apertus open access journals

Annals of Biomedical Studies

Open Access Research Article

The Effect of Gadolinium Chloride as Kupffer Cell Inhibitor on Cytokines Storm Induction by Diethylnitrosamine Induced Liver Tumor in BALB/c Mice

Mahmoud M. Elalfy[1] , Mona G. Elhadidy[2], Amer Abbass[2], Gad Abdelaziz[2] , Fayza Elmombawy[2]

[1]Department of Forensic Medicine & Toxicology, Faculty of Veterinary Medicine, Mansoura University

[2]Department Medical Physiology, Faculty of Medicine, Mansoura University

Corresponding Author: Mahmoud Elalfy: mahmoudelalfy@mans.edu.eg, Forensic Medicine and Toxicology Department, Faculty of Veterinary Medicine, Mansoura University, 60-Elgomhouria St., Mansoura City, Dakhalia Governorate , 35516, Egypt.

Received
19th May 2020

Accepted
15th June 2020

Available online
30th July 2020

Abstract

A Liver organ is rich with immune system cells and other cells not belonging to the immune system that have immunological functions. Kupffer cells are the hepatic tissue macrophages and play an important role in the immune mechanisms of the liver. However, their role in liver cancer remains controversial. In the present study we tested effect of gadolinium chloride (GdCl3) at dose of 10 mg/kg 3 times per week for 2 weeks as Kupffer cell function inhibition and on progression of liver injury induced by diethyl-nitrosamine (DEN). Notably, the electron microscope scanning revealed that mice treated with DEN plus GdCl3 displayed hypertrophied Kupffer cells. DEN treated mice shown significant increase of liver enzymes and reduction of albumin, total protein, glucose and urea serum levels. Also, GdCl3 enhanced collagen deposition as early as dysplastic and delay hepatocellular carcinoma occurrence and minimized the cytokines storm resulted from DEN treatment. Taken collectively, the depletion of Kupffer cell had significant role to removal of cytokines storm and delay liver tumorigenesis. Also, GdCl3 could use as therapeutic agent but in combination with other drugs as it alone couldn't improve the liver biochemical as albumin and total protein that share in tissue regeneration.

Keywords: Gadolinium chloride, Kupffer cell, BALB/c mice, Diethylnitrosamine, Liver tumor.

Introduction

Liver as an organ participating in biotransformation, is exposed to continuous effects of various antigens, reaching it principally through the portal vein system from the digestive system and via hepatic artery from the entire body (1).

It was stated that blood inflowing to liver is rich with nutrients, but also with bacterial degradation products, including pathogen associated molecular patterns, microbe-associated molecular patterns and lipopolysaccharides of Gram-negative bacteria, and toxins and antigens principally originating from the digestive system that could modulation of immunity (2).

Therefore, the liver organ, in order to have the immunological function, is rich with immune system cells and other cells not belonging to the immune system that have immunological functions. It must be mentioned that any substance reaching liver are metabolized there by hepatocytes, while various antigens and bacterial degradation products are removed by elements forming the liver immune system (2,3) and which after activation generate many pro and anti-inflammatory factors that modulate the body's immunological response.

Kupffer cells are the tissue macrophages resident in the liver and play an important role in the body defense mechanism. However, their role in liver cancer still controversial. The treatment of the $GdCl_3$ caused apoptosis of Kupffer cells and blocked the Kupffer cell function based on the decrease in CD68 expression and phagocytic activity (4).

The inhibition of Kupffer cell function by $GdCl_3$ or other agent appears to protects against liver injury from the alkylating agent melphalan (5), the mycotoxin fumonisin B1 (6), the industrial chemical thioacetamide (7), and the immunostimulantsconcanavalin A and Pseudomonas exotoxin (8). Kupffer cells also contribute to liver injury during ischemia followed by reperfusion (9). On the other hand, the depletion of Kupffer cells increases liver injury from partial hepatectomy (10), suggesting that these cells play a protective role in this circumstance. One possible interpretation of these data is that the Kupffer cell is the

primary target for the toxicants and carcinogenesis (11) but not in partial keratectomy (12). The rationale of this study to investigate the effect of $GdCl_3$ induced Kupffer cell inhibition on cytokines storm and liver injury induced by DEN in mice.

Materials and Methods

Animals and Treatments

30 adult male BALB/c mice, weighed from 25 to 30g were obtained from medical experimental research center (MERC), faculty of medicine, Mansoura university. The mice were housed, 3-5 per cage and maintained under constant room temperature (25°C), provided with free access to standard chow and tap water under a 12h light (08.00-20.00) and 12h darkness (20.00-08.00). Animals were treated in accordance with Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press). This study was approved by the animal welfare ethics committee of Mansoura University. 30 male mice divided randomly into 3 groups; Group one of ten mice were orally given saline i/p (0.2 ml) twice per week for 6 weeks. Second group, 10 mice given DEN at dose of 100 mg/kg (13), dissolved with saline, twice per week for 6 weeks. Third group treated with DEN at dose of 100 mg/kg, dissolved with saline, twice per week for 6 weeks and after that mice received gadolinium at dose of 10 mg/kg 3 times per weeks for 2 weeks and kept for observation until 32 weeks of age. All rats were euthanized by overdose of thiopental (0.5 ml/100 gm of thiopental sodium) at 32 weeks of age. Blood was collected from abdominal aorta for serum separation and liver was divided into 3 parts; first part stored at -80 for RNA isolation, second part stored in 2.5 buffered glutaraldehyde solution and third part preserved in buffered formalin for histopathological examination

Pathological examination

Specimens from liver in 10% formalin and 5µ thickness sections of specimens were prepared then stained with hematoxylin and eosin (H&E) and examined microscopically (14).

Biochemical analysis

Blood was collected from each mouse in a centrifuge tube and placed

at room temperature for 20 min. Serum was then separated by centrifugation at 3,000 rpm for 10 min. Serum sample was divided into aliquots, for determination of serum alanine transaminase (ALT) and serum aspartate transaminase (AST) (15), alkaline phosphatase (ALP) (16), glucose (17), urea concentration (18), total bilirubin (19), albumin (20), and total protein (21).

