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Original Research Article

The Histological Structure of the Liver after the Administration of Interferon α -2a in Adult Male Albino Rats and the Role of Ursodeoxycholic Acid (UDCA) Supplementation

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ABSTRACT

Objective, The aim of this study is to observe the histological structure of the liver of rats after an injection of (IFN-α) and to determine the role of UDCA supplementation. Materials and methods, 45 adult male albino rats were divided equally into three groups. Group I served as the control group. Group II included rats that received IFN-α alone (100000 IU/kg/ three times/week, intraperitoneally). Group III included rats that received both IFN-α (at the same previous dose) and UDCA at a dose of 25 mg/kg once daily. At the end of the experiment (8 weeks), all animals were sacrificed and liver was excised. Paraffin sections were prepared and stained with H&E, Masson trichrome stain and immunohistochemical staining for hepatocyte paraffin-1 (Hep Par-1). Morphometric and statistical analyses were carried out. Results, Liver of the group that received IFN-α showed most of hepatocytes had marked cytoplasmic vacuolations, vascular congestion with prominent sinusoidal Kupffer cells, congestion and dilatation of the portal veins. Morphometric and statistical analyses showed a significant marked decrease in the area% of the positive Hep par 1, indicating cytoplasmic degeneration. Changes were limited by concomitant UDCA supplementation with IFN-α. Conclusion, IFN-α exerted a deleterious effect on the histological structure of the liver in rats and concomitant supplementation of UDCA minimized these effects.

Keywords: IFN-α - UDCA, liver, rat.

INTRODUCTION

The primary goal of interferons (IFNs) therapy is eradication of HCV. However, it is possible that may improve hepatic, portal inflammation and fibrosis. In addition, IFNs reducing risk of hepatocellular carcinoma (Malik and Lee 2000). IFNs are new molecules with longer half-lives, making it possible to obtain more constant serum levels and to administer once weekly (Manns, McHutchison et al. 2001; Lindsay, Trepo et al. 2001). Furthermore, Numerous studies have since used different types of IFNs at different dosages for different periods to treat patients with chronic disease (Parmar and Platanias 2003). IFNs are classified as helical cytokines and are categorized as type I or type II, according to their physical and functional properties. Type (I) IFNs are further divided into α (leukocytes), β (fibroblasts)(Borden and Williams 2000;Parmar and Platanias 2003; Stubgen 2009).

IFN-α is approved in the United States for treatment of condyloma acuminatum, chronic hepatitis C, chronic hepatitis B, Kaposi's sarcoma in HIV-infected patients and other malignancies including leukemia, multiple myeloma, non-Hodgkin's lymphoma, and urogenital carcinomas such as renal cell cancer, bladder cancer and ovarian cancer (Hayden et al.2000;Nishiguchi, Shiomi et al. 2001). Administration of IFN-α had antifibrotic effects in rats and patients with hepatitis C virus (HCV) infection. It was reduced the degree of fibrosis. The histological and oxidative parameters were significantly improved compared to those of untreated rats ((Poynard, McHutchison et al. 2000; Shiratori, Imazeki et al. 2000;Vendemiale, Grattagliano et al. 2001).

IFN-α is a natural glycoprotein that is produced and secreted by immune cells in response to viral infection. IFN-α with antiviral, antiproliferative, anti-oncogenic and immunomodulatory effects, it has been widely used for treatment in many systemic disorders, especially of viral and neoplastic origins(Fontaine and Pol 2001;Yoshida, Arakawa et al. 2002; Gorur, Kandemir et al. 2003; Shiratori, Ito et al. 2005). The antiviral effect of IFN-α in patients with chronic hepatitis C was quickly recognized (Donato, Degott et al. 2005).However, the most common adverse events associated with IFN-α

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therapy are flu-like symptoms, leukopenia, thrombocytopenia, depression, and thyroid disorders(Derbala, Amer et al. 2005). IFN-α administration produce adverse effects, including pathologic autoimmune effects(Mehmet, Yilmaz et al. 2006). In addition, administration of IFN-α induced apoptosis in isolated hepatocytes from preneoplastic rat livers(de Luján Alvarez, Quiroga et al. 2006). The activity of IFN-α against oxidative damage has not been confirmed in a rat model of short-term liver injury (Fukuno, Inoue et al. 2002). However, others reported that IFN-α decreases the production of Reactive Oxygen Species (ROS) in stimulated hepatocytes and inhibits oxidative stress in patients with HCV infection (Parmar and Platanias 2003;Serejo, Emerit et al. 2003; Quiroga, de Lujan Alvarez et al. 2009). interferon-α decreased activin-A and increased follistatin significantly in serum and liver(Refaat, ElShemi et al 2015).

Clinically, long-term Ursodeoxycholic acid (UDCA) therapy improves liver function tests(Nishio, Keeffe et al. 2000;Lindor, Kowdley et al. 2009).In addition, UDCA delayed the development and progression of cirrhosis in primary biliary cirrhosis (PBC)(Corpechot, Carrat et al. 2000). UDCA is the only effective treatment for PBC, chronic cholestasis, primary sclerosing cholangitis(PSC) and in patients with liver disorders such as fibrosis, cirrhosis, eventually liver failure (Kaplan and Gershwin 2005). UDCA has been reported to have antifibrotic activity in bile duct ligation and carbon tetrachloride (CCl4) induced experimental liver fibrosis. Significantly higher effects were obtained in treatment with both IFN-α and UDCA (Tasci, Mas et al. 2006). Moreover, combinations of UDCA with immunomodulating drugs, have been tried but have not been successful according to limited clinical trials(Silveira and Lindor 2008).

