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SHORT AND LONG TERM CARDIOTOXICITY OF SOFOSBUVIR AND DACLATASVIR ASSOCIATED WITH LIPID PROFILE ABNORMALITIES

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Background: *Direct-acting antivirals (DAA) are relatively a new group of drugs. Different studies reported disturbances in lipid metabolism among HCV patients treated with DAA combinations* ***Objective:*** *This study tries to evaluate the short and long term effects of DDA on lipid profile, cardiac enzymes and oxidative stress, as well as, to determine if these effects are disease-related or drug-dependent.* ***Methods:*** *Male Wistar rats were treated with sofosbuvir with or without daclatasvir for four consecutive weeks. Five samples were collected at day 0 (baseline point), another ten samples were obtained after four weeks (end of treatment point) and finally, after six months of treatment, the last ten animals were assigned for follow up point. AST, ALT, lipid profile, serum creatine kinase and troponin were assessed colorimetrically. Moreover, liver tissue content of malondialdehyde was assessed.* ***Results:*** *Results revealed that, at the end of drug therapy period, sofosbuvir whether alone or combined with daclatasvir caused significant increase in total cholesterol, LDL and triglyceride compared to baseline data. These effects were persistent for 20 weeks after the end of treatments. This increase in lipid profile was also correlated with a significant deterioration of cardiac markers such as troponin and creatine kinase –MB and increased oxidative stress.* ***Conclusion:*** *Sofosbuvir and/or daclatasvir elevate lipid profile and cardiac enzymes. These changes are due to the effects of direct acting antiviral agents and independent of hepatitis C virus infection. Consequently, Lipid profile and cardiovascular markers should be monitored during and after drug cessation.*

Keywords: *Sofosbuvir –daclatasvir – lipid profile – troponin – CK-MB - MDA*

INTRODUCTION

Direct-acting antivirals (DAAs) are relatively a new group of drugs that was developed as a result of extensive study of hepatitis C virus (HCV) life cycle[1]. These drugs are designed to inhibit different nonstructural HCV proteins that enable virus replication[2]. According to their mechanism of action DAAs are divided into three types. The first is; NS3/4A inhibitors. The second category which includes daclatasvir (DCV) targets NS5A protein. Another important group of DAAs are the NS5B nucleotide inhibitor or polymerase inhibitors such as sofosbuvir (SOF)[3&4].

The regimens for HCV eradication are usually combination of two of more DAA agents and the selection is mainly genotype specific. Sofosbuvir/daclatasvir combination with or without ribavirin are assigned for all HCV genotypes[5]. The approval of direct acting antiviral is considered as a revolution in HCV eradication. The efficacy of these drugs

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evidenced to be more potent than pegylated interferon-based regimens. Moreover, treating naive patients with SOF/DCV achieved sustained viral response (SVR) reaching up to (96% to 100%)[6&7]. In addition, this combination achieved SVR 84.54% in cirrhotic patients and 87.01% in treatment-experienced patients[8].

Recent studies reported lipid metabolism disturbances in HCV patients treated with DAA combinations. In 2018, Chida and his coworkers reported rapid persistent increases in serum levels of total low density lipoprotein (LDL-cholesterol) and high density lipoprotein (HDL-cholesterol) in patients receiving a combination of asunaprevir and daclatasvir[9]. Similarly, HCV patients treated with sofosbuvir and simeprevir showed significant elevations in serum cholesterol, LDL-c and HDL-c as well as LDL/HDL ratio while serum triglyceride was decreased[10]. Moreover, sofosbuvir and ribavirin combination was shown to significantly induce an increase in serum LDL along treatment period[11]. It is not yet clear whether these reported changes in the lipid profile associated with DAA regimens, occurs as a result of HCV eradication or it is a direct pharmacological side effect of these drugs[12]. Upto date, the cardiovascular consequences of this effect are not yet assigned. In the current study, we hypothesize that the pharmacological profile of DAA regimens may include a modulation of lipid profile independent of HCV eradication activity. This work aimed to investigate the impact of sofosbuvir/daclatasvir combination on lipid profile in healthy rats and its cardiovascular consequences if any.

MATERIALS AND METHODS

Drugs and chemicals

Sofosbuvir and daclatasvir powders were kindly provided by Marcyrl Company for pharmaceutical industries, Cairo, Egypt. Sofosbuvir was suspended in 0.2% methylcellulose whereas daclatasvir was dissolved in normal saline. Unless stated, the rest of chemicals were purchased from Merck, Taufkirchen, Germany.

