ijlpr 2021; doi 10.22376/ijpbs/lpr.2021.11.4.L137-146

International Journal of Life science and Pharma Research

Research Article

Pharmacology For Better Treatment

# Vitamin C Ameliorates the Cefepime-Induced Changes of Liver Enzymes, Histopathology and Proinflammatory Cytokines in Male Albino Rats

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**Abstract:** Cefepime belongs to the fourth generation cephalosporin family of antibiotics, it showed side effects such as nephrotoxicity and neurotoxicity. However little reports showed its hepatotoxic effects. The aim of the current study was to evaluate cefepime-induced hepatotoxicity and its associated histopathological changes in the liver. Also, it aimed to examine the effects of cefepime treatment on the proinflammatory cytokines as well as the protective and recovering roles of vitamin C against the cefepime-induced effects. . Blood samples were collected and the liver enzymes in serum ALT, AST and Total Bilirubin (T. Bil.) were estimated biochemically; liver tissues were collected and divided into two parts: the first part were fixed for histopathological examination by H&E staining whereas the other part was processed for RNA extraction and further for real time PCR examination of the mRNA expression of IL1β and TNFα. Cefepime could significantly increase the concentration of ALT, AST, T. Bil. as well as the mRNA expression of proinflammatory cytokines IL1β and TNFα; histopathological changes in the form of hepatic lobules distortion, marked degeneration, hepatocellular vacuolation and necrosis, nuclear pyknosis, scattered apoptosis, distortion of portal areas and marked fibrosis were observed after two weeks of cefepime administration; co-administration of vitamin C with cefepime or administration administering it for 7 days after cefepime could successfully improve the levels of ALT, AST, T. Bil., histopathological and mRNA expression of IL1β and TNFα. The current study suggested that cefepime has hepatotoxic effects shown as alternation of the liver enzymes, changing in the histopathological features as well as stimulating the inflammation in the liver tissue. Vitamin C can be used as a protective or recovering drug against cefepime-induced hepatotoxicity.

**Keywords:** Antibiotics, cefepime, hepatotoxicity, proinflammatory cytokines, vitamin C.

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**Received On** 2 May, 2021
**Revised On** 15 July, 2021
**Accepted On** 23 July, 2021
**Published On** 30 July, 2021

**Funding** This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

**Citation** Ahmad M. Abdel-Mageed, Muaz Magzub1, Mohamed A. Abdein and Nabil S. Awad , Vitamin C ameliorates the cefepime-induced changes of liver enzymes, histopathology and proinflammatory cytokines in male Albino rats.(2021).Int. J. Life Sci. Pharma Res.11(4), L137-146 http://dx.doi.org/10.22376/ijpbs/lpr.2021.11.4.L137-146

## 1. INTRODUCTION

Patient's health is harmfully and mischievously affected by drugs administration. The excessive and prolonged usage of such medications are relatively common causes of vital organs toxicity[1-3]. Liver is commonly subjected to injury resulting from therapeutic drugs administrations [4,5] as it is the site in which many drugs and nutrients are metabolized and bio-transformed. Also, it is the organ where bile acids are synthesized, concentrated and secreted and excretion of other toxicants such as bilirubin.[6] Exposure to pharmacological drugs and other non-pharmacological agents may cause liver damage or hepatotoxicity[7] which is considered as an adverse reaction of the drugs.[8] All kinds of hepatotoxicity are idiosyncratic. [3, 9, 10] Depending on the predominant enzyme values, hepatotoxicity may be either hepatocellular injury or cholestatic in origin. In hepatocellular pattern, ALT and/or AST value is higher than ALP. On the other hand, cholestatic injury patterns show higher ALP value. [9, 11] In cholestatic diseases, hepatocellular apoptosis was stimulated with activated Kupffer cells associated with neutrophil infiltration leading to triggering an immune reaction in the form of inflammation mediated by reactive oxygen species[12], cytokines, chemokines and consequently bile production capacity of hepatocytes is decreased. [13] A wide range of drugs are stimulators of hepatotoxicity including antibiotics, non-steroidal anti-inflammatory drugs, anti-tubercular drugs, anti-retroviral drugs, anti-hyperlipidemic drugs, an aesthetic agents, anti-rheumatic drugs, neuroleptic drugs or antipsychotic drugs, drugs of abuse and anti-hypertensive drugs are the most common drugs to cause liver damage, however antibiotics are still the most common causes of liver damage.[2, 12, 14-21] Cephalosporins are a group of antibiotics associated with nephrotoxicity[22], however many observations reported that those groups are associated with little or no hepatotoxicity.[23] Cefepime is a member of the 4$^{th}$ generation cephalosporin family with broad spectrum of antimicrobial activities, it is recommended for a variety of infections.[24] It is characterized by well-tolerability with few side effects. It has been well reported that there is a possible relation between cefepime and nephrotoxicity and biochemical alterations, especially when administered at high doses [25, 26], however its hepatotoxicity has not been well focused and studied. Administration of cefepime resulted in production of oxidative stress which can be observed either by detection of oxygen free radicals such as reactive oxygen species (ROS) or other similar substances in the kidney or liver [12, 5], or by presence of chronic inflammatory immune response in the form of increasing inflammatory cytokine markers and fibrosis.[26] Recently, it is of great medical importance to find out a successful strategy to fight against the deleterious effects of antibiotics, especially those caused by cefepime. It was suggested that cellular antioxidant activity is enhanced by using dietary antioxidants or antioxidant drugs.[27, 28] Supplementation of antioxidants such as Vit. C can be considered as the alternative method for chelation therapy.[29] It has an ability to scavenge free radicals and ROS, which are synthesized during metabolic processes.[30] The aim of this study was to examine the cefepime-induced hepatotoxic effects, studying the histopathological changes in the liver caused by the cefepime as well as its effects on the pro-inflammatory cytokines. One of the main aims of the current study was to find out a successful strategy to minimize and recover the damage in the liver caused by administration of cefepime by using potential antioxidant such as vitamin C. Minor objectives in this study are summarized in the following specific questions: 1) Did the liver functions mediated by ALT, AST and bilirubin change in response to cefepime administration? 2) Did the mRNA expression of pro-inflammatory cytokines IL-1β and TNF-α change after cefepime treatment? 3) Did cefepime change the normal histological structure of the liver? 4) Did vitamin C improve the cefepime-induced hepatic injury?

## 2. MATERIALS AND METHODS

### *2.1 Drug and ascorbic acid*

Cefepime was purchased from a local Egyptian pharmacy and ascorbic acid was obtained from Al-Gomhouria Company for medicines and medical supplies Cairo, Egypt.