Transmission Electron Microscopy

Two freshly cut liver sections (size, about 1 mm^3 each) from the left lobe of the livers were taken. The liver samples were immersed in buffered 5 % glutaraldehyde for 2-24 hours. Then washed in cacodylate buffer (0.1 M, pH 7.2) 3-4 times for 20 minutes at every time and then post fixed in 1% osmium tetroxide for 2 hours. After repeated washing in cacodylate buffer (4 × 20 minutes), by using ascending grades of ethyl alcohol up to 100% (30, 50, 70, 80, 90 and 100% /2 hours) dehydration was done and using gelatin capsule they were embedded in Epon 812. For polymerization, the embedded samples were kept in incubator at 35 C° for one day, at 45 C° for another day and three days at 60 C°. From prepared blocks, using LKB ultra microtome, semithin sections in thickness of 0.5-were prepared. The sections were stained by toluidine blue, examined by light microscope, photographed and regions for preparation of ultrathin sections were oriented and by Leica ultramictotome the ultrathin sections at a thickness of 500-800 Å were made and fixed on copper grids (200μ meshes). The ultrathin sections were then contrasted in uranyl acetate for 15 minutes and lead citrate for 5 minutes and examined by a transmission electron microscope (Jeol, CX11) in the electron Microscope Unit, of Assiut University (22).

Immunohistochemistry

The standard immunohistochemically methods were adopted. The tissue sections were treated with microwave to produce unmasking of the epitopes of the antigen (23). The detection of tissues antigens by immunostaining is a two-step process. The first step is binding of the antigen with related primary antibody (Table 1) then visualization of this reaction by a universal secondary antibody. The primary antibody

measure the specificity of the reaction, while, the secondary antibody, with its linked enzyme, produces amplification of the reaction to increase the sensitivity of the test. A universal system used was the Biotin-Streptavidin (BSA) system to visualise the markers (24). Diaminobenzidine (DAB) was used as a chromogen since it produces a permanent preparation. Also, Hematoxylin counterstain was used. All slides were evaluated and classified semi-quantitatively by means of a four-degree score according to (25).

	Name	Code	Dilution
1	TGFβ1 (V)	sc-146-G	1:50
2	FAS (FL-335)	sc-7886	1:50
3	IL-2 (H-133)	sc-7896	1:50
4	CD68 (H-255)	sc-9139	1:50
5	IL-10 (A-2)	sc-365858	1:100
6	IL-4 (C-19)	sc-1260	1:50
7	IL-6 (M-19)	sc-1265	1:100
8	Rabbit anti-goat IgG-AP	sc-2771	1:100
9	Mouse anti-rabbit IgG-AP	sc-2358	1:100
10	Goat anti-mouse IgG1-AP	sc-2066	1:100

Table 1: Shown the antibody list.

RNA isolation and Real Time PCR

Levels of mRNA of cytokines were analyzed by the quantitative real time RT-PCR using a LightCycler (Roche, Germany). Total RNA was isolated by Trizol Reagent (Sigma) from liver tissue that was pooled (5 mice) from each treatment in the BALB /c experiments. Two µL of total RNA were reverse transcribed for preparation of cDNA using M-MLV reverse transcriptase (Promega, Madison, WI). Then the cDNA was subjected to real time PCR according to methods described by Hunecke et al. (26). In details, the specific primer pairs were used to amplify specific genes in the presence of 3 mM $MgCl_2$. PCR was performed in

triplicates in a total volume of 20 µL of the LightCycler, HotStart DNA SYBR Green I mix (Roche) bearing primers and 5 µL of cDNA. PCR cycle were as follow, 10 min at 95˚C followed by 35–50 cycles of 15s at 95˚C, 15s at 60˚C and 15s at 72˚C. Primers were listed as following; 5-gacacttgtgctccttgtca-3 5-tca attctgtggcctgcttg-3 for IL-2 (27), 5-tcggcatttttgaacgaggtc-35-gaaaagcccgaaagagtctc-3 for IL-4 (27), 5′ GCTCCCTACTTCACAAGTCC 3 5′ GCAGGTTTGCCGAGRAGATC 3′ for IL-6 (28), 5′-aacctcgtttgtacctct-3′5′-caccatagcaaagggc-3 for IL-10 (29), 5′-gaacccccattgctgt-3′,5′-gccctgtattccgtct-3′) for tgfb1 (29) ,5′-ctctgatcaattttg 3′ 5aggaatctaagacgt-3 for fas legend and 5-ggcattgttaccaactgggacg-3,. 3-ctctttgatgtcacgcacgatttc-5. For B-actin. RT-PCR was performed as previously described (26).

Statistical analysis

The means and SEs were calculated for all parameters determined in this study. Statistical analyses were performed by the SPSS version 13 using one-way ANOVA analysis and *t*-test was used in case gene expression after normalization with housekeeping gene. *P*-value at ≤0.05 was accepted as statistically significant (30). Image analysis was done by use lycor image studio lite version 5.2 and GraphPad version 8.

Results

Diethyl nitrosamine is hepatotoxic agent used extensively to study the chronic liver injury (13). The present study investigated the ultrastructure, biochemical and pathological alteration of hepatocellular injury induced by DEN, received once per week intraperitoneally for 6 weeks, at dose of 100 mg/kg.

Hereby, the present study found that group one given only DEN at dose of 100 mg/kg and group 2 which received DEN and GdCl3 had reduced the body weight non significantly increase especially when compared with control group. But Liver to body weight ratio was increase significantly of both DEN groups when compared with control group (Figure 1).

