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Original Article *Clinical Pathology*

Effect of cefepime on hematological, immunological and oxidant/antioxidant parameters in rats experimentally infected with *E. coli* ATCC 25922

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ABSTRACT

Objective: To evaluate the effect of cefepime on hematological changes, immunological disorders and hepatic oxidative damage in rats experimentally infected with *E.coli* ATCC 25922.
Design: Randomized controlled experimental study.
Animals: Thirty-two adult male albino rats weighting150-200 g.
Procedures: Rats used for this study were randomly assigned into 4 equal groups: the control one, *E.coli* infected group (1×10^8CFU/I/P/once), the cefepime treated group (45 mg/kg bw/I/M/day) for 5 days and the *E.coli* infected group that treated with cefepime 24h after bacterial inoculation as previously described. Hematological and immunological parameters, liver function biomarkers and hepatic oxidative stress and antioxidant markers were determined.
Results: Our result revealed that E.coli infection induced a significant elevation in the erythrocytes count, hemoglobin concentration, PCV% and total leukocytic count (TLC) ($P < 0.05$). In the same respect, liver function biomarkers, serum glucose, total cholesterol, and triglyceride levels as well hepatic malondialdehyde (MDA), nitric oxide (NO), TNF-α, IL-10, and lysozyme activity were significantly increased compared to the control rats ($P < 0.05$). In contrast, hepatic reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were decreased significantly ($P < 0.05$). Cefepime treatment in *E.coli* + CFPM group reduced the elevated eythrogram, TLC and liver function biomarkers. Cefepime also ameliorated the oxidative damage and inflammatory response induced by *E.coli* infection.
Conclusion and clinical relevance: Cefepime is safe when administered in a fixed-dose and possess antioxidant that contributes to improve efficacy against adverse effect induced by *E.coli* ATCC 25922 infection.

Keywords: Cefepime, E.coli ATCC 25922, Hepatic oxidative damage, Immunological parameters, Rats.

1. INTRODUCTION

Gram-negative bacteria have often been implicated in the pathogenesis of severe infections, which are important causes of death in critical cases [1]. *Escherichia coli* (*E. coli*) is one of the main species of bacteria that normal inhabitants lower intestines of warm-blooded animals, including birds and mammals [2]. There are many studies recorded physiological changes and host immune reactions occur during infection with *E.coli* such as inflammatory response initiation, and an increase in cellular permeability [3]. Many *Escherichia coli* isolates are sensitive to several antibiotics used for the treatment of their different complications, although other strains are becoming resistant to them, especially antibiotics that are broadly used [4].

Cefepime (CFPM) is a fourth-generation of semi-synthetic broad-spectrum cephalosporin one of a class of beta-lactam antibiotics that have a bactericidal action against most Gram-positive *Streptococcus pneumonia*, Gram-negative including *E.coli* and anaerobic bacteria but not against methicillin-resistant *Staphylococcus aureus*or*enterococcus* [5].

CFPM has been first approved by the FDA in 1997 to be used as first-line empiric therapy for serious infections, including lower respiratory tract infections, acquired and nosocomial pneumonia, complicated and uncomplicated urinary tract infections, skin and skin structure infections, bacterial otitis media, sepsis and bacterial septicemia[6, 7].Cefepime, like to other β-lactams, is a bactericidal agent that prevents bacterial cell wall biosynthesis by covalent attachment to penicillin-binding proteins and hinders the final transpeptidation phase of the peptidoglycan layer of bacterial cell walls synthesis[8]. Cefepime has a property to increase the antioxidant capacity of the body and provide

effective scavenging of free radicals that give rise to lipid peroxidation, impair cell membranes and induce oxidative damage of DNA[9]. Therefore, our study was designed to investigate the efficacy of treatment with cefepime on hematological disturbance, hepatic damage, immunological changes induced by *Escherichia coli* infection in rats.

2. MATERIALS AND METHODS

2.1. The Pharmaceutical Drug

Cefepime hydrochloride (Maxipime®-Vial) was purchased from Bristol-Meyers Squibb, USA

2.2. Bacterial strain

E.coli serotype ATCC 25922 was purchased from the Animal Health Research Institute, Cairo, Egypt.