### *2.2 Experimental animals*

Twenty-five adult males Wistar albino rats (100-140 g) were obtained from the Nile for Pharmaceuticals & Chemical Industries Cairo, Egypt. Animals were fed ad libitum and had free access to water and kept at room temperature for one week before starting the experiment for acclimatization. All of the experiments were performed after the author has been approved by the National Committee of Bioethics (NCB E), King Abdulaziz City for Science and Technology (KACST) with the approval No. 1009830.

### *2.3 Experimental Design*

Rats were divided into five different groups, five rats each. The hepatotoxic effects were measured after twenty-one days. The experimental groups are shown as follows:

- Group 1: received distilled water throughout the period of the experiment.
- Group 2: received 100mg/kg B.W /day of cefepime for 15 days.
- Group 3: received 100mg/kg B.W/day of cefepime + 1000mg/kg B.W/day of ascorbic acid at the same time for 15 days.
- Group 4: received 100mg/kg B.W/day of cefepime for 15 days followed by 1000mg/kg B.W/day of ascorbic acid for 7 days.

Group 5: received 100mg/kg B.W/day of cefepime for 15 days followed by slain for 7 days.

The drugs in all groups were given to rats orally.

### *2.4 Serum biochemical analysis*

Whole blood samples were collected using ocular puncture technique, left up right for 15 minutes and centrifuged at 3000 rpm for 10 minutes. Then the serum was used to estimate AST, ALT and total bilirubin.[31]

### *2.5 Tissue samples*

The liver tissues were quickly removed after scarification, washed with physiological saline, and separated into two parts: the first part was stored in Triazole reagent for real-time gene expression analysis and stored at -20 °C. The second part from hepatic tissue was fixed in 15 % formaldehyde solution for pathological examination.[32]

### 2.6 *Determination of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and serum Bilirubin*

The ALT, AST and serum bilirubin, as indicators for liver function, were estimated in all animals according to the standard procedure.[31, 33]

### 2.7 *Pathological examination*

Formalin fixed specimens were routinely dehydrated in ascending series of alcohol, cleared in xylol, and finally embedded in paraffin. 4-5 µm-thick tissues were sectioned and processed for H&E staining[32]. Tissue slides were examined and were compared to their corresponding controls.

### 2.8 *Gene expression analysis*

Total RNA was extracted from liver tissues by TRIZOL reagent according to the manufacturer's instructions. RT-qPCR was performed by the use of SYBR® Green dye (One-Step RT-PCR Kit, Bio Rad) on a Rotor-Gene Q real-time PCR cycler. The sequence of primer were as follow; IL1β F 5' -CAACAAAAATGCCTCGTGCTG-3' IL1β R 5' TCGTTGCTTGTCTCTCCTTGTA5' [34], TNFα R 5'- 5'-GGCTCTGAGGAGTAGACGATAA3' and β-actin F 5'-CGGTCAGGTCATCACTATCGG-3' β-actin R 5'- 5'-TCTTTACGGATGTCAACGTCACAC3'.[35] The amplification steps for IL1β were carried out in primary denaturation at 95 °C for 10 min. RT-qPCR was performed in 40 cycles (including secondary denaturation at 95 °C for 15 sec, annealing at 60 C for 20 sec, and extension at 72 C for 25 sec)[34]. The amplification steps for TNFα and β-actin were as follow: a first denaturation at 95 °C for 10 min; RT-qPCR of 40 cycles with a three-step program (15 sec at 95 °C for denaturation, 20 sec at 60 °C for annealing, and 25 sec at 72 °C for extension). β-actin was used as the internal control gene to normalize the gene expression data. [35] The 2^ ΔΔCT method was used for the analysis of gene expression.

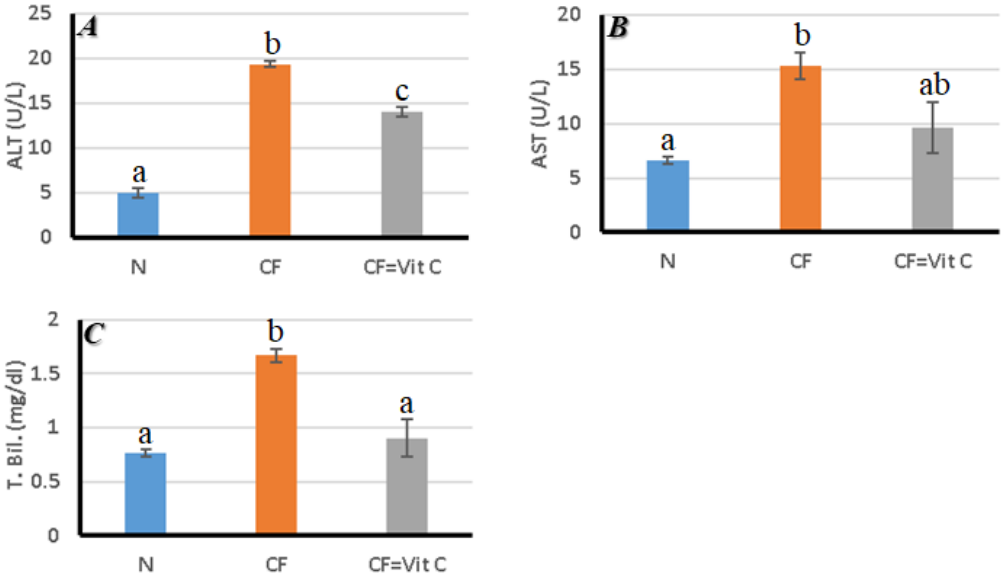
## 3. STATISTICAL ANALYSIS

Data were analyzed by Analysis of Variance (one-way ANOVA test) using Tukey post hoc test; SPSS version 21 software was used for statistical analysis tests. Values were presented as mean ± S.E.M. Values are considered significant at *P* value < 0.05 (Tukey post hoc test).[31]