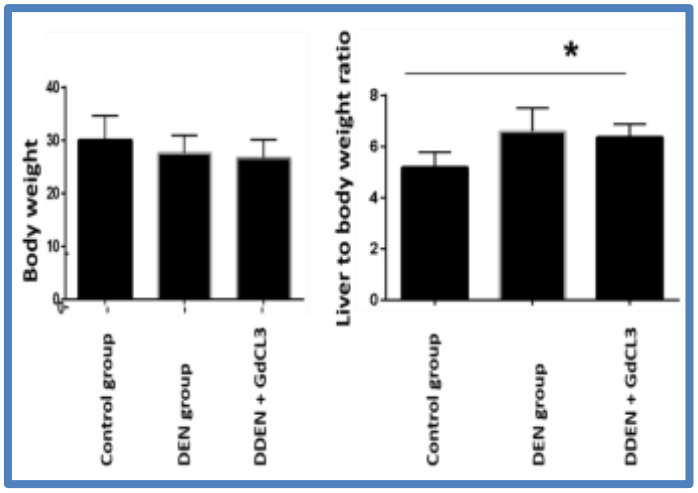

Figure 1 : The depicted figure displayed the body weight and relative liver to body weight ratio of treated. The body weight of DEN groups were reduced none significantly than control group. While liver to body weight ratio of DEN were increased significantly when compared with control group.

Notably, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were significantly increased in DEN groups which received DEN or DEN plus $GdCl_3$ when compared with control group (Table 2). Also, albumin, total protein, cholesterol, glucose and urea were reduced significantly in groups treated with DEN or DEN plus $GdCl_3$ when compared with control group. Moreover, there was little decrease non-significantly in level of bilirubin in both of DEN groups when compared with control group.

Parameters Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	Total bilirubin (mg/dl)	Total plasma protein (g/dl)	Serum albumin (g/dl)	Serum urea (mg/dl)	Serum glucose (mg/dl)
Control mice (B1) (n=10)	22.750^{A} ±3.25	51.20^{A} ±7.77	177^{A} ±2.097	0.621^{A} ±0.04	7.40^{A} ±0.39	1.62^{A} ±0.16	2.48^{A} ±0.09	172.60^{A} ±2.99
DEN treated mice (B2) (n=10)	54.75^{B} ±3.75	178.21^{B} ±2.85	212.20^{B} ±2.45	0.80^{B} ±0.04	5.56^{B} ±0.517	0.89^{B} ±0.079	1.38^{B} ±0.10	115.50^{B} ±1.32
DEN + $GdCl_3$ treated mice (B3) (n=10)	54.75^{B} ±3.40	179.50^{B} ±6.10	216.50^{B} ±2.327	0.80^{B} ±0.04	5.54^{B} ±0.52	0.91^{B} ±0.07	1.34^{B} 10±0.	115.75^{B} ±0.85

Table 2: The liver functions in DEN treated mice compared to DEN + gadolinium treated group and control mice. A,b,c significant at $p \leq 0.05$

Gross picture and Histopathological examination

Gross picture of liver treated with DEN or DEN plus $GdCl_3$ shown small foci on liver surface while normal liver shown normal architecture see figure 2. Histopathological Examination revealed that small foci of liver tumor show in both DEN groups while control one show normal parenchyma. (Data not shown).