Parenchymal cell damage of varying etiology is central to the initiation of fibrogenesis in the liver. Some agents recently been reported to have antifibrotic efficacy with different potency in the liver(Bataller and Brenner 2001). UDCA plays an important role in the treatment of PBC and exerts direct effects at cellular and molecular levels by stabilizing membranes, affecting signal transduction and regulating immune responses. Long-term UDCA therapy significantly delay the development of cirrhosis in PBC (Corpechot, Carrat et al. 2000;Qiao, Yacoub et al. 2002). Chronic cholestasis lead to liver necrosis, fibrosis, and cirrhosis, part of the cause an accumulation of toxic bile acids in the liver(Svegliati-Baroni, Ridolfi et al. 2005). Therapy with UDCA is based on its properties as a nontoxic hydrophilic bile acid that reduced the hydrophobicity of the bile acid pool and thereby reduced bile salt toxicity(Pusl and Beuers 2006). Furthermore, UDCA is known to have antiapoptotic effects in various cell systems (Bellentani 2005; Amaral, Viana et al. 2009).In addition, UDCA may act as a transcription factor modulator for pregnane X receptor and nuclear factor erythroid 2–related factor 2, which regulate the expression of genes involved in bile salt detoxification and redox signalling (Schuetz, Strom et al. 2001; Kawata, Kobayashi et al. 2010).

The aim of this study was to assess the histological structure of the liver after IFN-α which has better antiviral activity therapy alone and after a combination of IFN-α with ursodeoxycholic acid (UDCA) therapy to determine the possible protective effect of UDCA.

MATERIALS AND METHODS

Drugs

Commercially available injectable IFN-α-2a (Reiferon; Minapharm, Cairo, Egypt) 3 million IU/ml. UDCA available in hard capsules (ursofalk; cairo Egypt) 250mg/capsule. Both drugs were dissolved in saline.

Animals

This study was included 45 adult male albino rats weighing (150–200g) that were held in stainless-steel cages at Animal House, Faculty of Medicine, Al-Azhar University and maintained at room temperature. They were allowed water and were fed a standard laboratory diet. Animals were kept for 14 days before beginning the experiment for acclimatization. The rats were divided equally into 3 groups, Group (I) was the control group, 15 rats which were received saline treatment. Group (II) (IFN-α group) included 15 rats that received IFN-α alone. Group (III) (combined group) included 15 rats that received both IFN-α and UDCA. Rats of groups (II) and (III) received IFN-α at a dose of 100000 IU/kg three times a week (Hassan, El-Haleem et al. 2012). Rats of groups (III) received UDCA at a dose of 25 mg/kg once daily (Mas, Tasci et al. 2008). Both doses considered a normal human treatment dose. At the end of the experiment (8 weeks), all groups were fasted and sacrificed under anesthesia. The livers were taken from the sacrificed rats in all experimental groups. Specimens from liver were prepared for light microscopic.

HISTOLOGICAL PREPARATIONS

Preparation of paraffin sections

For the histological preparations, animals were anaesthetized under light diethyl ether and dissected to remove the livers at the end of the 8th week of treatment. Liver tissues were cut into small pieces and then fixed in 10% neutral buffered formalin for 24 hours. The tissue was routinely processed and sectioned at 4 to 5 um thickness with a microtome and stained with haematoxylin and eosin for histopathological studies and Masson trichrome stain for collagen (Bancroft and Gamble, 2008).

Immunohistochemistry Staining

For immunohistochemical study, the deparaffinised 5 µm paraffin sections on charged slides were used for localization of hepatocyte mitochondrial membrane antigen using hepatocyte paraffin-1 (Hep Par-1)(Lamps and Folpe 2003). Using avidin–biotin-complex (ABC) immunoperoxidase technique. The sections were incubated in hydrogen peroxide for 10 min to block the endogenous peroxidase then incubated with the primary anti-HepPar-1 mouse monoclonal antibody for 60 min at room temperature. The primary antibody used was Hep Par-1 mouse monoclonal antibody (Kiernan 2000). The antibody reaction was detected with HepPar-1 using diaminobenzidine (DAB) as chromogen. Sections were counterstained with haematoxylin for 15 seconds before checking under microscope. Normal liver tissues were used as positive control, omitting the primary antibody finally, the Hep Par-1 cytoplasmic sites of reaction were stained brown.

Morphometric measurements

Quantitative morphometric measurements were achieved by using the Image analyzer (Olympus, digital camera CH-9435 DFC 290, coupled to photomicroscope, Germany); Faculty of medicine, Ain shams University, Egypt. Measurements were performed with 10 non overlapping fields for each rat of four randomly chosen rats of each group. The area percent for Hep Par-1 immunoreaction in hepatocytes were measured using magnification 400 with measuring frame area 7286.78 l µm2(Bocci, Fasciani et al. 2001). The data obtained were subjected to statistical analysis using SPSS statistical software (SPSS for Windows, version 13.0). Comparison between groups was made using ANOVA, LSD (least significant difference). Data were expressed as mean (±) SD. Results considered significant when P value ≤0.05

RESULTS

A-Haematoxylin and Eosin results

Examination of transverse sections of liver in the control group (I) showed that a thin connective tissue capsule covered the liver which had flat cells with flat nuclei. The hepatic lobules revealed classic polygonal shape; they were in close contact without distinct interlobular connective tissue septa. The portal areas were found adjacent to hepatic lobules (Fig.1- a&b). Each hepatic lobule was formed of hepatocyte plates radiating from the central vein. Hepatocytes appeared with rounded vesicular nuclei and acidophilic cytoplasm. Blood sinusoids with their kupffer and endothelial cells lining were noticed between the hepatocyte plates. (Fig.2- a). The portal tract was formed of a branch of portal vein, a branch of bile duct and a branch of hepatic artery. The portal vein was lined with a simple squamous epithelium and the bile duct was lined with one layer of cubical epithelium. The hepatic artery was lined with a single layer of flattened endothelial cells resting on a basal lamina (Fig.2- b).