## 4. RESULTS

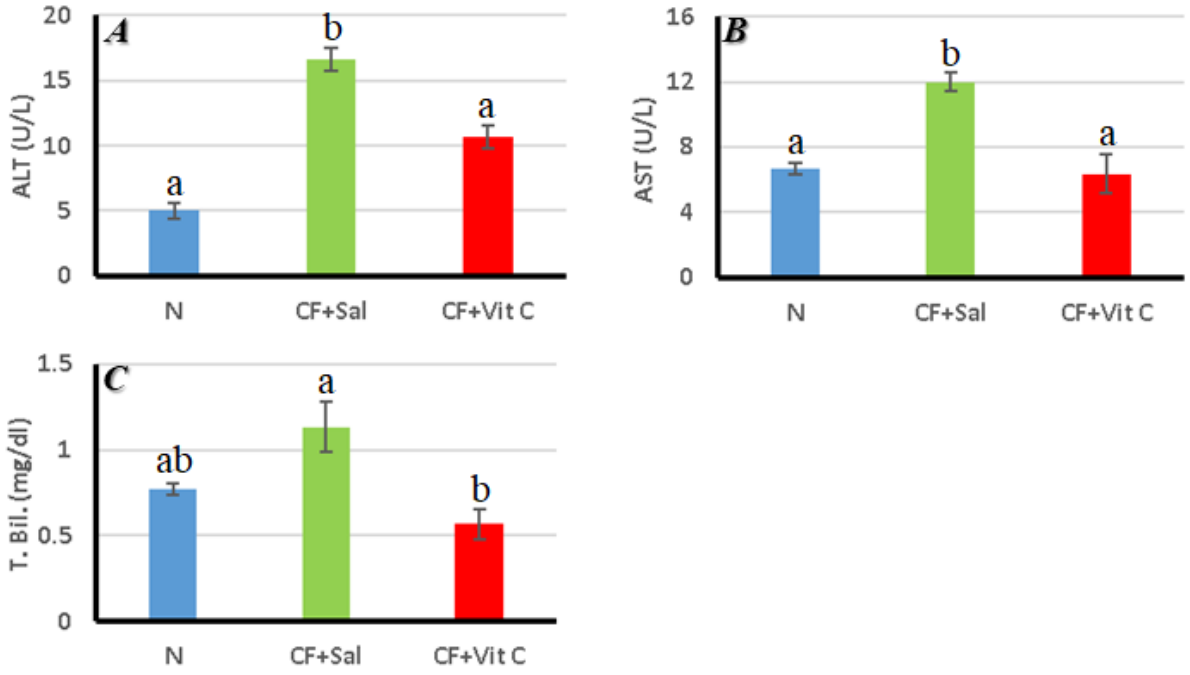
### 4.1 *Biochemical examination of liver enzymes and total bilirubin*

Fig. 1(A, B and C) shows significant increase in the concentrations of ALT, AST and T. Bil. compared to normal untreated control in response to cefepime treatment for two weeks, the concentration of ALT was decreased significantly by simultaneous treatment with vitamin C and cefepime, however such decrease is still significantly higher than the level of normal untreated group (Fig. 1A). The elevated concentration of AST by cefepime was not statistically affected by simultaneous administration of vitamin C, whereas the elevated concentration of T. Bil by cefepime was significantly downregulated by simultaneous treatment of vitamin C to a level statistically same as normal untreated group (Fig. 1C). Fig. 2 explains the effects of vitamin C administration for 7 days after following two weeks of cefepime administration on the concentration of liver enzymes and T. Bil. After two weeks of administration of cefepime and treatment with saline for another 7 days, the concentration of ALT, AST and T. Bil. was still increasing significantly; such increase is significantly decreased to a level comparable to the normal level of the untreated group (Fig. 2A, B and C).



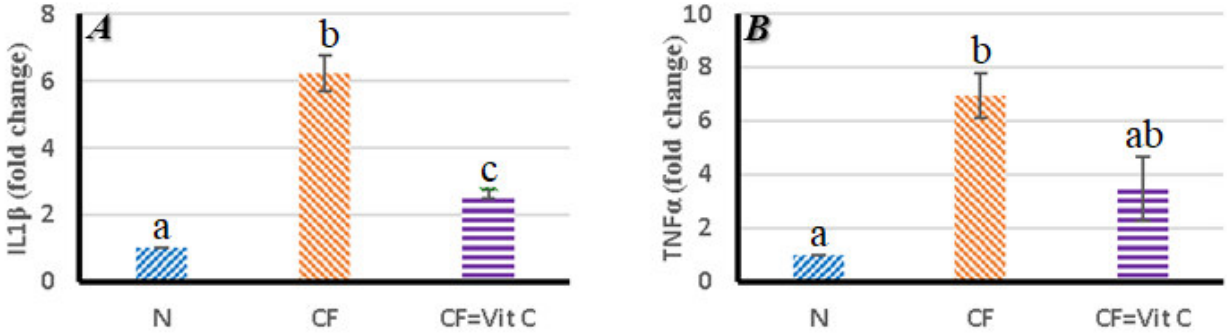

*Values are the mean ± SEM.; a, b and c represent the result of the statistical analysis of each experimental group. Different letters indicate significant differences between groups. Values were considered significant when P < 0.05.*

**Fig. 1. The change in the concentration of ALT (A), AST (B) and Total bilirubin (c) in response to cefepime treatment and the effect of the simultaneous administration of vitamin C with cefepime on the changes in their concentrations.**




*Values are the mean ± SEM; a, b and c represent the result of the statistical analysis of each experimental group. Different letters indicate significant differences between groups. Values were considered significant when P < 0.05.*

**Fig. 2. The effects of 7 days administration of vitamin C on the change in the concentration of ALT (A), AST (B) and Total bilirubin (C) following two weeks of cefepime treatment.**

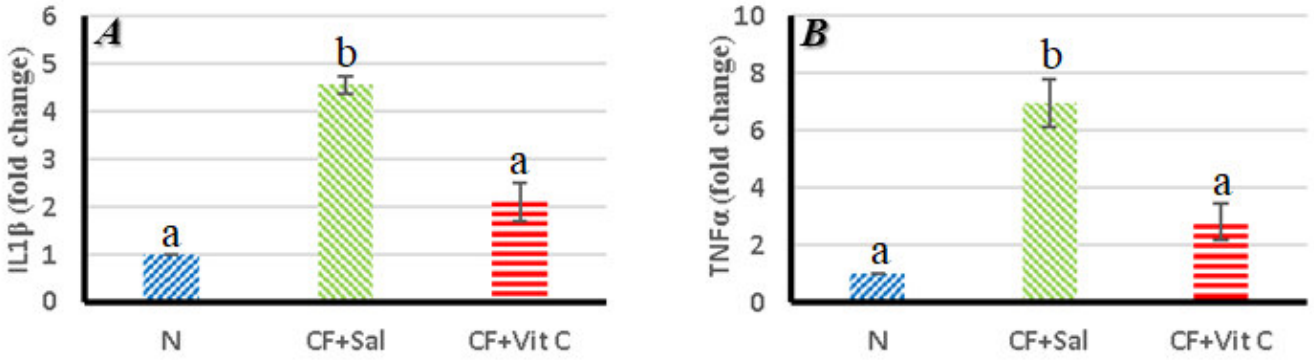



*Values are the mean ± SEM.; a, b and c represent the result of the statistical analysis of each experimental group. Different letters indicate significant differences between groups. Values were considered significant when P < 0.05.*

**Fig. 3. The change in the mRNA expression of proinflammatory cytokines IL1β (A) and TNFα (B) in response to treatment with cefepime and the effect of the simultaneous administration of vitamin C with cefepime on the changes in their mRNA expression.**

### *4.2 Examination of mRNA expression of proinflammatory cytokines*

In Fig. 3, cefepime treatment for two weeks increased the level of mRNA expression of proinflammatory cytokines IL1β and TNFα (Fig. 3A and B), such increase in the mRNA of IL1β was significantly decreased by vitamin C treatment, however the level is still higher than normal untreated groups (Fig. 3A) whereas the simultaneous treatment of vitamin C with cefepime did not change the level of mRNA of TNFα (Fig. 3B). In Fig. 4, treatment with cefepime for two weeks followed by another 7 days of saline treatment increased the mRNA expression of IL1β and TNFα. Treatment with cefepime for two weeks followed by 7 days of vitamin C significantly downregulated the mRNA expression of IL1β and TNFα to the untreated normal level (Fig. 4A and B).