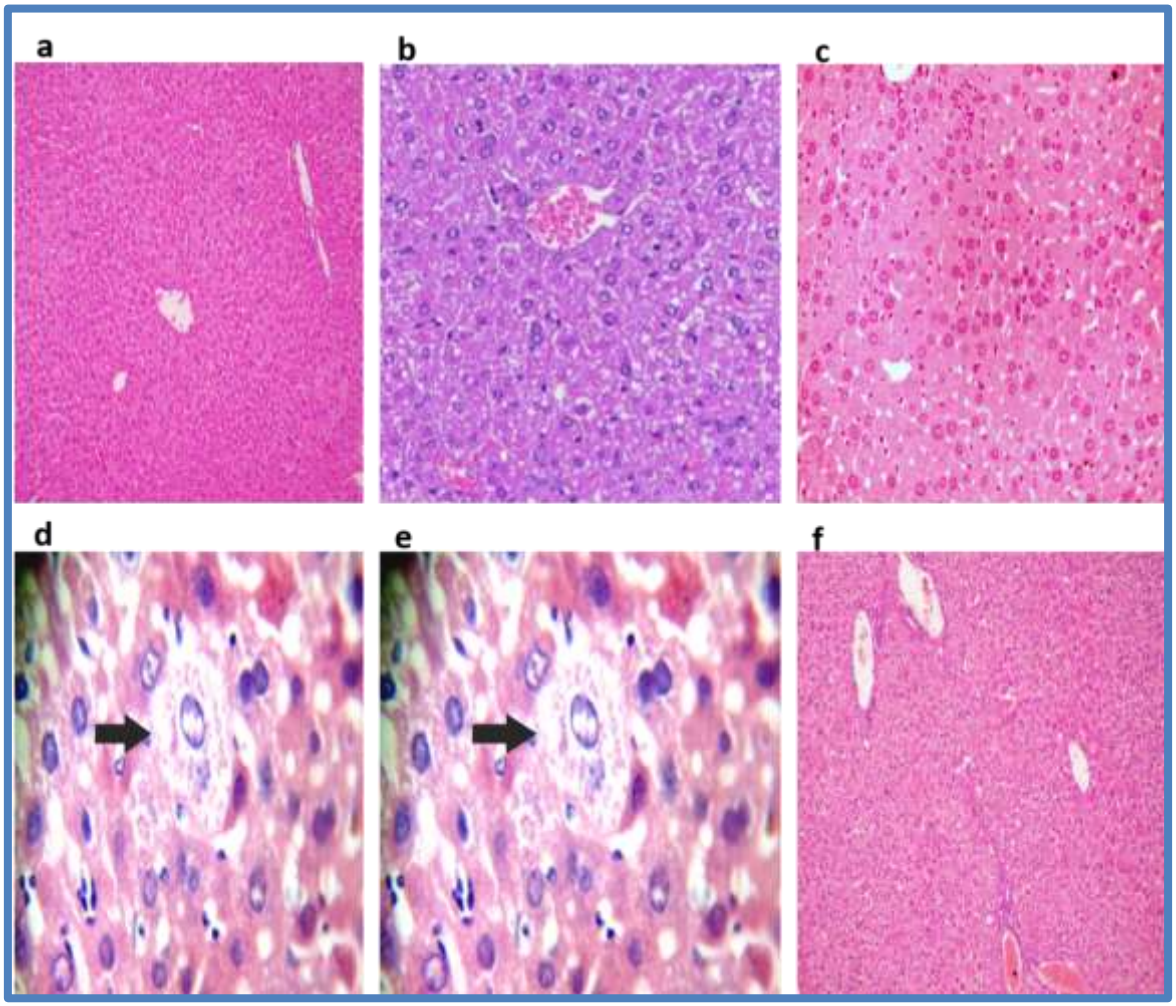

Figure 2 : The depicted figure showed the histopathological features of gadolinium chloride with or without DEN treatment (a) Normal liver parenchyma of control mice (b) The liver of DEN treated mice shown neoplastic changes were composed of liver cords (thin arrow) that were wider than the normal liver plate that was two cells thick. There is no discernable normal lobular architecture, though vascular structures were present (thick arrow) (H &E ×200). (c) Liver treated with DEN + shown dysplasia of hepatocyte with abnormal nucleus. (d) Liver treated with DEN+GdCl3 (B3) shows dysplasia hepatocyte indicated by enlarged nuclei and basophilic apoptotic bodies (right side) and (e) Liver treated with DEN + GdCl3 shown sever congested central vein and scattered necrosis all over liver tissue.

Transmission electron microscope scanning is advanced technique used to explore the ultrastructure in many studies. In control group, the hepatic cells (H) polygonal arranged in plats having large vesicular nucleus containing one or two nucleoli. The sinusoidal lumen narrow contained Kupffer cells in it wall. The central vein (cv) lined with endothelial cell, its cytoplasm contained mitochondria and glycogen

granules (G). Moreover, control group showing the normal morphological appearance of the hepatic cells (H) and the sinusoid (S) contain Kupffer cell (Kc) having nucleus (N), mitochondria (m) and small electron dens lysosomes.

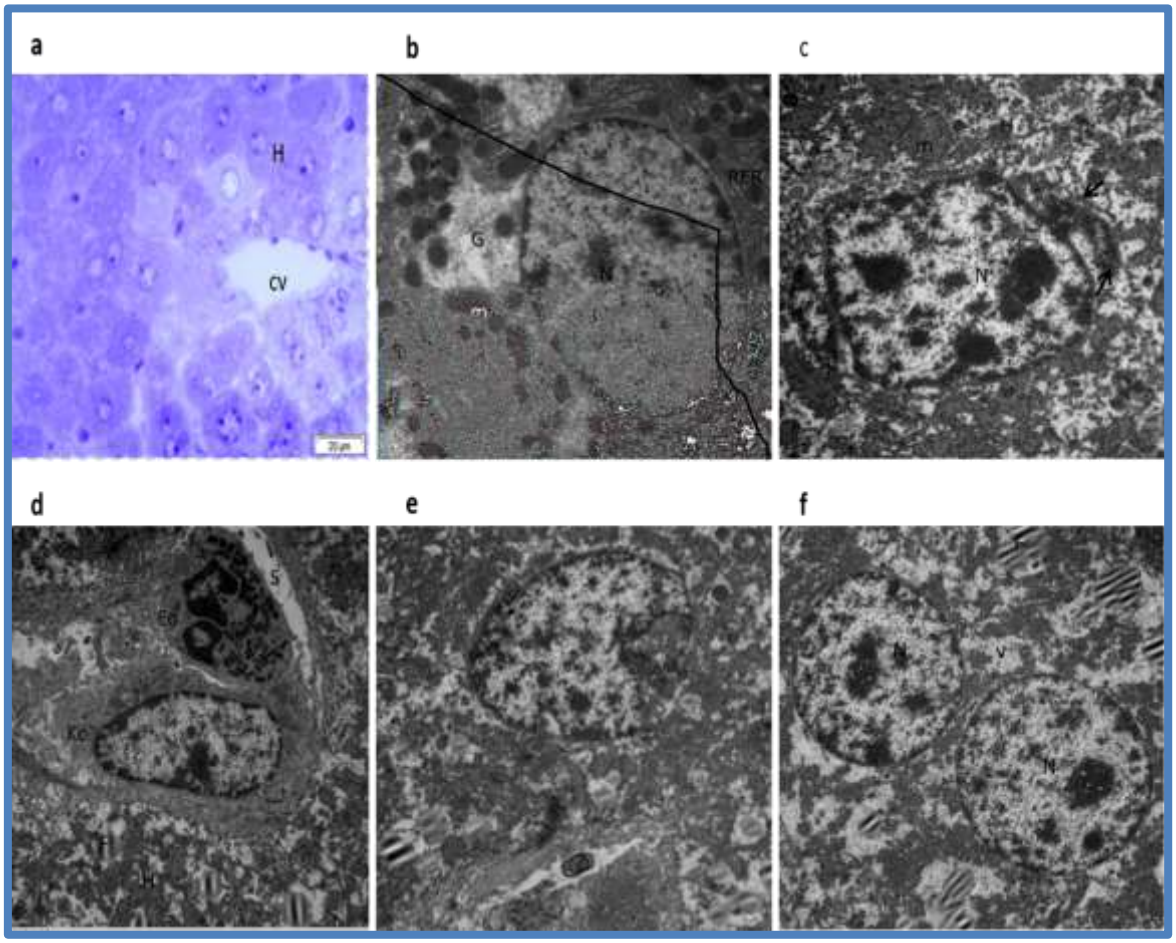

Figure 3 : The depicted figure shown (a) Semi-thin section of liver belonging to DEN treated mice stained with Toluidine blue stain shows neoplastic hepatic cells, the cells contain deformed nucleus with two or more nucleoli (arrow). The cytoplasm vacuolated and the sinusoids narrow and contain Kupffer cells in its wall. (b) T. E. micrograph of liver belongs to DEN treated mice shown hepatic cell (H) contain numerous fat globule (F) and the sinusoid(S) contain eosinophil cell (Eo) with its characteristic granules, the Kupffer cell hypertrophied (Kc) contain numerous electron dens lysosoms. (c) T.E. micrograph belongs to DEN treated mice shown neoplastic nucleus (N) of the hepatic cell (H). The nucleus appeared miss shaped, branched and containing more than two nucleoli. The cytoplasm contains mitochondria (m) were few in numbers, swollen and lost its cristi. Numerous large and small electron-dense granules (arrows) are present in the cytoplasm. (d) Liver belongs to normal control subgroup showed the hepatic cell have large vesicular nucleus and its cytoplasm contain mitochondria, RER, and glycogen granules. Normal morphological appearance of the sinusoid contains Kupffer cell having nucleus, mitochondria and small electron dens lysosomes. (e) Liver of DEN treated mice showed neoplastic hepatic cells, the cells contain deformed nucleus with two or more nucleoli in liver of treated group with both DEN and Gadolinium. (f) The cytoplasm vacuolated and contains fat globule,mitochondria were few, swollen and lost its cristi. Numerous large and small electron-dense granules were present in the cytoplasm. The sinusoids narrow and contain hypertrophied Kupffer cells contain numerous electron dens lysosomes in liver of treated group with both DEN and Gadolinium.