Animals

Hundred male Wister rats weighing (100-150 g) were purchased from National Research Center, Dokki, Giza, Egypt. The animals were kept under standardized conditions of at least two weeks before starting the experiments. Animals received standard rodent pellet diet and drinking water *ad libitum*. Rats were kept at 25 ± 2 °C, 60% humidity and a 12:12 hrs light/dark cycle. Animal care and experiments were conducted in accordance with the protocols approved by the ethical committee, Faculty of Pharmacy, Kafrelsheikh University. Additionally, the experimental procedures were performed in compliance with the ARRIVE guidelines in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments**.**

Experimental protocol and samples collection

Rats were randomly and equally divided into four groups (25 rats/group). The first one received only the vehicles and served as a control. The second group was treated orally with sofosbuvir (20 mg/kg). The third group received an oral daclatasvir (30 mg/kg). The fourth group received a combination of sofosbuvir and daclatasvir orally with same mentioned doses. Drugs were administered daily for four consecutive weeks. Five samples were collected at day 0 (baseline point), another ten samples were obtained after four weeks (end of treatment point) and finally, after six months of treatment, the last ten animals were assigned for follow up point (Figure 1).

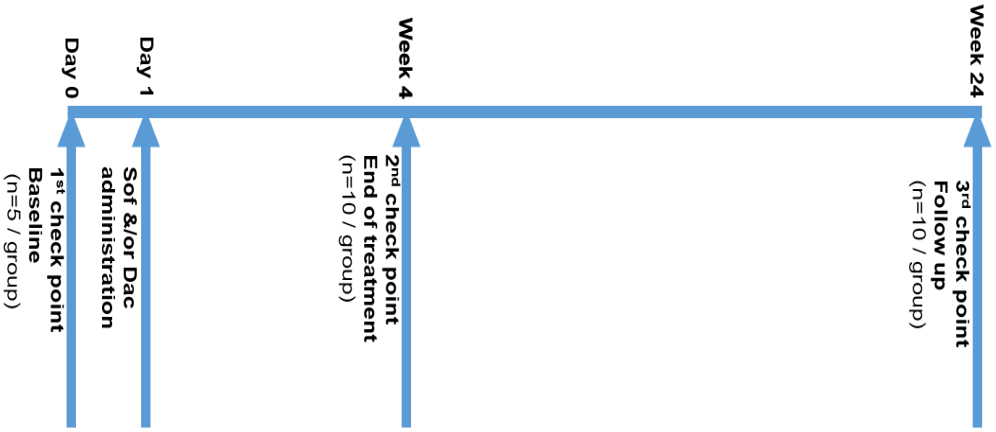

Fig. 1: Experimental design where samples were collected at day 0 and assigned for baseline point. One day later, sofosbuvir (Sof) and/or daclatasvir (Dac) were administered orally for four consecutive weeks, hence another samples were collected for analysis. Animals were left for another twenty weeks for follow up assessment. Samples were withdrawn via heart puncture.

Rats were anesthetized by diethyl ether and blood samples were obtained via cardiac puncture into non-heparinized tubes. Samples were allowed to clot at room temperature for 60 min. The samples were then centrifuged (3000 rpm, 10 min, 4ºC) and the resultant serum in each supernatant was separately aspirated and stored at -20°C for the time of analyses. After blood sample collection, animals were sacrificed by decapitation and liver tissues were collected then divided into two parts. The first part immersed in 10 % formol saline (PH 7.4) and embedded in paraffin blocks for histopathological examination. The second part was washed, weighed, and homogenized in 2 ml phosphate saline buffer under ice condition, The obtained homogenate was centrifuged at 5000 rpm at 4 ◦C for 15 min. The clear supernatant was separated kept at -80°c for biochemical analysis.

Indirect assay of liver functions

Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) activities were assayed using kits from Biodiagnostic (Giza, Egypt) that measured the amount of pyruvate and oxalo-acetate, respectively, produced from 2,4-dinitrophenylhydrazine.

Measurement of lipid profile

Total cholesterol and triglycerides were assayed according to kits' instructions (Spinreact, Barcelona, Spain). HDL were assayed using Biodiagnostic specific kit (Giza, Egypt) in compliance with producer instructions. LDL levels were calculated according to the formula LDL = Total Cholesterol-HDL-(Triglycerides/5).

Assay of creatine kinase and troponin

Serum Creatine kinase and troponin were measured according to manufacturer instructions of BioSystems (Barcelona, Spain) and Thermo Fisher Scientific, (Waltham, USA), respectively.

Assay of oxidative stress in serum

Liver tissue contents of malondialdehyde (MDA) were assessed as a measure for oxidative stress. MDA was assayed as previously described by Satoh using a Biodiagnostic specific kit (Giza, Egypt)[13].

Statistical analysis

All data were normally distributed, expressed as mean ± SD and statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey as a post-hoc test. Data are considered to be significant at $p < 0.05$. All analyses were performed using Prism software (v.6.0, Graphpad, San Diego, CA, USA).

RESULTS AND DISCUSSION

Results

Baseline characteristics of all groups

The base line data collected from all experimental groups showed non-significant differences at the level of liver enzymes (ALT and AST), lipid profile (cholesterol, LDL, triglyceride, and HDL), cardiac enzyme (troponin and CK-MB) and oxidative stress measured as MDA (Table1).