2.3. Animal and experimental design

Thirty-two adult healthy male albino rats, weighing 150-200 g, were purchased from Zagazig laboratory animal unit and housed under controlled conditions (25 ± 1 °C). Standard rodent pellet diet and water were provided *ad libitum*. The rats were handled in accordance with animal welfare and the protocol approved by the Animal Ethical Committee of the Faculty of Veterinary Medicine, Mansoura University, Egypt. After two weeks of acclimatization, rats were divided into the following experimental groups; control group received distilled water orally, *E.coli* infected group (infected with *E.coli ATCC 25922*, 1×10^8 CFU / I/P/once) according to Sánchez et al. [10], cefepime treated group (CFPM) (intramuscularly injected with cefepime at a dose of 45 mg/kg bw/ day for 5 days) according to Elsayed et al. [11] and the *E.coli* infected group treated with cefepime 24 h after infection (*E.coli* +CFPM) as was previously described.

2.4. Blood and tissue samples collection

At the 7th day post-treatment, two blood samples were withdrawn from the medial canthus of the eye, the first sample in Eppendorf tubes with EDTA for hematological examination and the second samples were collected in clean test tubes for serum separation. Serum was separated and stored in Eppendorf tubes at – 20°C to be used for biochemical and immunological analysis. After that, animals were cervically dislocated then one gram of liver tissues was immediately dissected from each rat then was perfused with chilled 0.85% NaCl solution and homogenized at 9ml iced phosphate buffer saline (PH 7.5). The homogenate was cold centrifuged for 15 minutes at 825 Xg to get the supernatant that collected carefully and used directly or stored into Eppendorf tubes at – 80°C for further use [12].Specimens from the liver were fixed in 10% neutral buffered formaldehyde for histopathological studies.

2.5. Hematological parameters

Whole blood was used for the total erythrocytic (RBCs) and leukocytic counts (TLC). Hemoglobin concentration (Hb cont.) and packed cell volume (PCV) were determined then red blood cell indices were evaluated [13].

2.6.Serum biochemical analysis

The serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by using diagnostic kits obtained from (Colorimetric Randox, UK). Meanwhile, alkaline phosphatase (ALP) was estimated with commercial diagnostic kits (Teco diagnostics, USA). The bilirubin was estimated by Diamond kits (Egypt), while total protein and albumin were detected using Stanbio Laboratory (USA) kits.Glucose, cholesterol, and triglycerides were measured by using ready-made kits provided by Spinract (Spain). All the parameters were spectrophotometrically detected (5010 photometer, BM Co., Berlin, Germany) according to the enclosed pamphlets.

2.7. Measurement of hepatic oxidative stress and antioxidant markers

The hepatic malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) were detected spectrophotometrically by the enzymatic colorimetric method using Bio-diagnostic kits (Egypt), referring to the manufacturer's protocols.

2.8. Measurement of some serum immunological parameters

Serum lysozyme was determined by the turbidimetric assay[14]. The lysozyme substrate was 0.75 mg of *Micrococcus Lysodeikticus* Lyophilized Cells (Sigma-Aldrich) which was suspended in 1 ml of PBS, pH 5.8. In the round bottom, a microtitre plate 25 µl of serum was added to each well with 175 µl of substrate solution at 25°C. The reduction in absorbance at 450 nm was read after zero and twenty minutes using microtitre plate ELISA reader. The unite of lysozyme in serum in µg /ml was obtained from the lysozyme curve made by Lyophilized hen egg-white lysozyme (Sigma-Aldrich).

Two hundred microliter of serum or Hank's Balanced Salt Solution as control was added to duplicate wells of 96 round bottom well microtiter plate and incubated for 2.5 h at room temperature with 50 µL of suspension live a 24 h culture of *E.coli* $3X10^8$CFU. To each well, 25 µL diphenyltetrazolium bromide solution (MTT; 2 mg/ml) (Sigma) was added and incubated for thirty minutes at room temperature to allow the formation of formazan. Then the supernatant was discarded and the precipitate was dissolved in two hundred microliters of dimethyl sulfoxide (DMSO). The absorbance was read at 560 mm with microtitre plate ELISA readers and reported as absorbance units [15].