Interferon treated group (II) showed most of hepatocytes had marked cytoplasmic vacuolations with variable in size, deeply stained nuclei and fragmented nucleoli while few hepatocytes appeared binucleated. Sinusoidal dilatation &congestion with prominent Kupffer cells were observed (Fig.1- c&d) as compared with the control one (Fig. 1-a&b). Central vein showed degeneration of its wall, Congestion and dilatation of the portal veins were observed (Fig. 2-c&d) as compared with the control one (Fig. 2-a&b). Interferon & UDKA treated group (III) showed preserved hepatic lobular architecture under the intact thick capsule with improvement of liver tissue representing normal hepatocytes with acidophilic cytoplasm, rounded vesicular nuclei Blood sinusoids with their kupffer and endothelial cells lining were noticed between the hepatocyte plates (Fig. 1-e&f) appeared as control one (Fig. 1-a&b). Normal size central vein & portal vein were observed (Fig. 2-e&f) as compared with the control one (Fig. 2-a&b).

B- Masson's trichrome stain results

Examination of transverse sections of liver in the control group (I) by Masson's trichrome stain showed the normal distribution of the collagen fiber in the capsule, in between hepatocytes and in portal area (Fig. 3-a&b). In Interferon treated group (II) Masson's trichrome stain showed marked decreased of the collagen fiber deposition in the capsule, in between the hepatocytes and in the portal area (Fig. 3-c&d) as compared with the control one (Fig.3-a& b). In Interferon & UDKA treated group (III) Masson's trichrome stain showed the collagen fiber deposition in the capsule, in between the hepatocytes and in the portal area appeared nearly similar to control (Fig. 3-e&f).

C-Immunohistochemical results

Immunoperoxidase technique for Hep Par-1 in the control liver group (I) revealed positive Hep Par-1 immunoreaction in the form of scattered granules throughout the hepatocytes cytoplasm. Negative immunoreaction observed in the most flat endothelial cells of the capsule and inside the central vein (Fig. 4-a&b). In Interferon treated group (II) Immunoperoxidase technique for Hep Par-1showed negative immunoreaction in most of hepatocyte cytoplasm and in central vein while positive immunoreaction in most of the capsule and around fatty vacuoles (Fig.4-c&d) as compared with the control one(Fig. 4-a&b). In Interferon & UDKA treated group (III) Immunoperoxidase technique for Hep Par-1showed marked increased immunoreaction in the most of hepatocytes cytoplasm, flat endothelial cells of the capsule and flat endothelial cells, which lined the central vein while negative immunoreaction inside the central vein were observed(Fig.4-e&f) as compared with the control one(Fig. 4-a&b).

D- Statistical analysis

The area % of Hep Par 1 immune reaction in interferon treated group (II) was significantly decreased in comparison with the control (P ≤0.05). In addition, the area % of immune reactions in interferon & UDKA treated group III was non-significant in comparison with the control (P≥ 0.05). Moreover, The area % of Hep Par 1 immune reaction in interferon treated group (II) was highly significantly decreased in comparison with the interferon & UDKA treated group (III) (P ≤0.05). (Table 1, Histogram 1).

DISCUSSION

In the present study Interferon treated group II showed most of hepatocytes had marked cytoplasmic vacuolations, with variable in size, deeply stained nuclei with fragmented nucleoli. Sinusoidal congestion and dilation with prominent Kupffer cells were observed. Central veins showed irregular wall with degeneration of the most of its endothelial cell lining. Portal veins showed Congestion and dilatation. This results in a line with several investigators reported that IFNα exacerbates cholestatic liver injury caused by bile duct ligation that induced hepatocyte damage. The liver sections showed slight dilatation with congestion of portal and central veins. Marked fibrosis extending from the portal areas and surrounding the hepatic lobule was observed. Many of the hepatocytes appeared normal, while a few hepatocytes showed pyknotic nuclei(Muriel and Castro 1998;Salam, Nofal et al. 2007).

Furthermore, some investigators have proposed that degeneration of hepatocytes by IFNs to oxidative stress (Shibata, Hirota et al. 2002). IFN-α mediated radical oxygen species (ROS) production that preceded the loss of mitochondrial transmembrane in preneoplastic cells. Loss of mitochondrial protein could be responsible of the release of cytochrome C during the initial hours of IFN-α-induced apoptosis (Nagamine, Suzuki et al. 2005). Additionally, IFNα suppressed DNA synthesis of human Hepatic stellate cells (HSCs). IFNα play a critical role in finalizing DNA fragmentation (Ogawa, Kawada et al. 2009).

In the present study Interferon treated group (II) Masson's trichrome stain showed marked decreased in the distribution of

the collagen fibers in the capsule, in between hepatocytes and in portal area compared to control group (I). This results in a agreement with many investigators showed that IFNα reduced the secretion of prolines and the mRNA expressions of collagens α1(I) and α1(III) (Inagaki, Nemoto et al. 2003). Moreover, other authors reported that IFNα may induced deactivation of human Hepatic stellate cels (HSCs) that are responsible for the synthesis of excessive collageneous matrix during fibrogenesis in the liver (Xu, Hui et al. 2005). The number of activated (HSCs) undergoing apoptosis was also significantly higher after treatment with IFNα (Tasci, Mas et al. 2006). In addition, In post-transplant recurrent HCV patients retreated with IFNα for 48 weeks, fibrosis score was improved in 65% of treated patients (Bizollon, Pradat et al. 2007).