Effect of sofosbuvir and daclatasvir on liver functions

Treating animals with sofosbuvir and daclatasvir whether alone or in combination showed non-significant changes in serum levels of ALT and AST compared to control group at baseline, neither after the 4th week of treatment period nor at the follow up point; the 24th week (Table 2).

Table 1: Baseline characteristics of all groups.

	Control	Sofosbuvir	Daclatasvir	Sof +Dac
ALT (U/L)	33.00 ± 3.90	35.60 ± 6.348	30.00 ± 4.55	29.80 ± 5.50
AST (U/L)	27.40 ± 6.80	30.40 ± 5.177	26.80 ± 2.60	27.20 ± 3.60
T. Cholesterol (mg/dl)	70.00 ± 6.30	72.00 ± 5.14	66.00 ± 4.98	73.00 ± 3.20
LDL (mg/dl)	23.30 ± 1.70	25.70 ± 1.98	22.90 ± 2.5	26.00 ± 2.10
TG (mg/dl)	55.30 ± 3.50	54.00 ± 9.20	59.70 ± 5.66	54.70 ± 2.98
HDL (mg/dl)	26.30 ± 2.30	23.00 ± 2.05	24.20 ± 2.50	26.70± 2.60
Troponin (ng/ml)	0.35 ± 0.04	0.33 ± 0.10	0.40 ± 0.09	0.40 ± 0.10
CK-MB (U/L)	50.10 ± 2.30	59.00 ± 7.50	49.50 ± 5.90	52.30 ± 4.60
MDA (mmol/dl)	30.60 ± 7.30	33.98 ± 2.98	34.30 ± 6.20	30.00 ± 3.70

Data are expressed as mean ± SD.

Table 2: Effects of sofosbuvir and daclatasvir on liver functions.

	Baseline	4th week	24th week
ALT (U/L)			
Control	33.00 ± 3.90	35.50 ± 3.73	38.80 ± 5.715
Sofosbuvir	35.62 ± 6.40	37.83 ± 2.32	37.50 ± 6.950
Daclatasvir	30.00 ± 4.60	38.67 ± 5.90	36.33 ± 8.140
Sof/Dac	29.80 ± 5.50	36.83 ± 5.50	37.83 ± 7.026
AST (U/L)			
Control	27.40 ± 6.81	31.00 ± 5.20	30.80 ± 6.648
Sofosbuvir	30.40 ±5.20	33.20 ± 4.88	32.60 ± 5.320
Daclatasvir	26.80 ±2.60	33.80 ± 7.10	30.40 ± 3.647
Sof/Dac	27.20 ± 3.60	35.00 ± 8.97	33.00 ± 3.606

Data are expressed as mean ± SD.

Effect of sofosbuvir and daclatasvir on lipid profile

Control rats showed non-significant changes in total cholesterol, LDL, Triglycerides and HDL along all experiment period starting from baseline until follow up after 6 months (Table 3). Sofosbuvir-challenged rats showed a significant increase in total cholesterol, LDL and triglycerides compared to control group at the 4th week of treatment as well as at the 24th week of the experiment ($p \leq 0.001$). On the other hand, sofosbuvir had a non-significant effect on HDL level compared to control group along all experimental periods (Table 3, Figure 2).

Daclatasvir significantly augmented serum triglycerides and LDL levels compared to control rats at the 4th and 24th weeks of the experiment while daclatasvir effects on total cholesterol and HDL were non-significant compared to control rats. In addition, the augmented effects of daclatasvir on triglycerides and LDL at the 4th and 24th weeks of the experiment were significant compared to the baseline data (Table 3, Figure 2). The serum levels of total cholesterol and HDL in

daclatasvir-treated rats were nearly constant along treatment period. Co-administration of sofosbuvir and daclatasvir resulted in a significant elevation of total cholesterol, LDL, triglycerides levels compared to control group at the 4th and 24th weeks of the experiment, whereas the serum level of HDL remained unchanged (Table 3, Figure 2).

The serum increased levels of LDL and triglycerides in response to combined therapy were significant when compared to the values achieved by sofosbuvir alone at the 4th week of the experiment. There were no significant differences at the 24th week of the experiment (Table 3).

Table 3: Effects of Sofosbuvir and Daclatasvir on lipid profile.