Estimation of serum nitric oxide

Nitric oxide (NO) was determined by a colorimetric assay spectrophotometrically (BM Co., Germany, 5010) by Bio-diagnostic kits [16].

Measurement of serum tumor necrosis factor-α (TNF-α) and interleukin-10 s (IL- 10)

TNF-α and IL-10 were estimated using Rat ELISA kit provided by Quantikine Company, using an automatic microplate ELISA reader (Human Diagnostic, Co., Germany)

according to the manufacturer's protocols. The values were expressed as pg/ml [17, 18].

2.9. Histopathological studies

Specimens from the hepatic tissue were fixed in 10% neutral buffered formalin then were embedded in paraffin. Section of 5-micron thickness was prepared and stained by hematoxylin & eosin (H &E) and examined microscopically [19].

2.10. Statistical Analysis

Results were expressed as the mean ± standard error and were analyzed were using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test, $P < 0.05$ was considered significant. All statistical analyses were performed using a statistical software program SPSS for Windows, version 23(IBM, Armonk, NY, USA) and Graphs were performed using GraphPad Prism version 5 (GraphPad Software Inc., La Jolla, CA, USA).

3. RESULTS

3.1. Hematological results

The RBC count, Hb concentration, PCV, and TLC were significantly increased in *E.coli* infected rats, compared to the control group. While MCV, MCH, and MCHC were insignificantly changed in between all groups ($P < 0.05$). The treatment with cefepime in the *E.coli* + CFPM group reduced the RBCs, Hb, PCV and TLC to their normal values. No noticeable differences were observed in the hematological picture in the CFPM group as compared to the control ($P < 0.05$) (Table 1).

3.2. Serum biochemical results

The ALT, AST and ALP serum activities as well the serum levels of total, direct, and indirect bilirubin were significantly raised in the *E.coli* infected rats in comparison with the control group ($P < 0.05$). On the other hand, all of the previous parameters were dramatically reduced upon the treatment with cefepime in the *E.coli* + CFPM group compared to the*E.coli* infected non-treated one ($P < 0.05$) (Table 2). No significant differences were recorded in the total protein, albumin, globulin, and A/G ratio in between all groups ($P < 0.05$) (Table 2).

Total cholesterol and triglyceride levels were significantly elevated in *E.coli* infected group comparing with the control meanwhile they were significantly reduced in the *E.coli* +CFPM compared to the *E.coli* infected group. The same direction was adopted by the glucose level in the serum (Table2).

3.3.Hepatic oxidative stress and antioxidant markers

As displayed in the Figure.1, experimentally *E.coli* infection in rats induced hepatic oxidative damage reflected by a significant elevation in the MDA level while SOD& CAT activities and GSH levels were significantly reduced compared with the control rats. These alterations were improved upon the treatment with cefepime in the *E.coli* +CFPM group. It was also noted that cefepime treatment in the CFPM group insignificantly altered the hepatic oxidative stress and antioxidant markers ($P < 0.05$).

Table 1. Hematological parameters in rat experimentally infected with *E. coli* ATCC 25922 and treated with cefepime.

Prameter	Treatment			
	Control	*E.coli*	CFPM	*E.coli*+CFPM
RBCs 0^6/µL	5.29±0.27^b	6.15±0.26^a	5.14±0.21^b	5.34±0.06^b
Hb g/dl	13.58±0.55^b	14.79±0.35^a	13.01±0.18^b	13.23±0.30^b
PCV %	46.20±0.37^b	50.40±0.87^a	46.60±0.50^b	46.40±1.02^b
MCV fl	89.44±4.86^a	82.48±3.73^a	91.03±3.08^a	86.80±2.08^a
MCH pg	21.30±2.13^a	17.77 ±0.9^a	21.3 ±1.12 ^{a}	17.70 ±0.74^a
MCHC%	29.05±1.26^a	29.86±0.60^a	27.96±0.66^a	28.54±0.54^a
TLC 10^3/µL	13.48±0.58^b	18.37±1.35^a	13.58±0.87^b	13.54±0.36^b