In current study, it was observed that the liver sections of the concomitant administration of UDCA with IFN-α treated group (III) revealed marked restoring of general hepatic lobular architecture with plates of normal hepatocytes as compared with IFN-α treated group (II). Normal size blood sinusoids, central vein& portal vein were observed. These results coincide with many authors who studied the protective role of UDCA in liver tissue have shown that UDCA hade hepatoprotective effects in various liver diseases (Okan, Astarcioglu et al. 2002). UDCA improve liver histological results and delay the time to liver transplantation in patients with cholestatic liver diseases (Paumgartner and Beuers 2002).In addition, UDCA alleviates hepatocyte organelle injury in CCl4-induced liver fibrosis(Mas, Tasci et al. 2008). UDCA treatment is beneficial for liver regeneration and the reduction of inflammation(Ishizaki, Iwaki et al. 2008).

Moreover, Treatment with UDCA and retinoic acid significantly reduced liver fibrosis, bile duct proliferation and nearly eliminated liver necrosis after bile duct ligation (BDL) (He, Mennone et al. 2011). Hepatocytes of UDCA-treated animals showed retained of their metabolic activity as evidenced by increased amount of glycogen storage (Buryova, Chalupsky et al. 2013) UDCA monotherapy significantly improved liver fibrosis (Xiang, Chen et al. 2013). On the other hand the long-term administration of UDCA after discontinuation of IFN-α had no beneficial effect on the clinical course of hepatitis C virus infection. Histological evaluation of fibrosis and necrotic inflammatory activity in liver were similar in the two groups. (Boucher, Guyader et al. 2000).

However, the role of UDCA in the protection of hepatocytes against interferon-induced apoptosis is not clear and the mechanism of the UDCA impact is still fragmentary. In the present study the improvement of liver tissue by UDKA may be due to activation of antiapiptotic pathway to limit cell death. Some authors have proposed that to a major survival pathway in hepatocytes that the activation of the transcription factor that nuclear factor (NF)-B causes inhibition of apoptosis. (Schoemaker, Ros et al. 2002). Furthermore, other cell survival pathways, like the activation of mitogen-activated protein kinases (MAPK), could be involved. (Johnson and Lapadat 2002). UDCA was found suppress proapoptotic Bax translocation to the mitochondria(Tsuruta, Masuyama et al. 2002).

In addition the hepatoprotective effect of UDCA has been ascribed to membrane-stabilizing action, free radicals scavenging properties and inhibition of lipid peroxidation. They postulated that UDCA stimulates biliary secretion of bile acids, toxic compounds and thereby reduced bile salt toxicity (Paumgartner and Beuers 2004; Pusl and Beuers 2006)). Therefore, UDCA is known to have antiapoptotic effects in various cell systems(Bellentani 2005; Amaral, Viana et al. 2009). On the other hand, combinations of UDCA with immunomodulating drugs, have been tried but have not been successful according to limited clinical trials(Silveira and Lindor 2008). Other theory demonstrated that UDCA also increases the expression of matrix metalloproteinase inhibitor TIMP- 1, thereby preventing matrix metalloproteinase (MMPs) from their deteriorative proteolytic activity in the liver(Buryova, Chalupsky et al. 2013).

In the current study, in Interferon & UDKA treated group (III) Masson's trichrome stain showed marked increased of the collagen fibers deposition in the capsule, in between the hepatocytes and in the portal area as compared with IFN-α treated group (II) while the results nearly similar the control one. These results in agreement with many researches which approved that UDCA has been shown to reduce hepatocellular damage in liver disease. However, UDCA does not seem to be an effective modulator of HSC activation and cell proliferation (Fickert, Zollner et al. 2002). Compared with others who reported that UDCA had decreases the deposition of perisinusoidal collagen in treated patients of PBC (Neuman, Cameron et al. 2002). Also, livers from BDL rats treated with UCDA for 1 month significantly decreased collagen accumulation in livers (Yang, Huang et al. 2009).

In the present study, immunohistochemical staining for Hep Par-1 of control group revealed positive immunoreaction appeared as scattered granules throughout the hepatocytes cytoplasm without zonal preference. Negative immunoreactive appeared in flat cells of the capsule,inside the central veins and in the bile ducts. The current findings of were in agreement with other studies that showed who approved the antigen for Hep Par-1 antibody is Carbamoyl Phosphate Synthetase 1 (CPS1) which is an abundant hepatocellular protein predominantly localizes in the mitochondria. Hep Par-1 has a high sensitivity and specificity for hepatocytes. There is no immunohistochemical staining of bile ducts or other hepatic non-parenchymal cells (Lugli, Tornillo et al. 2004; Butler, Dong et al. 2008 ; Shehata, Mohamed et al. 2013).

All the examined groups showed a negative immunoreactive in the central veins. That explained by previous studies which stated that the relative cellular hypoxia that affects mitochondrial metabolic pathways in the layer of hepatocytes adjacent to the central vein might explain the negative expression of this area(Chu, Ishizawa et al. 2002). Interferon treated group (II) revealed negative immunoreaction in the most of hepatocytes with stronger expression around fat vacuoles. The flat cells of capsule showed with positive immunoreactive for Hep Par 1. This results in a harmony with many studies reported that Hep Par 1 immunoreactivity in fatty livers was maintained in the cytoplasm adjacent to fat vacuoles. This finding is of great importance as it is an indicator that the hepatic fatty infiltration in interferon treated group (II) is endogenous hepatocellular in origin and not due to cell proliferation or infiltration(Lamps and Folpe 2003;Shehata, Mohamed et al. 2013). The area % of immune reaction in this group was significantly decrease compared to control group (I) and interferon treated group (II) $P \leq 0.05$.