	Control	Sofosbuvir	Daclatasvir	Sof + Dac	n
Total cholesterol (mg/dl)					
Baseline	70± 6.33	72 ± 5.14	66 ± 4.98	73 ± 3.2	5
4th week	69.00 ± 3.34	87.20 ± 4.55*¥	67.00 ± 2.60	80.00 ± 3.18*α	10
24th week	66.00 ± 4.98	94.20 ±10.1*¥	67.90 ± 8.70	88.00 ± 6.40*α	10
LDL (mg/dl)					
Baseline	23.36 ± 1.20	25.70 ± 1.70	22.90 ± 2.10	26.00 ± 2.90	5
4th week	22.80 ± 3.80	45.20 ± 5.63*¥	27.60 ± 2.10*β	38.80 ± 2.60	10
24th week	24.00 ± 4.60	47.20 ± 7.90*¥	31.20 ± 2.11*β	41.40 ± 3.11*α	10
TG (mg/dl)					
Baseline	55.30 ± 3.50	53.98 ± 9.2	59.68 ± 5.66	54.68 ± 2.98	5
4th week	49.60 ± 5.82	87.60 ± 5.8*¥	75.20 ± 3.96*β	82.60 ± 10.14*α	10
24th week	57.2 ± 4.15	89.20 ± 6.76*¥	68.60 ± 7.70*β	83.20 ±7.40*α	10
HDL (mg/dl)					
Baseline	26.25 ± 2.3	22.98 ± 2.05	24.20 ± 2.50	26.68 ± 2.60	5
4th week	24.60 ± 3.21	24.40 ± 3.98	23.60 ± 2.97	27.00 ± 3.65	10
24th week	29.60 ± 4.45	27.80 ± 2.68	26.20 ± 3.35	30.20 ± 3.11	10

Data are expressed as mean ± SD.* marked significant differences from control. ¥ marked significant differences from sofosbuvir baseline. β marked significant differences from daclatasvir baseline. α marked significant differences from sof/ dac baseline.

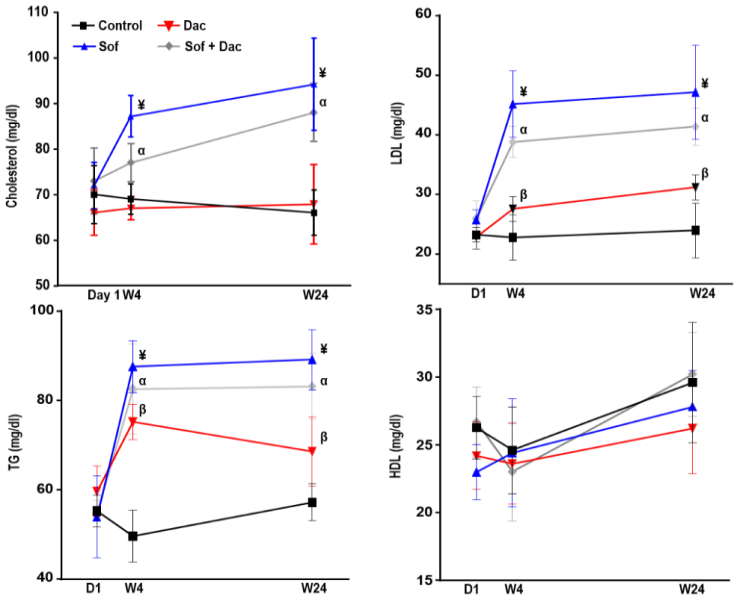

Fig. 2: Effect of sofosbuvir (Sof) &/or daclatasvir (Dac) on lipid profile. Apart from HDL, oral Sof and/or Dac significantly increases lipid profile values (cholesterol, LDL and triglycerides). The effects seem to be due to sofosbuvir more than daclatasvir. Data are expressed as mean ± SD. ¥ marked significant differences from sofosbuvir baseline. β marked significant differences from daclatasvir baseline. α marked significant differences from sof/ dac baseline. Samples were collected at three different check points, day 1 of the experiment (D1; n = 5), after four weeks (W4; n =10) and after 24 weeks (W24; n = 10).

Effect of sofosbuvir and daclatasvir on cardiac enzymes

Control rats showed non-significant changes in troponin and creatine kinase-MB (CK-MB) along the whole experimental period, starting from the baseline until follow up after 6 months (Table 4 and Figure 3). Compared to the control group, sofosbuvir-treated rats showed a significant increases in troponin and CK-MB levels after four weeks. At the last check point (24th week), both enzymes were significantly higher than control and sofosbuvir baseline as well (Table 4 and Figure 3). On the other hand, daclatasvir treated rats showed a significant increase in troponin serum level compared to control rats at the 4th and 24th weeks of the study. There were no significant differences between both values (Table 4 and Figure 3).

It is important to report that daclatasvir had no impact on CK-MB serum level compared to the control group at the baseline, 4th week and 24th week of the study. The combination of sofosbuvir and daclatasvir significantly elevated the serum level of troponin and CK-MB compared to the control group at the 4th and 24th week of the study. Similarly, the levels of these parameters were significantly increased at the 4th and the 24th week compared to the baseline data (Table 4 and Figure 3).

Table 4: Effects of sofosbuvir and daclatasvir on cardiac enzymes.