E.coli (infected with *E.coli*), CFPM (cefepime treatment 45 mg/kg bw/day), *E.coli* +CFPM (*E.coli* infected and treated with cefepime)
Data are expressed as Mean ± standard error of the mean (n=8). The different letters show significant difference between groups ($P<0.05$).
RBCs, Red blood cell count; *Hb*, Hemoglobin; *PCV*, Packed cell Volume;*MCV*,mean corpuscular volume;*MCH*, mean corpuscular hemoglobin;*MCHC*, mean corpuscular hemoglobin concentration;*TLC*, Total leukocytic count

3.4. Serum immunological results

As presented in Figure 2, the serum lysozyme activity and NO level were significantly increased in the *E.coli* infected group ($P < 0.05$). Following the treatment with cefepime in the *E.coli* +CFPM, the above-mentioned parameters significantly decreased compared with the *E.coli* non treated group.

The serum bactericidal activity was insignificantly altered between all tested groups. Meanwhile, the serum level TNF-α and IL-10 were significantly elevated in the *E.coli* infected group with respect to control one ($P < 0.05$). But, their levels were significantly decreased in the *E.coli* +CFPM treated group compared to the *E.coli* non treated one ($P < 0.05$) .

3.5 Histopathological results

E.coli infected group showed marked hepatocyte necrosis and intense lymphocytic infiltration in the hepatic lobules. Meanwhile, the *E.coli* +CFPM group showed mild lymphocytic infiltration in the hepatic tissue with normal hepatocytes. The hepatic sections of the CFPM group showed normal hepatocytes with normal histological architecture similar to the control rats (Figure 3).

Table 2. Serum biochemical parameters in rat experimentally infected with *E. coli ATCC 25922* and treated with cefepime.

Prameter	Treatment			
	Control	*E.coli*	CFPM	*E.coli* + CFPM
ALT U/L	25.08±0.72^{c}	38.54±0.91^{a}	25.94±1.15^{c}	29.00±1.10^{b}
AST U/L	±0.54 ^{c}60.19	73.81±1.38^{a}	60.92±0.43^{c}	63.45±0.29^{b}
ALP U/L	524.26±8.69^{c}	720.26±11.01^{a}	530.60±9.74^{c}	596.72±9.44^{b}
Total bilirubin (mg/dl)	0.48±0.00^{c}	0.58±0.00^{a}	0.50±0.01^{c}	0.53±0.00^{b}
Direct bilirubin (mg/dl)	0.30±0.01^{b}	0.41±0.02^{a}	0.32±0.00^{b}	0.38±0.02^{a}
Indirect bilirubin (mg/dl)	0.18±0.01^{a}	0.16±0.02^{a}	0.18±0.01^{a}	0.15±0.02^{a}
Total Protein g/dl	7.28±0.36^{a}	6.44±0.19^{a}	6.40±0.45^{a}	6.42±0.28^{a}
Albumin g/dl	3.01±0.08^{a}	3.04±0.11^{a}	3.06±0.04^{a}	2.95±0.19^{a}
Globulin g/dl	4.27±0.37^{a}	3.39±0.12^{a}	3.33±0.46^{a}	3.47±0.31^{a}
A/G ratio	0.73±0.07^{a}	0.90±0.03^{a}	0.98±0.11^{a}	0.88±0.12^{a}
Glucose mg/dl	73.40±2.31^{b}	89.23±3.43^{a}	76.30±1.85^{b}	75.05±2.47^{b}
Cholesterol mg/dl	60.31±186^{b}	68.80±0.86^{a}	60.80±1.82 ^{b}	65.20±0.58^{a}
Triglyceride mg/dl	138.60±3.50^{b}	179.20±5.64^{a}	132.20±3.08^{b}	139.20±1.59 ^{b}