Cytokines modulation by gadolinium chlorides in DEN induced liver injury.

DEN treatment enhanced the expression of cytokines as il-2,4,6 and 10 while had little expression of CD68, FAS and TGFb1 when compared to control group or group treated only with DEN. Notably gadolinium treatment with DEN had no expression of most cytokines except TGFb1 when compared to control group or group treated only with DEN (figure 4)

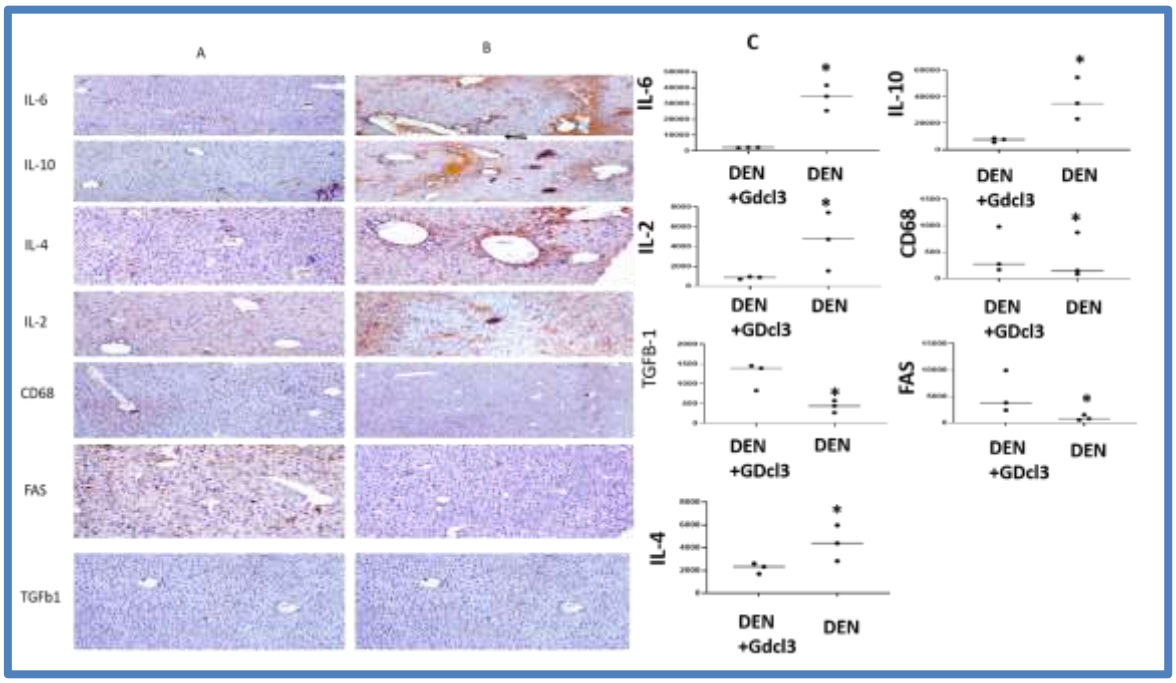

Figure 4 : The depicted figure show IHC of cytokines expression. The Gdcl3 treatment reduced bothinflammatory and anti-inflammatorycytokines treated either with DEN + Gdcl3 with the exception of tgfb1 and fas expression (A) while DEN (B) alone enhanced expression of both inflammatory and anti-inflammatory cytokines with the exception of tgfb1 and fas expression. The quantitative analysis of cytokines expression in liver tissue of rtats treated either with DEN alone or both DEN +Gdcl3 (C).

Gene expression regulation by Gdcl3 treatment in DEN induced liver injury

Both DEN group increased the cytokines expression as IL-2, IL-4, IL-6, IL-10, FAS and tgfb-induced factor but all in non-significant manner. While DEN treatment with gadolinium was lower than group treated with DEN only except fasand tgfb-induced apoptosis factors (figure 5).