	Control	Sofosbuvir	Daclatasvir	Sof + Dac	n
Troponin (ng/ml)					
Baseline	0.35 ± 0.03	0.33 ± 0.10	0.39 ± 0.095	0.40 ± 0.10	5
4th week	0.31 ± 0.15	1.48 ± 0.39*¥	0.65 ± 0.26*	1.50 ± 0.27*α	10
24th week	0.28 ± 0.09	1.53 ± 0.39*	0.78 ± 0.27*	1.50 ± 0.32*α	10
CK-MB (U/L)					
Baseline	50.10 ± 2.30	59.00 ± 7.50	49.50 ± 5.90	52.30 ± 4.60	5
4th week	55.80 ± 9.30	178.40 ± 19.30*¥	50.20 ±7.90	153.60 ± 19.50*α	10
24th week	46.00 ± 15.70	251. 80 ± 36.13*¥	64.20 ± 17.30	240.60 ± 37.30*α	10

Data are expressed as mean ± SD.* marked significant differences from control. ¥ marked significant differences from sofosbuvir baseline. α marked significant differences from sof/ dac baseline.

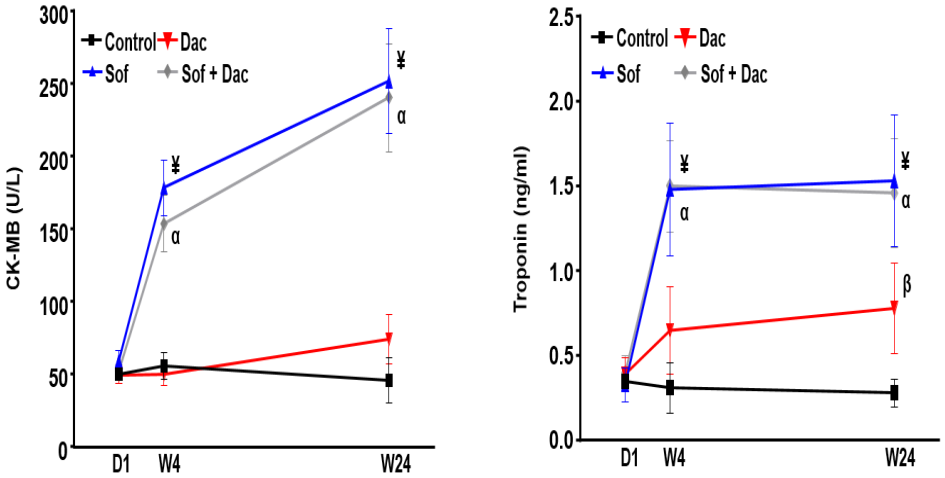

Fig. 3: Effect of sofosbuvir (Sof) &/or daclatasvir (Dac) on cardiac enzymes. Left panel represents the effect on CK-MB where right one represents troponin. Sofosbuvir seems to have the most deteriorating impact after the fourth week as well as at the end of experiment. Data are expressed as mean ± SD. ¥ marked significant differences from sofosbuvir baseline. β marked significant differences from daclatasvir baseline. α marked significant differences from sof/ dac baseline. Samples were collected at three different check points, day 1 of the experiment (D1; n=5), after four weeks (W4; n=10) and after 24 weeks (W24; n=10).

Effect of sofosbuvir and daclatasvir on oxidative stress

The oxidative stress was measured as malondialdehyde level in serum. The results showed that sofosbuvir either alone or combined with daclatasvir showed an increase in MDA serum level compared to the control group at the end of the 4th and 24th week of the study. These increases were also significant when compared to the baseline data (Table 5 and Figure 4).

The increase in the oxidative stress induced by daclatasvir therapy was slightly different. The drug induced a significant increase in MDA level at the end of the 4th week compared to the control. Following up, the rats revealed that the increase in MDA level was not persistent at the 24th week when compared to control group and even to the baseline data of this group.

Histopathological examination of liver tissues

A liver section from control rats showed normal liver architectures (Figure 5A). Liver section from sofosbuvir-treated rats showed fatty changes detected in the hepatocytes adjacent and surrounding the congested central vein (Figure 5B). On the other hand, daclatasvir-treated rats showed normal hepatic parenchyma (Figure 5C). Lastly, rats treated with sofosbuvir and daclatasvir showed fatty changes in the hepatocytes surrounding and adjacent to the dilated central vein (Figure 5D)

Table 5: Effect of sofosbuvir and daclatasvir on oxidative stress.

	Control	Sofosbuvir	Daclatasvir	Sof + Dac	n
MDA (mmol/dl)					
Baseline	30.60 ± 7.33	33.98 ± 2.98	34.30 ± 6.20	30.80 ± 3.70	5
4th week	32.60 ± 2.50	40.90 ± 4.50*¥	37.50 ±4.90*	38.20 ± 1.90*α	10
24th week	33.60 ± 6.70	44.40 ± 5.03*¥	38.80 ± 4.90*	42.70 ± 4.90*α	10

Data are expressed as mean ± SD.* marked significant differences from control. ¥ marked significant differences from sofosbuvir baseline. α marked significant differences from sof/ dac baseline.