E.coli (infected with *E.coli*), CFPM (cefepime treatment 45 mg/kg bw/day)), *E.coli* +CFPM (*E.coli* infected and treated with cefepime) Data are expressed as Mean ± standard error of the mean (n=8). The different letters show significant difference between groups (P<0.05).
ALT: Alanine aminotransferase, *AST*:Aspartate aminotransferase, *ALP:*Alkaline Phosphatase, *A/G ratio*: Albumin/Globulin ratio

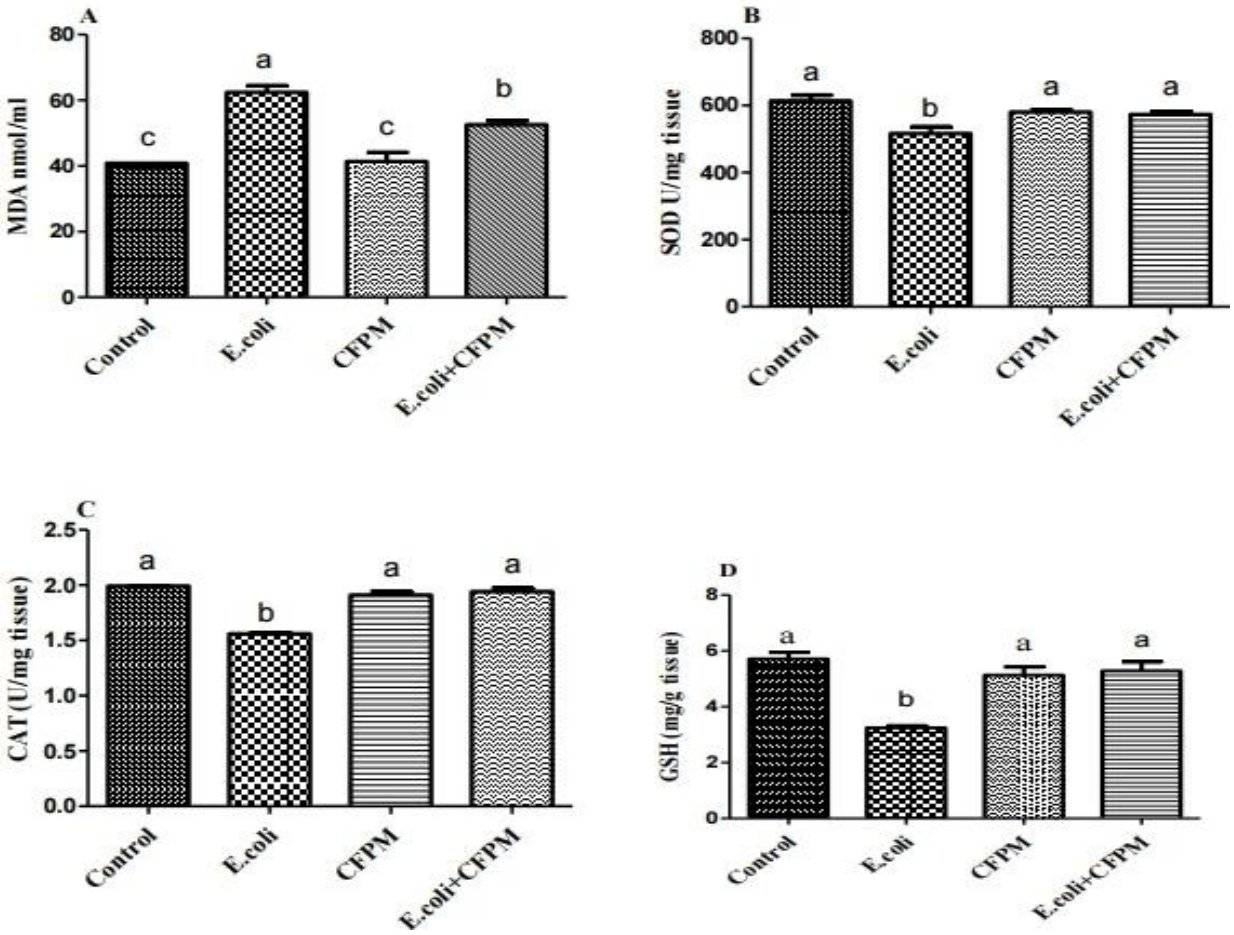

Figure 1. The hepatic (**A)** MDA level (**B)** SOD activity (**C)**CAT activity (**D)** GSH level in rat experimentally infected with *E. coli ATCC 25922* and treated with cefepime