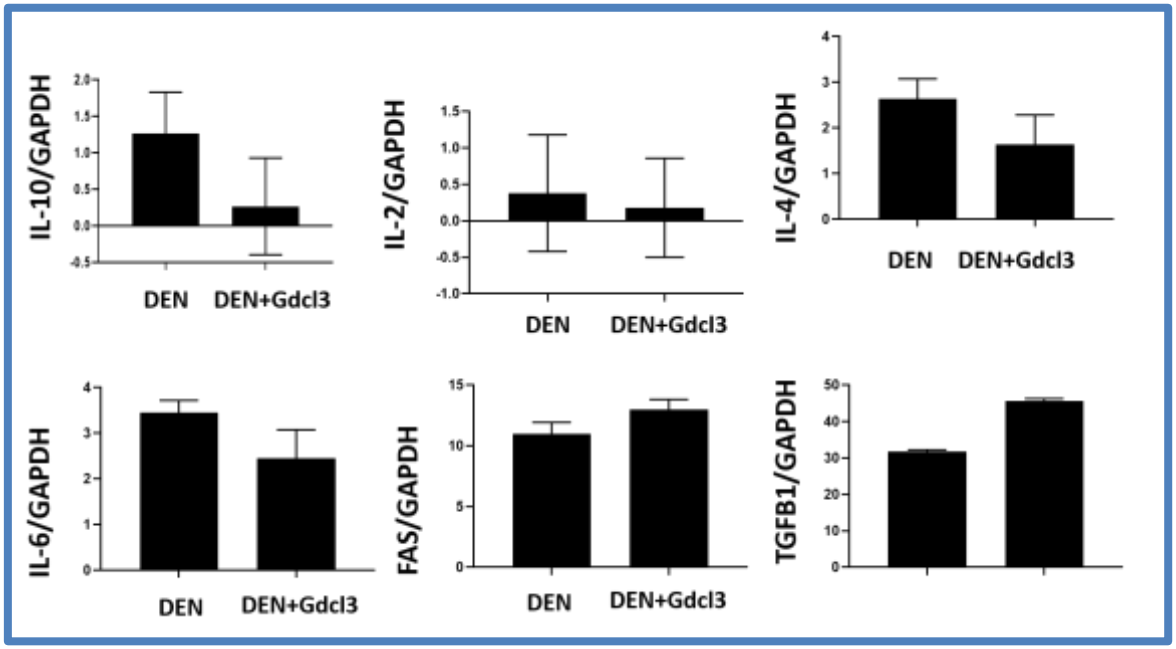

Figure 5 : The depicted figure displayed the gene expression of cytokines. All cytokines of gadolinium treated group plus DEN were reduced except FAS and TGFB1when compared to DEN treated group. While DEN enhanced the expression of all cytokines under this experiment of both inflammatory and non-inflammatory except FAS and TGFB1.

Discussion

Recently, there are many hundred literature published in natures and other publishers regard the role of immunity in progression of cancer in human data and body defense could have several roles in the tumor progression, regression and resistance to anticancer therapy (31-34).

The liver being the organ that often exposed to various toxins, coming by way of blood around the body and has developed mechanisms to ensure its protection through biotransformation and immune response. This organ is rich in immune cells, which in addition to the phagocytic action, secretes numbers of pro-and anti-inflammatory cytokines which also take part in the defense of the liver. The administration of suitable doses of $GdCl_3$ blocked the effector function of Kupffer cells selectively, but did not cause liver parenchymal cell toxicity (4). Also, we previously studied the cytotoxicity of $Gdcl_3$ for 6 weeks at different doses and the dose here in the current study at 10mg/kg didn't had any adverse effects on biochemical or liver parenchyma in albino rats (35). To better understand the role of immunity on liver cancer progression, we use $GdCl_3$ for inhibition of Kupffer cell in mice treated with DEN at dose of 100 mg/kg twice

weekly for 6 weeks (13).

Body weight of mice treated with DEN or DEN plus $GdCl_3$ decreased non-significantly when compared with control group. While liver to body weight ratio was increased significantly in DEN groups than control groups. In this regard, Shiota et al. explain the toxicity of DEN that responsible for decrease body weight but increase proliferation of dysplastic cells was related to increase of liver to body weight ratio (13). Mice treated with DEN or DEN plus $GdCl_3$ displayed significantly an increase of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase when compared with control group. In this regard, Shiota et al. who studied effect of glycyrrhizin for inhibition of hepatocellular carcinoma in mice. Similarly, DEN increased significantly the enzymes in earlier study (13). But $GdCl_3$ had no effect on hepatic cells as it selectively toxic for Kupffer cells (4) and this dose didn't revealed biochemical or pathological alteration in similar studies (35). In consistent with our finding findings, the activities of serum ALT and AST were drastically increased by DEN exposure in accordance with other protective studies (36). The activity of ALT and AST available in serum may indicate hepatic dysfunctions as these enzyme activities are increased alongside liver dysfunction.

Notably, the hepatotoxicity of DEN was recorded here by alteration of liver enzymes and biochemical as recorded earlier (13). Moreover, in group treated with DEN and $GdCl_3$ showed the ultra-thin section of collagen fibrin was induced and Kupffer became swollen and hypertrophied besides alteration of the cellular organelles as mitochondori. Similarly, the collagen deposition due to inhibition Kupffer cell and collagen resolution by matrix-degrading proteases (37). Similarly, the $GdCl_3$ pretreatment reduced the hepatotoxicity of cadmium (38) and carbon tetrachloride (35).

Notably, TEM of DEN group revealed no collagen deposition in liver parenchyma. While $GdCl_3$ enhanced collagen deposition as early as dysplastic and neoplastic cell occurrence. In this regards, the macrophage promotion of matrix regression may be direct with macrophage release of matrix-degrading proteases, or by stimulating either the release or activation of proteases by other cell types, including HSCs and macrophages could be the source of enzymes that

degrade the fibrillar interstitial matrix during fibrosis (37).

In the current study, we found that DEN enhanced cytokines deposition in liver tissue while gadolinium treated group show low level of cytokines when compared to other groups. These phenomena called cytokines storm which resulted in stoppage and induction of death in animals bearing tumors. Similarly, the $GdCl_3$ treated group in hemorrhagic pancreatitis rats , there was no increase in hepatic or systemic cytokine levels and less lung injury was observed (39). Also, the gadolinium significantly reduced the serum concentration of IL-6 increased by thioacetamide treatment (7). In liver diseases, there is accumulating evidence proof that Kupffer cells may act as effector cells in the destruction of liver tissue by producing harmful soluble mediators (40). Also, Kupffer cell induced chronic mitochondrial dysfunction and more aggressive ROS production that trigger tumorigenesis through JNK activation (41).

In the current study, Kupffer cell was hypertrophied by Electron microscope scanning before its death and leakage of FAS as reported in IHC of liver tissue of rats treated by DEN and gadolinium. In this regard, Gadolinium significantly reduced the elastase-induced the expression of FasL and FasL mRNA but had low effect on Fas in liver injury (42) and the Kupffer cells were expressed B7-H1 in tumor tissues compared with surrounding non-tumor liver tissues in patients with HCC and this correlation with associated with poorer survival (43).

Notably, $Gdcl_3$ restored the hepatocyte gap junction intercellular communications after liver injury (44), combination of Gdcl3 and sorafenib, a multiple tyrosine kinase inhibitor reduced the liver fibrosis induced by dimethyl-nitrosamine in rats through suppressing the collagen accumulation (45), Gadolinium chloride can effectively retained the parameters of liver dysfunction in Niemann–Pick type C (NPC) mice (46) and $Gdcl_3$ could suppress the hepatocellular carcinoma by inhibiting the regulation of CD206 in tumor associated macrophages (47) .