*Values are the mean ± SEM.; a, b and c represent the result of the statistical analysis of each experimental group. Different letters indicate significant differences between groups. Values were considered significant when P < 0.05.*

**Fig. 4. The effects of 7 days administration of vitamin C following cefepime treatment on the change in the mRNA expression of IL1β (A) and TNFα (B).**

### *4.3 Histopathological examination*

The effects of cefepime treatment as well as the effects of vitamin C on the cefepime histopathological-induced liver are illustrated in Fig. 5. Livers of control animals showed normal histological structural features of the central vein, portal areas and hepatic cells (A). Histopathological changes in the liver of cefepime treated animals were explained (Fig. 5B, C and D). A distortion of the portal areas with dilated vessels, intense inflammatory cells infiltration and fibrous tissue proliferation were observed in the liver of cefepime treated animals (Fig. 5A, B and C). Also, distortion in architecture of the cefepime treated liver was seen represented by irregular arrangement of hepatic strands and marked degeneration, vacuolation of the hepatic cells with fat cyst formation with sometimes cytoplasmic reticulation and containing pyknotic or fragmented nuclei as well as hepatocellular necrosis and scattered apoptosis (Fig. 5D). The histopathological changes in the liver of cefepime treated rats followed by treatment with saline for 7 days are shown in Fig. 5E and F; the liver of this group showed diffuse hepatocellular vacuolar degeneration and portal areas enlargement with fibrous proliferation and inflammatory cells infiltration (Fig. 5E). The vacuoles in the diffuse hepatocellular vacuolar degeneration were well-circumscribed of variable sizes, with signet ring appearance of most cells at which the vacuole pushed the nucleus to the periphery. Sometimes scattered fat cysts were observed with necrotic cells (Fig. 5F). Regarding livers of Cefepime and vitamin C co-administered rats (Fig. 5G and H), the examination of which showed moderate degree of hepatocellular vacuolar degeneration, nuclear pyknosis and scattered necrosis with marked protection of the portal tracts, few of which showing very mild inflammatory cells infiltration. While livers of rats given Cefepime for 2 weeks followed by vitamin C (Fig. 5I and K) showed good restoration of the hepatic parenchymal cells with mild scattered vacuolar degeneration and necrosis of the hepatic cells, notice mononuclear inflammatory cells infiltration in some portal areas (Fig. 5I). Some livers showed very mild hepatic cells degenerative and necrotic changes and few nuclear pyknosis (Fig. 5K).

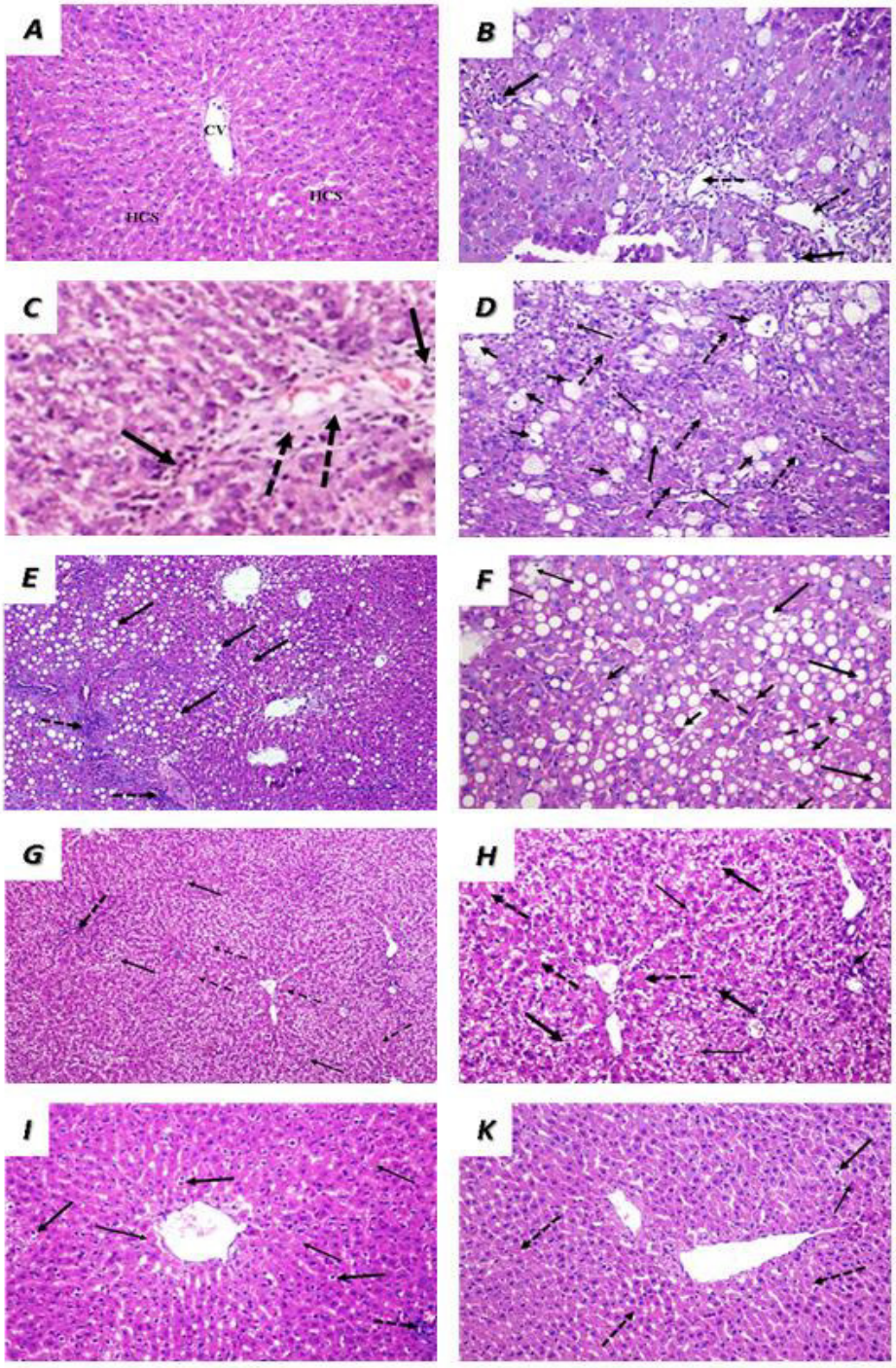



**Fig. 5 (A-K). Photomicrographs for the histopathological changes in the liver describing the cefepime-induced damage in the liver and the recovering effects of vitamin C on the damage occurred.**