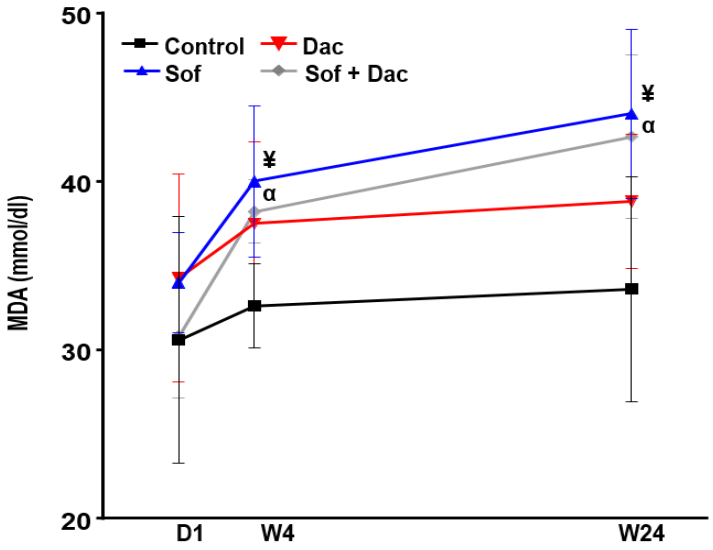

Fig. 4: Effect of sofosbuvir (Sof) &/or daclatasvir (Dac) on MDA content. Sofosbuvir either alone or in combination with daclatasvir significantly increases MDA contens as an oxidative stress measure after 4th and 24th weeks of treatment. Data are expressed as mean ± SD. ¥ marked significant differences from sofosbuvir baseline. α marked significant differences from sof/ dac baseline. Samples were collected at three different check points, day 1 of the experiment (D1; n = 5), after four weeks (W4; n = 10) and after 24 weeks (W24; n = 10).

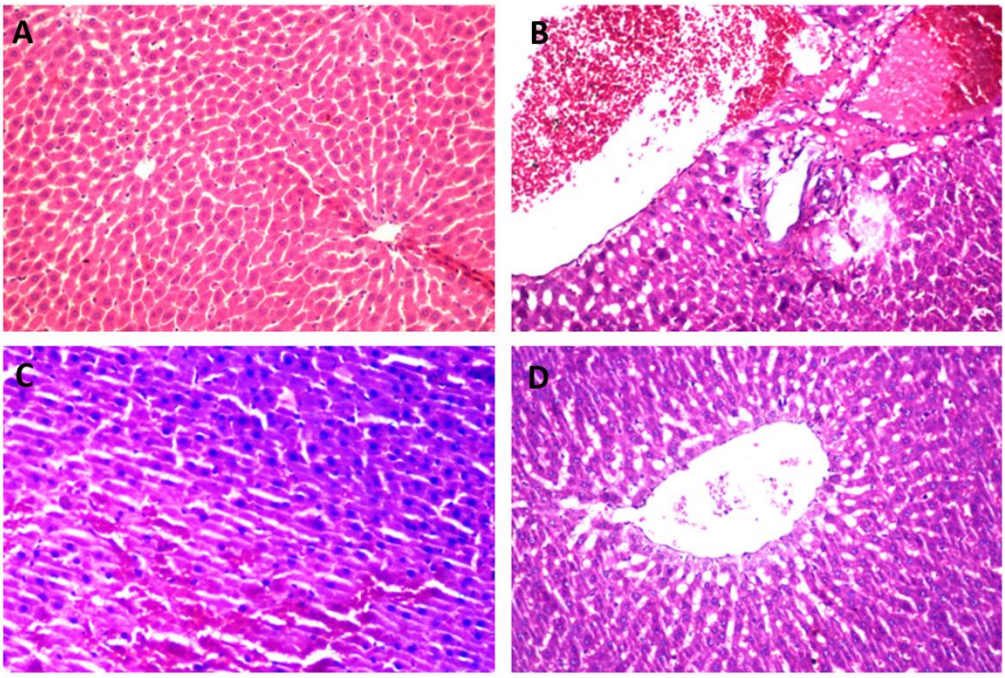

Fig. 5: Histopathological changes in liver architectures. (A) Showed normal liver architectures of control rats. (B) Represents liver section from sofosbuvir treated rats showing fatty change detected in the hepatocytes and congested central vein. (C) Represents liver section from daclatasvir treated rats showing normal hepatic parenchyma. (D) Represents liver section from sofosbuvir and daclatasvir treated rats showed fatty change in the hepatocytes surrounding and adjacent the dilated central vein.

Discussion

Hepatitis C virus is one of the most dangerous hepatic disorders. According to World Health Organization, about 71 million people have chronic HCV infection worldwide. Among those patients, about 15 - 30% have a higher risk for the development of liver cirrhosis. Furthermore, 399.000 people died from cirrhosis and hepatocellular carcinoma induced by HCV in 2016. Accordingly, HCV represents a potential threat. Fortunately, antiviral medications are able to cure effectively about 95% of cases with HCV[14].