In conclusion, the depletion of Kupffer cell had important role to resolve cytokines storm and delay liver tumorigenesis. Also, GdCl3 could use as therapeutic agent but in combination with other drugs as it alone couldn't affect or improved liver biochemical as albumin and

total protein that share in tissue regeneration reported. Intracranial tumors especially lymphomas have been shown to masquerade uveitis. Further studies are warranted.

Acknowledgments

This work was supported by grants-in-aid from academy of scientific research and technology of Egypt. We thank the director of MERC institute who help us to conduct the experiment in his lab. We thank prof. drAlamNefady director of electron microscope unit of Asuit University who help us in electron microscope scanning of liver samples and histopathology.

Author Contributions

Mona Elhadidy designed, carried out and approved the manuscript. Mahmoud Elalfy, associate professor of forensic medicine and toxicology, faculty of medicine, Mansoura university, did the further gene expression, analysis, of IHC, wrote, revised the manuscript and approved the submission. All other professor supervised the research study and approved the submission

Conflict of Interest

All author had no conflict of interest

References

1. Sevior D.K., Pelkonen O., Ahokas J.T. (2012) Hepatocytes: the powerhouse of biotransformation. The international journal of biochemistry & cell biology 44:257-261.
2. Kern M., Popov A., Kurts C., Schultze J., Knolle P. (2010) Taking off the brakes: T cell immunity in the liver. Trends in immunology 31:311-317.
3. Hickey M.J., Kubes P. (2009) Intravascular immunity: the host–pathogen encounter in blood vessels. Nature reviews immunology 9:364-375.
4. Ding H., Peng R., Reed E., Li Q.Q. (2003) Effects of Kupffer cell inhibition on liver function and hepatocellular activity in mice. International journal of molecular medicine 12:549-557.
5. Kresse M., Latta M., Künstle G., Riehle H.-M., Van Rooijen

N., Hentze H., Tiegs G., Biburger M., Lucas R., Wendel A. (2005) Kupffer cell-expressed membrane-bound TNF mediates melphalan hepatotoxicity via activation of both TNF receptors. The Journal of Immunology 175:4076-4083.

6. He Q., Kim J., Sharma R.P. (2005) Fumonisin B1 hepatotoxicity in mice is attenuated by depletion of Kupffer cells by gadolinium chloride. Toxicology 207:137-147.
7. Andrés D., Sánchez-Reus I., Bautista M., CascalesM.a. (2003) Depletion of Kupffer cell function by gadolinium chloride attenuates thioacetamide-induced hepatotoxicity: expression of metallothionein and HSP70. Biochemical pharmacology 66:917-926.
8. Schümann J., Wolf D., Pahl A., Brune K., Papadopoulos T., van Rooijen N., Tiegs G. (2000) Importance of Kupffer cells for T-cell-dependent liver injury in mice. The American journal of pathology 157:1671-1683.
9. Qiao Y.-l., Qian J.-m., Wang F.-r., Ma Z.-y., Wang Q.-w. (2014) Butyrate protects liver against ischemia reperfusion injury by inhibiting nuclear factor kappa B activation in Kupffer cells. Journal of Surgical Research 187:653-659.
10. Prins H.A., Meijer C., Boelens P.G., Diks J., Holtz R., Masson S., Daveau M., Meijer S., Scotté M., van Leeuwen P.A. (2004) Kupffer cell–depleted rats have a diminished acute-phase response following major liver resection. Shock 21:561-565.
11. Roberts R.A., Ganey P.E., Ju C., Kamendulis L.M., Rusyn I., Klaunig J.E. (2007) Role of the Kupffer cell in mediating hepatic toxicity and carcinogenesis. Toxicological Sciences 96:2-15.
12. Zeini M., Hortelano S., Través P.G., Gómez-Valadés A.G., Pujol A., Perales J.C., Bartrons R., Boscá L. (2005) Assessment of a dual regulatory role for NO in liver regeneration after partial hepatectomy: protection against apoptosis and retardation of hepatocyte proliferation. The FASEB journal 19:995-997.
13. Shiota G., Harada K., Ishida M., Tomie Y., Okubo M., Katayama S., Ito H., Kawasaki H. (1999) Inhibition of

hepatocellular carcinoma by glycyrrhizin in diethylnitrosamine-treated mice. Carcinogenesis 20:59-63.

14. Bancroft J.D., Gamble M. (2008) Theory and practice of histological techniques Elsevier health sciences.
15. Reitman S., Frankel S. (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American journal of clinical pathology 28:56-63.
16. Kind P. (1954) Estimation of plasma phosphatase by determination of hydrolysed phenol with aminopyrines. J ClinPathol 7:332-337.
17. Murray R., Kaplan A. (1984) ClinChem The CV Mosby Co. St Louis. Toronto, Princeton.
18. Patton C., Crouch S. (1977) Enzymatic determination of urea. Anal. Chem 49:464-469.
19. Jendrassik L., Grof P. (1938) Colorimetric method of determination of bilirubin. Biochem Z 297:81-82.
20. Doumas B.T., Watson W.A., Biggs H.G. (1971) Albumin standards and the measurement of serum albumin with bromcresol green. Clinicachimicaacta 31:87-96.
21. Yatzidis H. (1987) A colorimetric method for detection of total protein. J Clinica l Chemistry Journal 23:908.
22. Gandhi C.R., Chaillet J.R., Nalesnik M.A., Kumar S., Dangi A., Demetris A.J., Ferrell R., Wu T., Divanovic S., Stankeiwicz T. (2015) Liver-specific deletion of augmenter of liver regeneration accelerates development of steatohepatitis and hepatocellular carcinoma in mice. Gastroenterology 148:379-391. e4.
23. Cattoretti G., Becker M.H., Key G., Duchrow M., Schlüuter C., Galle J., Gerdes J. (1992) Monoclonal antibodies against recombinant parts of the Ki-67 antigen (MIB 1 and MIB 3) detect proliferating cells in microwave-processed formalin-fixed paraffin sections. The Journal of pathology 168:357-363.
24. Hsu S.-M., Raine L., Fanger H. (1981) A comparative study of the peroxidase-antiperoxidase method and an avidin-biotin

complex method for studying polypeptide hormones with radioimmunoassay antibodies. American journal of clinical pathology 75:734-738.