A)Liver of control rat showing normal histological structure of the central vein (CV) and hepatic cells (HCs). (H&E, X200). B) Liver of cefepime administered rat showing distortion of the portal areas with dilated vessels, inflammatory cells infiltration (arrow) and fibrous tissue proliferation. (H&E, X200). C) Liver of cefepime administered rat showing distortion of the portal areas with intense inflammatory cells infiltration (arrows) and fibrous tissue proliferation (dashed arrows). (H&E, X200). D) Liver of cefepime administered rat showing distortion of the hepatic lobules and marked degeneration, vacuolation (arrow) of the hepatic cells with fat cyst formation and cytoplasmic reticulation (short arrow) that containing pyknotic or fragmented nuclei as well as hepatocellular necrosis (dashed arrows) and scattered apoptosis (thin arrow). (H&E, X400). E) Liver of cefepime administered rat for 2 weeks followed by saline showing diffuse hepatocellular vacuolar degeneration (arrow) and portal areas enlargement with fibrous proliferation with inflammatory cells infiltration (dashed arrow). (H&E, X100). F) Liver of cefepime administered rat for 2 weeks followed by saline showing diffuse hepatocellular vacuolar degeneration of variable sizes well-circumscribed vacuoles (arrow), signet ring appearance of most cells (dashed arrow), scattered fat cyst formation (thin arrow) and necrotic cells (short arrow). (H&E, X200). G) Liver of cefepime and vitamin C co-administered rat showing moderate degree of hepatocellular vacuolar degeneration (thin arrows) and scattered necrosis (thin dashed arrows) with marked protection of the portal tracts (thick dashed arrow), few of which showing very mild inflammatory cells infiltration. (H&E, X100). H) Liver of cefepime and vitamin C co-administered rat showing

moderate hepatocellular vacuolar degeneration *(arrow), necrosis (dashed arrow), nuclear pyknosis (thin arrow) and very few inflammatory cells infiltrating some portal areas (short arrow). (H&E,* X200). J) Liver of rat administered cefepime for 2 weeks followed by vitamin C showing good restoration of the hepatic parenchymal cells with mild scattered vacuolar degeneration (arrow) and necrosis (thin arrow) of the hepatic cells, notice mononuclear inflammatory cells infiltration (dashed arrow) in some portal areas. (H&E, X200). K) Liver of rats administer cefepime for 2 weeks followed by vitamin C showing good restoration of the hepatic tissue with very mild hepatic cells degenerative (arrow) and necrotic (dashed arrow) changes and few nuclear pyknosis. (H&E, X200).

## 5. DISCUSSION

The current investigation intended to study some of the cefepime-induced hepatotoxicity and the effects of vitamin C as a potent antioxidant on the cefepime-induced hepatotoxicity. The most important findings in this study are i) two-weeks administration of cefepime could significantly increase the concentration of ALT, AST, T. Bil. as well as the mRNA expression of proinflammatory cytokines IL1β and TNFα; ii) histopathological changes in the form of hepatic lobules distortion, marked degeneration, hepatocellular vacuolation and necrosis, nuclear pyknosis, scattered apoptosis, distortion of portal areas and marked fibrosis were observed after two weeks of cefepime administration; iii) co-administration of vitamin C with cefepime could partially improve the concentration of ALT, AST, mRNA expression of IL1β and TNFα; and completely improved the concentration of T. Bil.; iv) administration of vitamin C for 7 days after two weeks of cefepime administration completely improved the concentration of ALT, AST, T. Bil. as well as the mRNA expression of proinflammatory cytokines IL1β and TNFα; v) good restoration of normal histological structure of the liver was obtained after vitamin C administration. Liver is a very important organ in metabolizing drugs and elimination of drug metabolites, thus it may be subjected to some damage or hepatotoxicity when drugs such as NSAIDs and antibiotics such as augmentin were administered. [9, 14, 36, 37] Although many reports suggested that cefepime causes neurotoxicity and nephrotoxicity [38, 39], many reports showed that cefepime has little or no hepatotoxic effects, however other reports stated that cefepime-induced hepatotoxicity is rare and even it might not cause hepatotoxicity. [23, 40] The results of the current study showed that cefepime had severe hepatotoxic effects at the levels of histological structures, liver enzymes as well as the mRNA expression of proinflammatory cytokines; such conflict whether cefepime causes severe liver injury, mild injury or even it is safe may be attributed to the idiosyncratic drug hepatotoxicity.[41] In the current study, the histopathological examinations of the liver of the cefepime treated rats as well as those treated with saline after cefepime showed marked hepatocellular degeneration, vacuolation, necrosis, nuclear pyknosis, fat cells formation, apoptosis, inflammatory cells infiltration and marked fibrosis in the portal areas. Some of the above-mentioned histopathological changes such as lymphatic inflammatory infiltration and presence of fat vacuoles were observed in the liver of 14-days infants rats whose mothers were treated with maxipime (cefepime hydrochloride).[42] The cause of such liver injury was attributed to an immunoallergic reaction against the structural β-lactam 4-ring.[40, 43] The liver enzymes are very important factors indicating the liver functions. They are localized in the cytoplasm and mitochondria of the hepatocytes. Any change in the hepatocyte permeability may lead to the releasing of those enzymes. Necrosis also may be a cause of such increase in the level of ALT and AST.[44, 45] Using antibiotics such as augmentin, cyclosporin, ciprofloxacin was proven to increase the serum levels of ALT and AST through changing the permeability of mitochondrial and cellular membranes. [46] In a case treated with cefepime, it was suggested that liver biochemistry showed elevation in the concentration of serum total bilirubin, ALT and AST. [40, 47, 48] In our work, hepatocellular necrosis was observed in liver cells of rats treated with cefepime, thus we agree with the point of view which attributes the increase of liver enzymes to the necrosis and alternation of membrane permeability of hepatocytes. In the current study, lipid vacuolations were observed in the hepatocytes of cefepime treated rats, such phenomenon was supported by the findings which suggested that cefepime interferes with the correlation of RNA with ribosomes, leading to lacking of protein building information which is a reason for the depletion in the synthesis of lipid acceptor protein in the hepatocytes, leading to inability of triglycerides to leave the hepatocytes resulting in accumulation of lipid inside the hepatocytes.[42, 49] Histopathological examination of the liver of cefepime-treated rats showed scattered apoptotic cells, the cause of such apoptosis may be attributed to the drug which stimulated an intrinsic intracellular pathway which may cause inhibition of DNA and RNA synthesis and delayed or inhibited the cell division [50, 51], thus causing nuclear DNA damage. IL1β and TNFα cytokines are endowed with an outstanding tissue-damaging potential, including induction of apoptosis. [52, 53] Marked upregulation in the mRNA expression of proinflammatory cytokines IL1β and TNFα was observed in the cefepime-treated rats. Such upregulation in the level of proinflammatory cytokines may be a reason for the apoptosis in the hepatocytes observed. A close relation between the upregulation of the proinflammatory cytokines IL1β and TNFα and apoptosis was confirmed in the kidney by Rama Falk et al (2005) who suggested that apoptosis in the renal cells was appeared in response to upregulation of IL1β and TNFα which are potential mechanism of tissue toxicity. [53] It was also noticed that apoptosis was induced in leukemic HL-60 and K 562 cell lines by IL1β and TNFα through activation of caspase [54] and in primary cultured thyroid cells by IL1β adenylyl cyclase and ERK1/2 inhibition. [55] The increased proinflammatory cytokines together with the presence of inflammatory cells infiltration in the cefepime-treated rats indicate a stage of developing hepatic inflammation, such observations were supported by the previous reports which focused on the hepatic inflammatory diseases. [56, 57] It was suggested that the inflammatory cells infiltration can be progressed into fibrous tissue through overproduction of ROS after triggering high levels of IL1β, 2, 6, 7 and TNFα. [58, 59] Similar findings were demonstrated in the current work, histopathological demonstration of the liver of cefepime-treated rats revealed the presence of inflammatory cells infiltrations, and marked production of fibrotic tissues in accordance with the elevated levels of proinflammatory cytokines IL1β and TNFα. Thus, we believe that the observed liver fibrosis may be attributed to the upregulated IL1β and TNFα and the inflammatory cell infiltrations. It was suggested that the acetaminophen-induced upregulation of ALT and AST was downregulated to a level statistically equivalent to the normal levels by using vitamin C [60, 61], the findings of our work showed similar results of improving the