In the present study Interferon &UDKA treated group (III) revealed positive immunoreaction of the most of hepatocytes and endothelial cell lining of the central vein. Most flat cells of capsule showed the negative immunereaction of Hep Par 1. The area % of immunereaction in this group was non-significant $p \geq 0.05$ compared to control group (I) while it's highly significantly increased when compared to interferon treated group (II) $P \leq 0.05$, with little data about the UDKA & Hep Par 1.

CONCLUSION

In conclusion, the results of this study showed that the IFN-α induced marked structural changes in the liver of albino rats. These changes could explain the hepatic complications and impairment associated with IFN-α therapy. Moreover, therapy with UDCA provided significant protection against these changes, which was confirmed histologically, morphometrically and statistically. Thus, Therapy with UDCA better is used as a promising therapy in hepatoprotection strategies with IFN-α therapy.

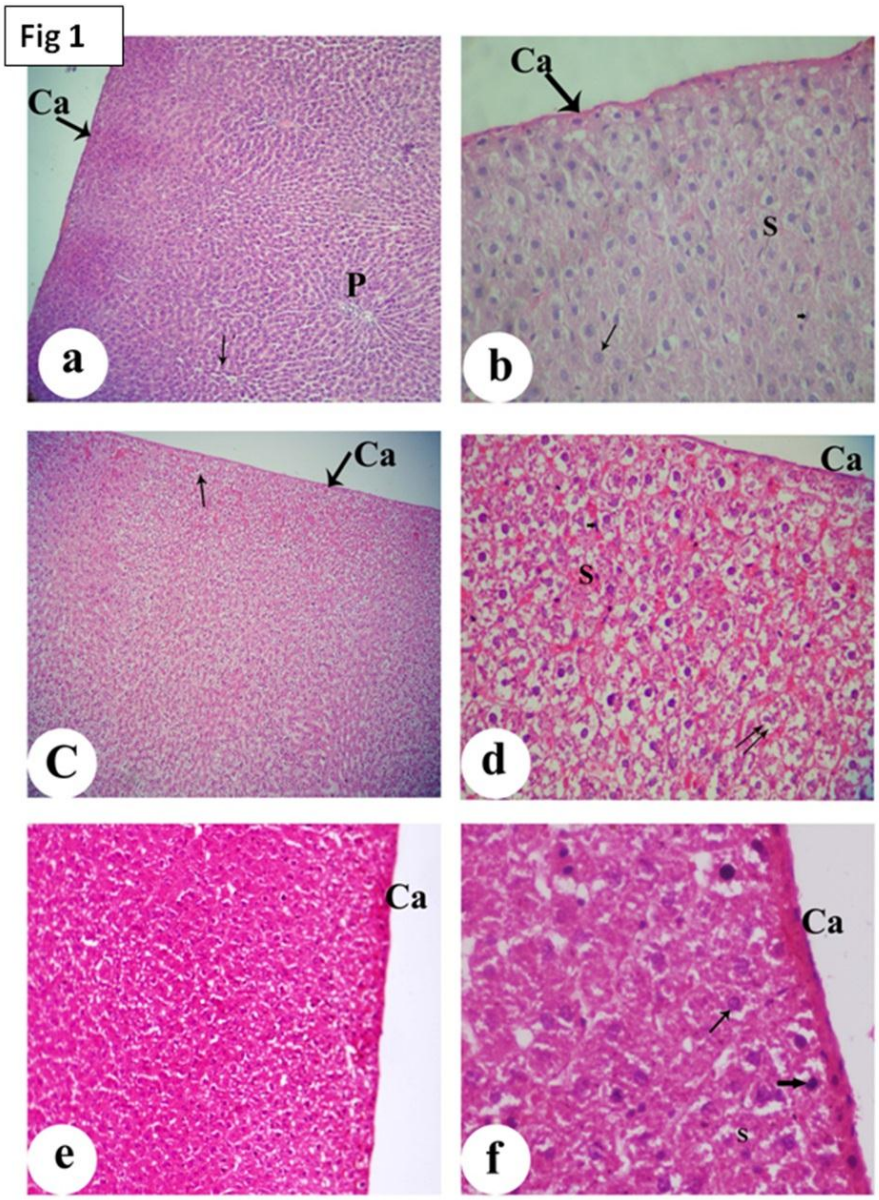


Figure 1: Photomicrograph of the control liver group (I) (a): shows the liver is covered by a thin capsule (Ca), classic hepatic lobules with plates of hepatocytes (arrow) and the portal area (P) is noticed (H&E X100). (b): High magnification of (a) shows hepatocytes with rounded vesicular nuclei and acidophilic cytoplasm (arrow) under capsule (Ca). Blood sinusoids (S) and kupffer cells (short arrow) are observed in between hepatocytes plates (H&E X400). Photomicrograph of interferon treated liver group (II) (c): shows hepatocytes have marked cytoplasmic vacuolation (arrow) under intact capsule (Ca) (H&E X100). (d): High magnification of (c) shows congestion of sinusoids (S), with prominent Kupffer cells (short arrow). Few hepatocytes were binucleated (double arrows), most of hepatocytes nuclei variable in size, deeply stained with fragmented nucleoli under intact capsule (Ca) (H&E X400). Photomicrograph of interferon &UDCA treated liver group (III) (e): shows preserved hepatic lobular architecture under intact thick capsule (Ca) (H&E X100). (f): Higher magnification (e) shows plates of normal hepatocytes with rounded vesicular nuclei (arrow) under intact thick capsule (Ca) and few of them have deeply stained nuclei (short arrow). Notice that blood sinusoids (S) appeared nearly as the control one (H&E X400).