Unfortunately, Egypt is classified as one of the highest prevalence rate of HCV. This is primarily due to the widespread administration of tartar emetic injections as anti-schistosomal agent at the last century[15]. Obviously, the therapeutic protocols of direct-acting antiviral agents (DAA) or interferon-free regimen have been considered as a revolution in the eradication of HCV. The sustained viral response reached more than 90% in different HCV genotypes using DAA[16&17]. Moreover, oral DAA agents are even able to cure HCV patients with advanced liver disease including decompensated liver disease[18&19].

Recent studies documented evidences of disturbance in both glucose and lipid metabolism associated with DAA regimen[20&21]. For instance, it was noticed that combination therapy (Sofosbuvir/Ribavirin) was associated with increased serum LDL level and decreased serum triglycerides level in patients infected with HCV[21&22]. On the other hand, eradication of HCV by sofosbuvir increased serum triglycerides along the treatment period and return to normal level in non-responsive patients[23&24]. Apparently, the toxic effect of DAA on lipid profile is a controversial issue between authors. At the present study, we aimed to clarify the modulatory effects of sofosbuvir and daclatasvir in rats –regardless of HCV eradication– on the lipid profile and its subsequent implications on CVS within short (4 weeks) and long term treatments (6 months)[24].

In 2018, Chida group demonstrated that lipid disturbances are common in hepatitis C patients, these effects were because HCV virus molecules used lipid droplet LDL and VLDL to

circulate within body leading to hypocholesterolemia and hypobetalipoproteinemia[25]. Eradication of HCV virus is then associated with more lipid disturbance and hepatic steatosis, defined as excessive triglyceride deposition in hepatocytes, which incidence is relatively high especially in those who attain SVR. The steatosis found in HCV patients is multifactorial including nutritional status, alcohol, presence of other metabolic disease[26]. However, there is paucity of data that document the sole role of DAA regimens as inducers of lipid metabolism disturbance and its associated liver steatosis. Although the study shed a light about the pharmacological intervention of sofosbuvir and daclatasvir in lipid metabolism and induction of hepatic steatosis, more details at cellular and molecular levels are needed. The effect of different DAA regimen is not clear enough.

This study revealed that combination of sofosbuvir/daclatasvir induced an increase in total cholesterol, LDL and triglyceride serum levels after 4 weeks of treatments. These elevations in LDL and TG levels were in accordance with the clinical data showed by Tada group[27]. This findings are supported by the human data where HCV-patients who were treated by a sofosbuvir-containing combination (SOF/ribavirin/interferon, SOF/simeprevir or SOF/ledipasvir ± ribavirin); all patients developed marked and significant increase in LDL and TG[28]. Another German clinical study showed similar results with increased total cholesterol and LDL whereas HDL and TG remained unchanged during and after DAA treatment[29].

Most recently, in 2018, Inoue and his co-workers illustrated that HCV patients received different regimens of DAAs developed significant increase in lipid profile[30]. Another clinical study showed that DAAs have significant reduction in pre-elevated TG (before the start of treatment) with marked increase in LDL, HDL and LDL/HDL ratio[10]. The variation in lipid profile changes – especially TG- among different groups may be referred to different genotypes[31].

Since sofosbuvir was implicated in all regimens, it seems that SOF is the main contributor in lipid profile changes[28&29]. That was in harmony with our data where rats treated with sofosbuvir alone showed increased total cholesterol, LDL and triglyceride serum levels. On the other hand, daclatasvir increased only LDL and triglyceride levels with no effect on total cholesterol. Moreover, the effect of sofosbuvir/daclatasvir combination on lipid profile of treated rats was not significantly different from those treated with sofosbuvir only. The superiority of sofosbuvir over daclatasvir was previously shown in different clinical studies[30-32].

According to the screened lipid profile of rats treated with sofosbuvir/daclatasvir, this study tried to explain the effect of the used drugs on liver tissues. Rats treated with sofosbuvir and daclatasvir showed fat accumulation in their liver tissues. This was also very clear in sofosbuvir- as well as combination-treated animals while not clear enough in daclatasvir treated rats. These findings are augmented by human data showing hepatic steatosis and stiffness[27]. HCV infection is recognized as a risk factor for clinical cardiovascular disease. Many studies have shown increased prevalence of cardiac and inflammatory biomarkers in patients with chronic HCV infection[33]. In 2000, Sato group showed that HCV patients have elevated serum levels of CK-MB and troponin. They interpreted this elevation that its due to the presence of positive-plus strands of HCV RNA in the patient's myocardium. They also accompanied the reduction in their serum levels with the positive response to interferon therapy[34]. However, little if any data are available for the cardiovascular consequences of HCV eradication using DAAs. Most recently a meta-analysis study showed that DAAs therapy for HCV infection is associated with a reduced risk of cardiovascular diseases[35].