cefepime-induced high level of ALT, AST and T. Bil. either by co-administration of vitamin C with cefepime or by administration of vitamin C after finishing the course of cefepime. Such improvement in the levels of liver enzymes together with the high degree of restoration of the normal histological appearance of the liver indicated the potent capability and the role of vitamin C to restore the normal functions of the hepatocytes, such effects were explained depending on the anti ROS effects of vitamin C. [62] Vitamin C is considered as a potent anti-inflammatory drug through the inhibition of ROS which is a mediator for production of proinflammatory cytokines in several inflammatory diseases. [63-65] Also, it has a protective effect against hepatotoxicity. [59] The anti-inflammatory effects of vitamin C were confirmed in our current study which showed a restoration of the normal levels of the mRNA expression of IL1β and TNFα either by simultaneous administration of vitamin C with cefepime or by its administration for a week after finishing the cefepime treatment. It was suggested that hepatocellular damage by inflammatory diseases such as hepatitis can be minimized by vitamin C through regulating the immune response. Thus, administration of suitable doses of vitamin C during such diseases or hepatic injury is a successful therapeutic option. [65]

## 6. CONCLUSION

The current study concluded that cefepime induced hepatotoxicity for rats represented in the form of biochemical alteration of the liver functions mediated by enzymes. Such alternation in the biochemical functions was associated with histopathological changes and induction of inflammatory reactions mediated by proinflammatory cytokines in the liver tissue. Administration of vitamin C had potential recovering effects against the damaged liver structure and functions. Thus, it can be considered as protective or/and recovering drug against cefepime-induced hepatotoxicity as well as restoring the normal functions of the liver.

## 7. AUTHOR CONTRIBUTION STATEMENT

Ahmad M. Abdel-Mageed supervised the teamwork, focused the idea of the work, planned the experimental design, analyzed the data, performed the statistical analysis and wrote the manuscript; Nabil S. Awad shared in selecting the point of the study and carried out and followed the experiments of the current study; Muaz Magzub and Mohamed A. Abdein reviewed the experimental design and the manuscript. All authors shared in interpreting and discussing the experimental data. All the authors read and approved the final version of the manuscript.

## 8. ACKNOWLEDGEMENT

We would like to acknowledge and appreciate approving this research work supported by the grant No. SAR-2018-3-9-F-7949 which was presented from the Deanship of Scientific Research at Northern Border University, Arar, K.S.A.

## 9. CONFLICT OF INTEREST

Conflict of interest declared none.

## 10. REFERENCES

1 M.A. Perazella, Pharmacology behind Common Drug Nephrotoxicities, Clinical Journal of the American Society of Nephrology (2018) CJN. 00150118.

2 A. Pandit, T. Sachdeva, P. Bafna, Drug-induced hepatotoxicity: a review, J Appl Pharm Sci 2(5) (2012) 233-43.

3 A.C. Paniagua, P. Amariles, Hepatotoxicity by Drugs, Pharmacokinetics and Adverse Effects of Drugs-Mechanisms and Risks Factors (2017).

4 I.G. McFarlane, Autoantibodies in alcoholic liver disease, Addiction Biology 5(2) (2000) 141-151.

5 S. David, J.P. Hamilton, Drug-induced liver injury, US gastroenterology & hepatology review 6 (2010) 73.

6 M. García-Cortés, R. Andrade, M. Lucena, R. González-Grande, R. Camargo, E. Fernández-Bonilla, J. Martos, R. Alcántara, Hepatotoxicidad secundaria a fármacos de uso común, Gastroenterología y hepatología 28(8) (2005) 461-472.

7 F. Tejada Cifuentes, Hepatotoxicidad por fármacos, Revista Clínica de Medicina de Familia 3(3) (2010) 177-191.

8 R.J. Andrade, M. Robles, A. Fernández-Castañer, S. López-Ortega, M.C. López-Vega, M.I. Lucena, Assessment of drug-induced hepatotoxicity in clinical practice: a challenge for gastroenterologists, World journal of gastroenterology: WJG 13(3) (2007) 329.

9 N. Sandhu, V. Navarro, Drug-Induced Liver Injury in GI Practice, Hepatology Communications 4(5) (2020) 631-645.

10 M. Biolato, C. Araneo, G. Marrone, A. Liguori, L. Miele, F. Ponziani, A. Gasbarrini, A. Grieco, Liver transplantation for drug-induced acute liver failure, Eur Rev Med Pharmacol Sci 21(1) (2017) 37-45.

11 R.J. Andrade, P.M. Tulkens, Hepatic safety of antibiotics used in primary care, Journal of Antimicrobial Chemotherapy 66(7) (2011) 1431-1446.