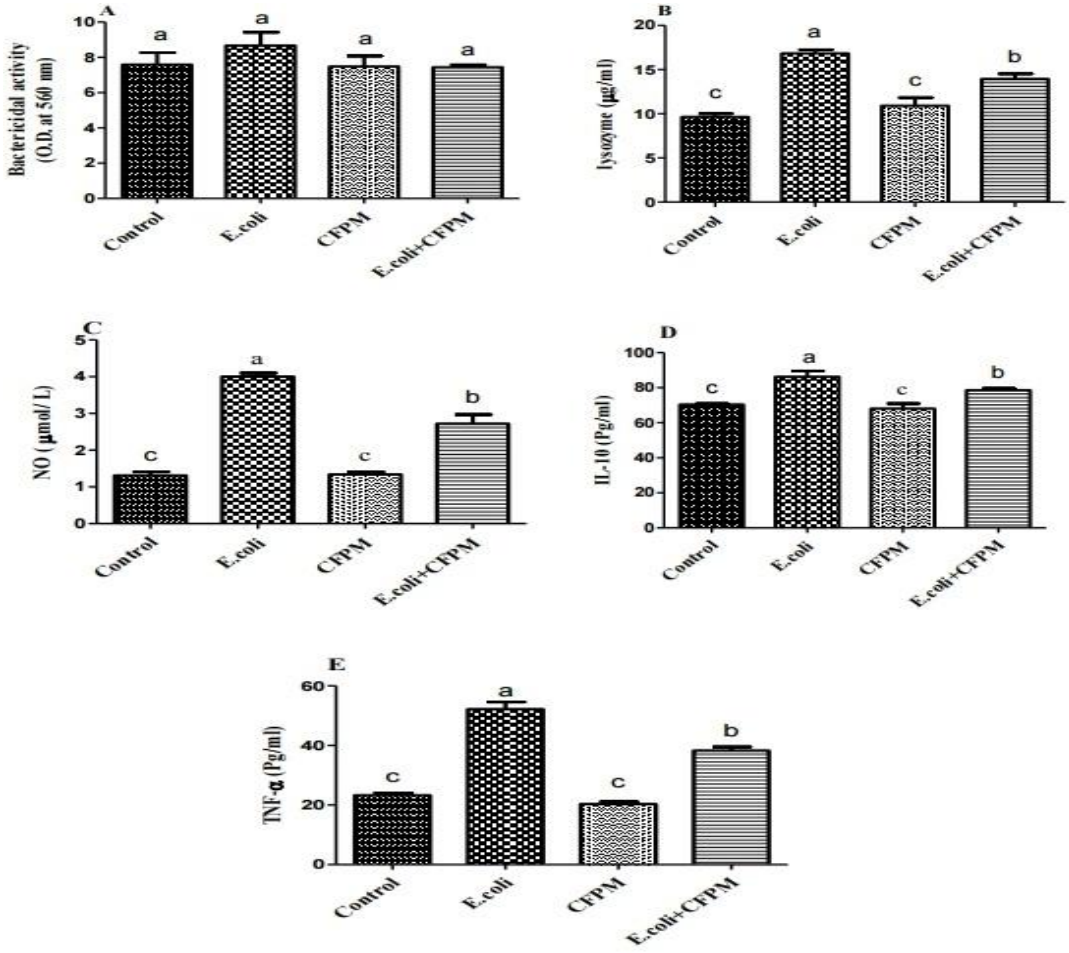

Figure 2. The serum (**A)** Bactericidal activity, **(B)** Lysozyme activity, (**C)** Nitric oxid level (**D)** IL-10 level (E) TNF-α level in rat experimentally infected with *E. coli* . *ATCC 25922* and treated with cefepime