To our knowledge, this is the first study focusing on the effects of sofosbuvir and/or daclatasvir on the cardiac enzymes CK-MB and troponin. Our data revealed that administration of sofosbuvir significantly increased serum levels of CK-MB and troponin during the treatment and most interestingly it was significantly higher at the end of the 24th week whereas daclatasvir and the combination showed a significant increase in both enzymes during and after drug termination without differences between the two check points. These findings could be supported by the left

ventricular heart disorders accompanied with sofosbuvir treatment in human[36]. In addition, another study showed a significant lowered heart rate with elongated RR and QT-intervals in the electrocardiogragh. However, none of the patients developed severe bradycardia or syncope[37]. An invitro study showed a bradycardiac effect of the combination of sofosbuvir and amiodarone[38].

Recent study proposed that the chemical structures of SOF may play a role in the lipid profile disturbance induced by the drug. The phosphoramidate side chain is degraded into phenolate ion and propan-2-yl 2-aminopropanoate. These cleaved products may promote β-lipoprotein synthesis and secretion[39]. Subsequently, increasing cholesterol and triglyceride may explain SOF-induced cardiotoxicity. However, more studies are required to validate this concept and extensive studies are needed to confirm the pathophysiological events that may be responsible for the toxicity profile of sofosbuvir.

In conclusion, the recent study suggested that the DAAs-induced lipid profile changes which are drug dependent and not related to the disease itself. Moreover, the data recommended that patients receiving DAAs should be monitored for cardiovascular consequences during and after cessation of the drugs.

Competing interests

The authors declare that there are no conflicts of interest

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السمية القلبية لعقارى سوفوسبوفير وداكلاتاسفير و الناتجة عن خلل في مستويات الدهون على المدى الزمنى القصير والطويل

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المقدمة : مضادات الفيروسات الكبدية ذات المفعول المباشرهي مجموعة جديدة نسبيًا من الأدوية. اظهرت دراسات عديدة حدوث اضطرابات في التمثيل الغذائي للدهون بين مرضى الالتهاب الكبد الفيروسي سى الذين عولجوا بتركيبات مختلفة من مضادات الفيروسات الكبدية ذات المفعول المباشر.

الهدف: هذه الدراسة تحاول تقييم التأثيرات الحادة والمزمنة لمضادات الفيروسات الكبدية ذات المفعول المباشر على مستويات الدهون ،الإنزيمات القلبية و مؤشرات الاكسدة. وكذلك لتحديد ما إذا كانت هذه التأثيرات ناتجه عن الاصابة المرضية نفسها او نتيجة لتناول هذه الادوية. **الطرق البحثية** : عولجت ذكور فئران من نوع ويستار بعقار سوفوسبوفير أو عقار داكلاتاسفيراو كلاهما معا لمدة أربعة أسابيع متتالية. تم جمع عينات من خمسة فئران في اليوم الاول للتجربة كمجموعة حاكمة لنتائج التجربة ثم تم الحصول على عشر عينات أخرى بعد أربعة أسابيع (نقطة نهاية العلاج) ، وأخيراً وبعد ستة أشهر من انتهاء العلاج تم تجميع عينات من عشرة حيوانات اخرى كنقطة لتحديد التاثير الاجل للعلاج. تم تقييم مستويات مؤشرات وظائف الكبد ، مستويات الدهون فى الدم ، مؤشرات سلامة القلب . و قد تم القياس بطريقة قياس لونية. علاوة على ذلك ، تم تقييم محتوى أنسجة الكبد من مؤشر الاكسدة (مالون داى الداهيد).

النتائج: أوضحت نتائج هذه الدراسة أنه في نهاية فترة العلاج الدوائي ، تسبب عقار سوفوسبوفير سواء بمفرده أو مع عقار داكلاتاسفير في زيادة معنوية في مستويات الكوليسترول الكلي ، البروتينات الدهنية منخفضة الكثافة والدهون الثلاثية مقارنة بنتائج الفئران فى المجموعة الحاكمة. و قد استمرت هذه التأثيرات لمدة ٢٠ أسبوعًا بعد انتهاء العلاج. ارتبطت هذه الزيادة في مؤشرات الدهون بتدهور ملحوظ في مؤشرات السلامة القلبية مثل التروبونين والكرياتينين كيناز و زيادة مؤشرات الاكسدة.

الخلاصة: ان تناول عقار سوفوسبوفير سواء بمفرده أو مع عقار داكلاتاسفير يرفع مستوى الدهون وأنزيمات القلب و ترجع هذه التغييرات إلى تأثير الادوية المضادة للفيروسات ذات المفعول المباشر والمستقلة عن عدوى فيروس اللاتهاب الكبد الوبائي سي. وبالتالي ، يجب مراقبة مستوبات الدهون وعلامات سلامة القلب أثناء وبعد التوقف عن تناول الادوية المضادة للفيروسات ذات المفعول المباشر.