12 P. Jobanputra, R. Amarasena, F. Maggs, D. Homer, S. Bowman, E. Rankin, A. Filer, K. Raza, R. Jubb, Hepatotoxicity associated with sulfasalazine in inflammatory arthritis: a case series from a local surveillance of serious adverse events, BMC musculoskeletal disorders 9(1) (2008) 48.

13 H. Jaeschke, G.J. Gores, A.I. Cederbaum, J.A. Hinson, D. Pessayre, J.J. Lemasters, Mechanisms of hepatotoxicity, Toxicological sciences 65(2) (2002) 166-176.

14 H.M. SHALABI, I.H. REFAAT, A.S. IBRAHIM, ABDEL-MAGEED1, A.M. 2, THE ROLE OF FOLIC ACID AS A PROTECTIVE DRUG ON SOME OF AUGMENTIN-INDUCED TOXIC EFFECTS IN MALE ALBINO RATS, (2019).

15 D. Girling, The hepatic toxicity of antituberculosis regimens containing isoniazid, rifampicin and pyrazinamide, Tubercle 59(1) (1977) 13-32.

16 F.W. Wit, G.J. Weverling, J. Weel, S. Jurriaans, J.M. Lange, Incidence of and risk factors for severe hepatotoxicity associated with antiretroviral

combination therapy, The Journal of infectious diseases 186(1) (2002) 23-31.

17 D.E. Cohen, F.A. Anania, N. Chalasani, An assessment of statin safety by hepatologists, The American journal of cardiology 97(8) (2006) S77-S81.

18 B. Ricaurte, A. Guirguis, H.C. Taylor, D. Zabriskie, Simvastatin–amiodarone interaction resulting in rhabdomyolysis, azotemia, and possible hepatotoxicity, Annals of Pharmacotherapy 40(4) (2006) 753-757.

19 A. Ansari, T. Elliott, B. Baburajan, P. Mayhead, J. O'donohue, P. Chocair, J. Sanderson, J. Duley, Long-term outcome of using allopurinol co-therapy as a strategy for overcoming thiopurine hepatotoxicity in treating inflammatory bowel disease, Alimentary pharmacology & therapeutics 28(6) (2008) 734-741.

20 E. Björnsson, Hepatotoxicity associated with antiepileptic drugs, Acta Neurologica Scandinavica 118(5) (2008) 281-290.

21 L. Jeffrey, T. Allan, Handbook of psychiatric drugs, JohnWiley and Sons Ltd., Chichester UK (2006) 34.

22 J. Quin, The nephrotoxicity of cephalosporins, Adverse drug reactions and acute poisoning reviews 8(2) (1989) 63.

23 A.A.N. Al Haboob, Cefixime-induced hepatotoxicity and acute renal failure: a case report, European Journal of Medical Case Reports 2(2) (2018) 55-58.

24 W. Chaijamorn, T. Charoensareerat, N. Srisawat, S. Pattharachayakul, A. Boonpeng, Cefepime dosing regimens in critically ill patients receiving continuous renal replacement therapy: a Monte Carlo simulation study, Journal of intensive care 6(1) (2018) 61.

25 M.G.A. Elsayed, A.A.A. Elkomy, M.S. Gaballah, M. Elbadawy, Nephrotoxicity of cefepime: a new cephalosporin antibiotic in rats, Journal of pharmacology & pharmacotherapeutics 5(1) (2014) 33.

26 K. Mac, R. Chavada, S. Paull, K. Howlin, J. Wong, Cefepime induced acute interstitial nephritis–a case report, BMC nephrology 16(1) (2015) 15.

27 R.L. Prior, G. Cao, Antioxidant phytochemicals in fruits and vegetables: diet and health implications, HortScience 35(4) (2000) 588-592.

28 J. Pincemail, K. Bonjean, K. Cayeux, J.-O. Defraigne, Mécanismes physiologiques de la défense antioxydante, Nutrition clinique et métabolisme 16(4) (2002) 233-239.

29 I.B. Amara, N. Soudani, A. Troudi, H. Bouaziz, T. Boudawara, N. Zeghal, Antioxidant effect of vitamin E and selenium on hepatotoxicity induced by dimethoate in female adult rats, Ecotoxicology and environmental safety 74(4) (2011) 811-819.

30 M. Yimcharoen, S. Kittikunnathum, C. Suknikorn, W. Nak-On, P. Yeethong, T.G. Anthony, P. Bunpo, Effects of ascorbic acid supplementation on oxidative stress markers in healthy women following a single bout of exercise, Journal of the International Society of Sports Nutrition 16(1) (2019) 2.

31 HANAN M. A. SHALABI, INAS H. REFAAT, ASMAA S. A. IBRAHIM AND AHMAD M.

32 ABDEL-MAGEED. THE ROLE OF FOLIC ACID AS A PROTECTIVE DRUG ON SOME OF

33 AUGMENTIN-INDUCED TOXIC EFFECTS IN MALE ALBINO RATS. International Journal of Biology, pharmacy and Allied Science 8(11) (2019), 2028.

34 A.M. Abdel-Mageed, A.K.E. Osman, N.S. Awad, M.A. Abdein, Evaluation of Antidiabetic Potentiality of Truffles and Balanites Aegyptiaca among Streptozotocin Induced Diabetic Rats, International Journal of Pharmaceutical Research & Allied Sciences 8(1) (2019).

35 O. Omodamiro, Hepatotoxicity Activity of Broad Spectrum Antibiotics (Amoxicillin and Ciprofloxa-cin) after Prolonged Administration in Wistar Albino Rats, SAJ Pharma Pharmacol 5 (2018) 205.

36 M. Esmaeilzadeh, E. Heidarian, M. Shaghaghi, H. Roshanmehr, M. Najafi, A. Moradi, A. Nouri, Gallic acid mitigates diclofenac-induced liver toxicity by modulating oxidative stress and suppressing IL-1β gene expression in male rats, Pharmaceutical Biology 58(1) (2020) 590-596.

37 R. Ahmadi-Naji, E. Heidarian, K. Ghatreh-Samani, Evaluation of the effects of the hydroalcoholic extract of Terminalia chebula fruits on diazinon-induced liver toxicity and oxidative stress in rats, Avicenna journal of phytomedicine 7(5) (2017) 454.

38 K. Oyedeji, A. Bolarinwa, S. Ojeniran, Effect of paracetamol (acetaminophen) on haematological and reproductive parameters in male albino rats, IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 4(6) (2013) 1-6.

39 N. Chalasani, H.L. Bonkovsky, R. Fontana, W. Lee, A. Stolz, J. Talwalkar, K.R. Reddy, P.B. Watkins, V. Navarro, H. Barnhart, Features and outcomes of 899 patients with drug-induced liver injury: the DILIN prospective study, Gastroenterology 148(7) (2015) 1340-1352. e7.