25. Jonker A.M., Dijkhuis F.W., Hardonk M.J., Moerkerk P., Kate J.T., Grond J. (1994) Immunohistochemical study of hepatic fibrosis induced in rats by multiple galactosamine injections. Hepatology 19:775-781.
26. Hunecke D., Spanel R., Länger F., Nam S.W., Borlak J. (2012) MYC-regulated genes involved in liver cell dysplasia identified in a transgenic model of liver cancer. The Journal of pathology 228:520-533.
27. Louis H., Le Moine A., Quertinmont E., Peny M.O., Geerts A., Goldman M., Le Moine O., Devière J. (2000) Repeated concanavalin a challenge in mice induces an interleukin 10–producing phenotype and liver fibrosis. Hepatology 31:381-390.
28. Natsume M., Tsuji H., Harada A., Akiyama M., Yano T., Ishikura H., Nakanishi I., Matsushima K., Kaneko S.i., Mukaida N. (1999) Attenuated liver fibrosis and depressed serum albumin levels in carbon tetrachloride-treated IL-6-deficient mice. Journal of leukocyte biology 66:601-608.
29. Luger D., Dayan M., Zinger H., Liu J.-P., Mozes E. (2004) A peptide based on the complementarity determining region 1 of a human monoclonal autoantibody ameliorates spontaneous and induced lupus manifestations in correlation with cytokine immunomodulation. Journal of clinical immunology 24:579-590.
30. Snedecor G.W., Cochran W.G. (1989) Statistical Methods, eight edition. Iowa state University press, Ames, Iowa.
31. David C.J., Massagué J. (2018) Contextual determinants of TGFβ action in development, immunity and cancer. Nature reviews Molecular cell biology 19:419-435.
32. Netea M.G., Domínguez-Andrés J., Barreiro L.B., Chavakis T., Divangahi M., Fuchs E., Joosten L.A., van der Meer J.W., Mhlanga M.M., Mulder W.J. (2020) Defining trained immunity and its role in health and disease. Nature Reviews

Immunology:1-14.

33. Taniguchi K., Karin M. (2018) NF-κB, inflammation, immunity and cancer: coming of age. Nature Reviews Immunology 18:309-324.
34. Topalian S.L., Taube J.M., Anders R.A., Pardoll D.M. (2016) Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. Nature Reviews Cancer 16:275.
35. Elalfy M.M. (2016) Cytotoxicity of Gadolinium Chloride and its Effect on Carbon Tetra Chloride Induced Liver Disorder in Albino Rats. Journal of Environmental Sciences 45:187-195.
36. Fukumasu H., Avanzo J.L., Heidor R., Silva T., Atroch A., Moreno F.S., Dagli M.L.Z. (2006) Protective effects of guarana (Paulliniacupana Mart. var. Sorbilis) against DEN-induced DNA damage on mouse liver. Food and chemical toxicology 44:862-867.
37. Henderson N.C., Iredale J.P. (2007) Liver fibrosis: cellular mechanisms of progression and resolution. Clinical Science 112:265-280.
38. Kyriakou L.G., Tzirogiannis K.N., Demonakou M.D., Kourentzi K.T., Mykoniatis M.G., Panoutsopoulos G.I. (2013) Gadolinium chloride pretreatment ameliorates acute cadmium-induced hepatotoxicity. Toxicology and industrial health 29:624-632.
39. Gloor B., Blinman T.A., Rigberg D.A., Todd K.E., Lane J.S., Hines O.J., Reber H.A. (2000) Kupffer cell blockade reduces hepatic and systemic cytokine levels and lung injury in hemorrhagic pancreatitis in rats. Pancreas 21:414-420.
40. Kolios G., Valatas V., Kouroumalis E. (2006) Role of Kupffer cells in the pathogenesis of liver disease. World journal of gastroenterology: WJG 12:7413.
41. Yuan D., Huang S., Berger E., Liu L., Gross N., Heinzmann F., Ringelhan M., Connor T.O., Stadler M., Meister M. (2017) Kupffer cell-derived Tnf triggers cholangiocellular tumorigenesis through JNK due to chronic mitochondrial dysfunction and ROS. Cancer Cell 31:771-789. e6.
42. Gallagher S.F., Yang J., Baksh K., Haines K., Carpenter H.,

Epling-Burnette P., Peng Y., Norman J., Murr M.M. (2004) Acute pancreatitis induces FasL gene expression and apoptosis in the liver1, 2. Journal of Surgical Research 122:201-209.

43. Wu K., Kryczek I., Chen L., Zou W., Welling T.H. (2009) Kupffer cell suppression of CD8+ T cells in human hepatocellular carcinoma is mediated by B7-H1/programmed death-1 interactions. Cancer research 69:8067-8075.
44. Yang L., Dong C., Tian L., Ji X., Yang L., Li L. (2019) Gadolinium Chloride Restores the Function of the Gap Junctional Intercellular Communication between Hepatocytes in a Liver Injury. International journal of molecular sciences 20:3748.
45. Liu C., Yang Z., Wang L., Lu Y., Tang B., Miao H., Xu Q., Chen X. (2015) Combination of sorafenib and gadolinium chloride (GdCl3) attenuates dimethylnitrosamine (DMN)-induced liver fibrosis in rats. BMC gastroenterology 15:159.
46. Klein A.D., Oyarzún J.E., Cortez C., Zanlungo S. (2018) Gadolinium Chloride Rescues Niemann–Pick Type C Liver Damage. International journal of molecular sciences 19:3599.
47. Zhu F., Li X., Jiang Y., Zhu H., Zhang H., Zhang C., Zhao Y., Luo F. (2015) $GdCl_3$ suppresses the malignant potential of hepatocellular carcinoma by inhibiting the expression of CD206 in tumor-associated macrophages. Oncology reports 34:2643-2655.