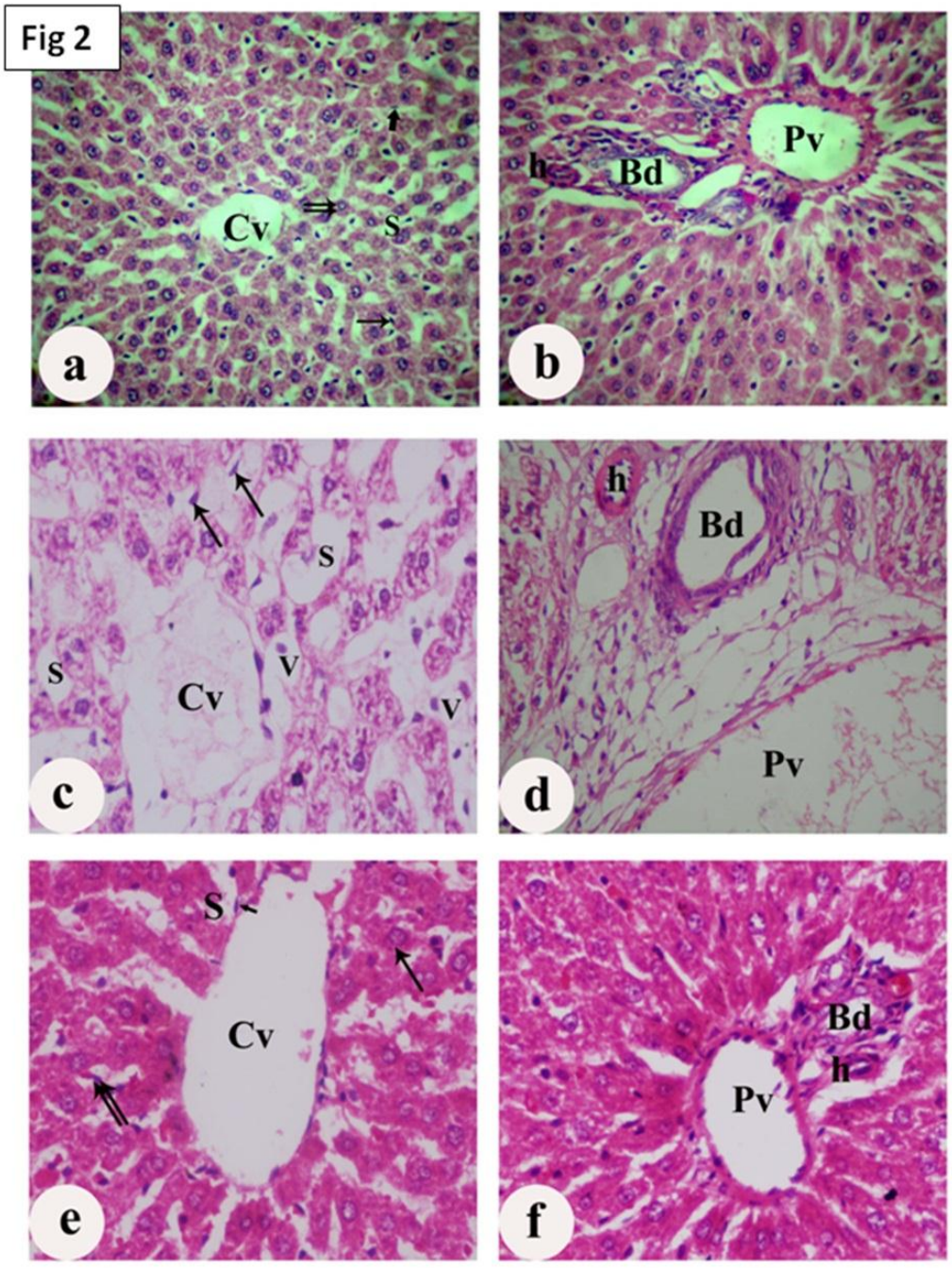


Figure 2: Photomicrograph of the control liver group (I) (a): shows classic hepatic lobules with plates of hepatocytes radiating from the central veins (Cv). Hepatocytes with rounded vesicular nuclei and acidophilic cytoplasm (double arrow). Blood sinusoids (S), their endothelial cell (short arrow) and kupffer cells (arrow) are observed in between hepatocytes plates. (b): the portal area is containing a branch of portal vein (Pv), a branch of bile duct (Bd) and a branch of hepatic artery (h). Photomicrograph of interferon treated group (II) liver (c): shows central vein with partially degenerated wall (Cv) and many vacuoleated hepatocytes (v). Notice dilated blood sinusoids with prominent kupffer cells (arrow). (d): shows the portal area is containing dilated branch of portal vein (Pv), a branch of bile duct with irregular thick wall (Bd) and a branch of hepatic artery (h). Photomicrograph of interferon &UDCA treated group (III) liver (e): shows central vein (Cv) and hepatocytes (arrow) appeared as control one. Notice normal size blood sinusoids (S) with their endothelial cell (short arrow) and kupffer cells (arrows) are observed in between hepatocytes plates. (f): shows portal area is containing normal size portal vein (Pv), a branch of thick wall bile duct (Bd) and a branch of hepatic artery (h) appeared as a control one (H&E X400).

