/kg body weight) and sofosbuvir (0.01 mg/kg body weight) for 75 days caused no histopathological changes in the liver. Other studies showed long term improvement of some histopathological changes such as inflammation and fibrosis in HCV patients and no adverse effect of both ribavirin and sofosbuvir on the histological structure of the liver **(Shiffman *et al*., 2014).** In consistence with the results of the above results of the present study showed that, the number of AgNORs count was normal in all groups of the study as well as no differences in the count were observed between groups. The present study concluded that treatment with sofosbuvir has no side effect on the liver or kidney tissues of rats.

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الملخص العربي

عنوان البحث: دراسة نسيجية علي تأثيرات والسوفوسبوفير والريبافيرين على الجردان الطبيعية

جمال عبدالرحيم عبدالله ، قدري البكري، نجلاء العربانى، سيق التمتام

قسم علم الحيوان- كلية العلوم- جامعة دمياط

اجريت الدراسة الحالية لقياس مدي تأثير السوفوسبوفير والريبافيرين علي انسجة الكبد والكلي في الجردان الطبيعية تم معالجة الجرذان بجرعة قدرها 0.01 مج/كج من عقار السوفوسبوفير يوميا لمدة 12 اسبوع وجرعة قدرها 0.0227 مج/كج يوميا لمدة 12 اسبوع من الريبافيرين. وقد تم معاملة مجموعة من الجرذان بخليط من السوفوسبوفير والريبافيرين يوميا لمدة 12 اسبوع . بنهاية فترة العلاج تم ذبح الجرذان واستئصال الفص الاكبر من الكبد و الكلي من جرد واجراء الدراسة النسيجية عليهم و اوضحت النتائج ان لا توجد اي تغيرات نسيجية في انسجة الكبد او الكلي للجرذان المعاملة بالسوفوسبوفير و/ او الريبافيرين وتأكيدا لدلك فقد وجد انه ليس هناك اي فروق معنوية في عد مناطق المنضمات النووية في كل الجرذان المعاملة مقارنتا بجرذان المجموعة الغير طبيعة الغير معاملة. ومن الدراسة الحالية يمكن الاستنتاج ان العلاج بالسوفوسبوفير والريبافيرين لا يسببان اي اتار جانبية او تلف لأنسجة الكبد او الكلي.