40 C. Lau, D. Marriott, M. Gould, D. Andresen, S.E. Reuter, J. Penm, A retrospective study to determine the cefepime-induced neurotoxicity threshold in hospitalized patients, Journal of Antimicrobial Chemotherapy 75(3) (2020) 718-725.

41 W.C. Rutter, J.N. Cox, C.A. Martin, D.R. Burgess, D.S. Burgess, Nephrotoxicity during vancomycin therapy in combination with piperacillin-tazobactam or cefepime, Antimicrobial agents and chemotherapy 61(2) (2017).

42 P.-F. Liao, Y.-K. Wu, K.-L. Huang, H.-Y. Chen, A rare case of cefepime-induced cholestatic liver injury, Tzu-Chi Medical Journal 31(2) (2019) 124.

43 N. Kaplowitz, Idiosyncratic drug hepatotoxicity, Nature reviews Drug discovery 4(6) (2005) 489-499.

44 S.O.A.B. Balubaid, Study the Adverse Effects of Antibiotic Maxipime (Cefepime Hydrochloride) on the Evolution of Composition of the Liver in Albino Rats

During Pregnancy and Lactation, Biosciences Biotechnology Research Asia 6(2) (2016) 455-464.

45 S.A. Alqahtani, D.E. Kleiner, M. Ghabril, J. Gu, J.H. Hoofnagle, D.C. Rockey, D.-I.L.I.N.D. Study, Identification and characterization of cefazolin-induced liver injury, Clinical Gastroenterology and Hepatology 13(7) (2015) 1328-1336. e2.

46 A. Ricart, Drug-induced liver injury in Oncology, Oxford University Press, 2017.

47 S. Kalender, A. Ogutcu, M. Uzunhisarcikli, F. Açikgoz, D. Durak, Y. Ulusoy, Y. Kalender, Diazinon-induced hepatotoxicity and protective effect of vitamin E on some biochemical indices and ultrastructural changes, Toxicology 211(3) (2005) 197-206.

48 O.T. Oyebode, O.R. Adebiyi, O.O. Olorunsogo, Toxicity of some broad-spectrum antibacterials in normal rat liver: the role of mitochondrial membrane permeability transition pore, Toxicology mechanisms and methods 29(2) (2019) 128-137.

49 P. Thangaraju, S. Varthya, M. Guruthalingam, S. Venkatesan, Cephalosporin's induced hepatic enzyme derangement-An educational report, Journal of Family Medicine and Primary Care 9(4) (2020) 2143-2143.

50 M. Abozenah, Drug-induced liver injury: case report, Reactions 90(11) (1752) 2019.

51 R. Mitchell, R. Cotran, Cellinjery, Adaptation and Death p. 6, Kumar V, Cotran RS, Robbins SL. Robin basic pathology. 7th edition. Saundersn of Elsevier science. USA (2003).

52 H. Fritzsche, U. Waehnert, J.B. Chaires, N. Dattagupta, F.B. Schlessinger, D.M. Crothers, Anthracycline antibiotics. Interaction with DNA and nucleosomes and inhibition of DNA synthesis, Biochemistry 26(7) (1987) 1996-2000.

53 I. Grattagliano, L. Bonfrate, C.V. Diogo, H.H. Wang, D.Q. Wang, P. Portincasa, Biochemical mechanisms in drug-induced liver injury: certainties and doubts, World journal of gastroenterology: WJG 15(39) (2009) 4865.

54 D.L. Eizirik, T. Mandrup-Poulsen, A choice of death–the signal-transduction of immune-mediated beta-cell apoptosis, Diabetologia 44(12) (2001) 2115-2133.

55 R. Falk, M. Hacham, A. Nyska, J.F. Foley, A.J. Domb, I. Polacheck, Induction of interleukin-1b, tumour necrosis factor-a and apoptosis in mouse organs by amphotericin B is neutralized by conjugation with arabinogalactan.

56 P. Taburee, D. Siripin, N. Wongjindanon, S. Fucharoen, D.I. Tanyong, Interleukin-1 beta and tumor necrosis factor-alpha induced apoptosis via caspase in leukemic K 562 and HL-60 cell lines, Songklanakarin Journal of Science & Technology 33(5) (2011).

57 H. El Btaouri, G. Rath, H. Morjani, C. Schneider, E. Petitfrere, F. Antonicelli, L. Martiny, Interleukin-1β-induced apoptosis through adenylyl cyclase and ERK1/2 inhibition in primary cultured thyroid cells, Biochemical and biophysical research communications 339(2) (2006) 469-476.

58 Y.H. Oo, S. Shetty, D.H. Adams, The role of chemokines in the recruitment of lymphocytes to the liver, Digestive Diseases 28(1) (2010) 31-44.

59 S. Shetty, P.F. Lalor, D.H. Adams, Lymphocyte recruitment to the liver: molecular insights into the pathogenesis of liver injury and hepatitis, Toxicology 254(3) (2008) 136-146.

60 I. Hassan, H. Ebaid, I.M. Alhazza, J. Al-Tamimi, S. Aman, A.M. Abdel-Mageed, Copper mediates anti-inflammatory and antifibrotic activity of gleevec in hepatocellular carcinoma-induced male rats, Canadian Journal of Gastroenterology and Hepatology 2019 (2019).

61 E. Wallace, L. Gewin, Imatinib: novel treatment of immune-mediated kidney injury, Journal of the American Society of Nephrology 24(5) (2013) 694-701.

62 A.M. Abdulrazzaq, M. Badr, O. Gammoh, A.A. Abu Khalil, B.Y. Ghanim, T.M. Alhussainy, N.A. Qinna, Hepatoprotective Actions of Ascorbic Acid, Alpha Lipoic Acid and Silymarin or Their Combination Against Acetaminophen-Induced Hepatotoxicity in Rats, Medicina 55(5) (2019) 181.

63 S. Sabiu, T.O. Sunmonu, E.O. Ajani, T.O. Ajiboye, Combined administration of silymarin and vitamin C stalls acetaminophen-mediated hepatic oxidative insults in Wistar rats, Revista Brasileira de Farmacognosia 25(1) (2015) 29-34.

64 S.M. Elshazly, M.A. El-Moselhy, W. Barakat, Insights in the mechanism underlying the protective effect of α-lipoic acid against acetaminophen-hepatotoxicity, European journal of pharmacology 726 (2014) 116-123.

65 B. CL, rose nr. Autoimmune thyroiditis and rOS, Autoimmun rev 7 (2008) 530-537.63- L.I. Filippin, R. Vercelino, N. Marroni, R.M. Xavier, Redox signalling and the inflammatory response in rheumatoid arthritis, Clinical & Experimental Immunology 152(3) (2008) 415-422.