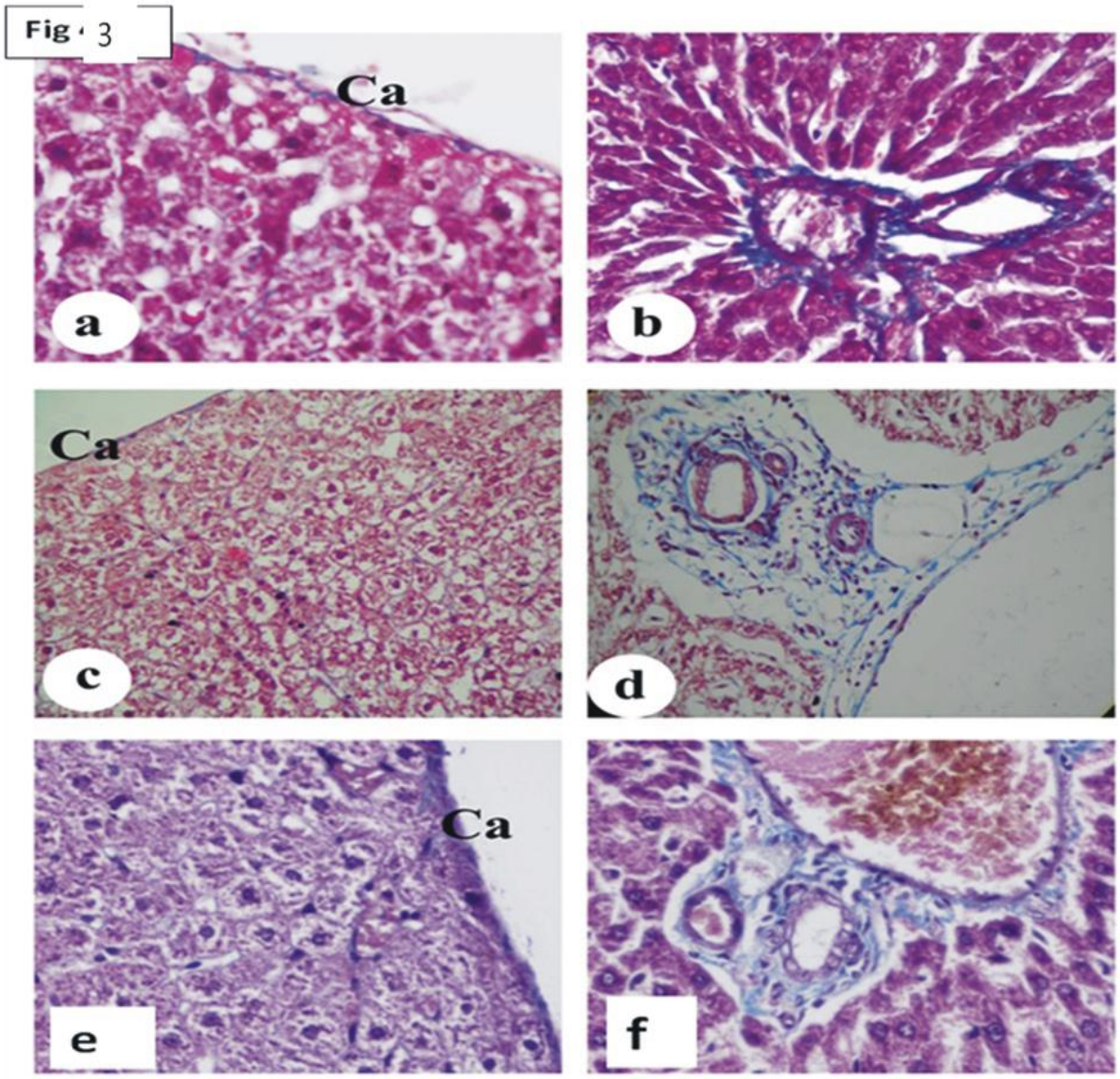


Figure 3: Photomicrograph of the control liver group (I) (a): shows the normal distribution of the collagen fibers in the capsule (Ca) and in between the hepatocytes. (b): shows the normal distribution of the collagen fibers in the portal area. Photomicrograph of interferon treated group (II) liver (c): shows marked decrease in the distribution of the collagen fibers deposition in the capsule (Ca) and in between the hepatocytes compared with control. (d): shows marked decrease in the collagen in the portal area compared with control one. Photomicrograph of interferon &UDCA treated liver group (III) (e): shows the collagen fibers deposition in the capsule (Ca) and in between the hepatocytes appeared nearly similar the control one (f): shows the collagen distribution in the portal area appeared nearly similar the control one (Masson's trichrome X 400).

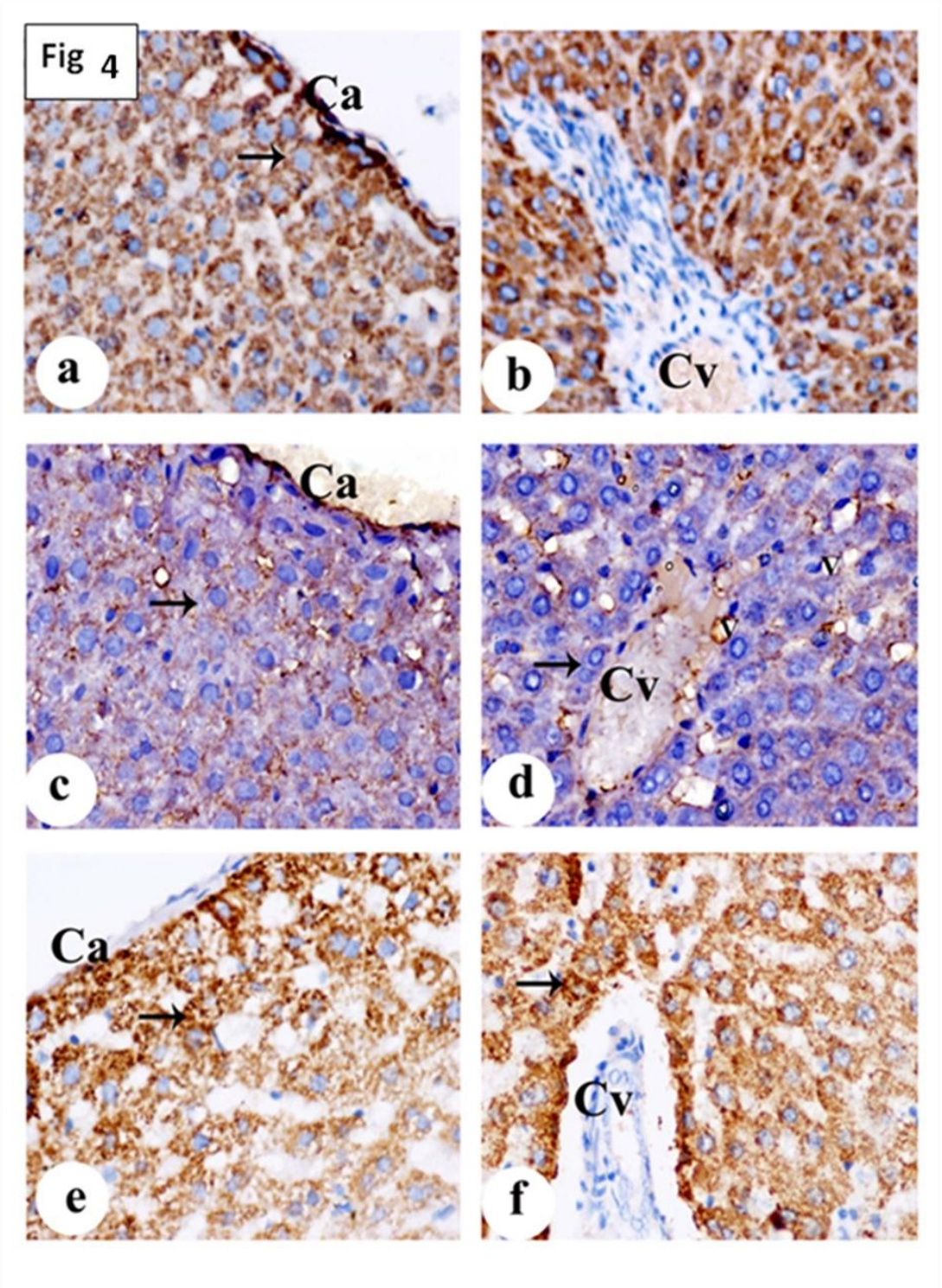
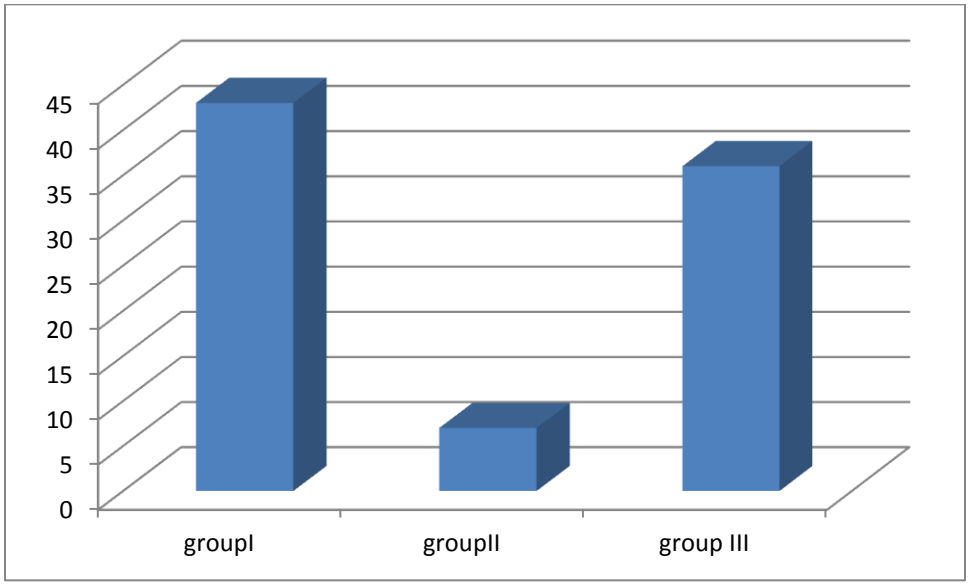


Figure 4: Photomicrograph of the control liver group (I) (a): Shows positive Hep Par-1 immunoreaction in the form of scattered granules throughout the hepatocytes cytoplasm (arrow) under intact thin area of negative immunoreaction capsule (Ca). (b) Show positive Hep Par-1 immunoreaction in hepatocytes cytoplasm with negative immunoreaction central vein (Cv). Photomicrograph of interferon treated liver group (II) (c): Shows negative Hep Par-1 immunoreaction of hepatocytes (arrow) under intact positive Hep Par-1 immunoreaction capsule (Ca). (d): Show negative immune expression is seen in both hepatocytes (arrow) & central vein (Cv). Notice also stronger expression around fatty vacuoles (v). Photomicrograph of interferon &UDCA treated liver group (III) (e): Most of hepatocytes show strong positive immune reaction (arrow) under intact thin area of negative e Hep Par-1 immunoreaction capsule (Ca).(f): Most hepatocytes show strong positive immune reaction (arrow) around negative immune reaction in the central vein (Cv) (immunoperoxidase technique for Hep Par-1x400).

Table 1: comparison of Mean area % of Hep Par I immunoreactivity among studied groups.

Groups	Group 1	Group II	Group III	F	P
Parameters	Mean ±SD	Mean ±SD	Mean ±SD	**74.72123894**	**0.05**
Area %	**43.25 ± 5.909032634**	**7.75 ± 1.707825128**	**36 ± 4.320493799**		



Histogram I: Mean area % of Hep Par I immunoreactivity

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