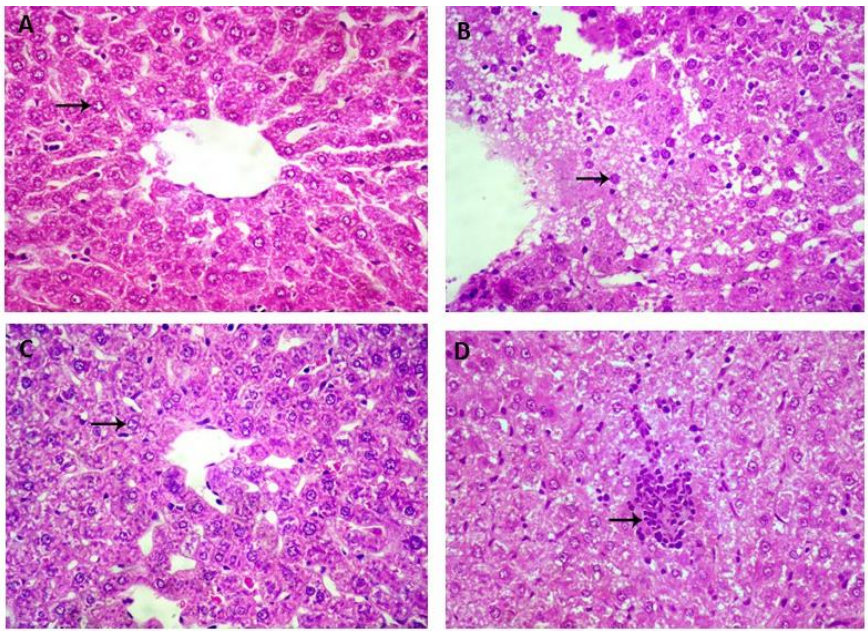

Figure 3. Hepatic micrograph (H&E, × 400) of the **A)** Control group shows normal hepatocytes (arrow) with normal hepatic architecture**. B)** The micrograph of *E.coli* rat shows necrosis of hepatic tissue and dysplastic alteration of hepatocytes (arrow). **C)** CFPM group shows normal hepatocytes (arrow) with normal hepatic architecture. **D)**The micrograph of *E.coli* +CFPM group shows histiocytic infiltrate in hepatic tissue (arrow).

4. DISCUSSION

E.coli has been shown to worldwide induce outbreaks of diarrhea, hemorrhagic colitis [20], pyelonephritis and its recurrent infections can be associated with fibrosis [21]. Cefepime is a newer semisynthetic, broad-spectrum fourth-generation oxyimino-cephalosporin agent being used progressively for treatment of infections caused by resistant gram-negative bacteria[22] as it has long elimination half-life, contribute in its potent protective properties [23].

The erythrogram result of this work revealed, that the *E.coli* infection led to a significant increase in RBCs count, Hb concentration and PCVcompared to control. This may be an indication of the occurrence of relative polycythemia, as a result of acute enteritis induced by *E.coli* colonization in the small intestine, resulting in severe diarrhea and extensive dehydration [24]. In the existing study, the treatment of rats with cefepime alone has an insignificant effect on the arthrogram result compared to control and these findings agreed with Abou-Samra [25]. On the other hand, the *E.coli* infected group treated with CFPM revealed improvement in the erythrogram results, which may be a good indication for its bactericidal activity, and this is agreed with Arhoumah et al. [26].

In our study, leukocytosis was detected in *E.coli* infected rats which gives a signal that infection has been established and there is the production of more white blood cells by the animal to fight *E. coli*[27]. Concerning the treatment with cefepime, there was an improvement in TLC induced by *E.coli* infection in comparison with *E.coli* non-treated rats. This is supported by Arhoumah et al. [26]who reported that the feverish rats infected with lipopolysaccharide from *E.coli* and treated with cefepime revealed a significant decrease in the TLC.

The biochemical analysis of liver function can provide some valuable data on the condition of the liver. The elevation in the serum activity of ALT and AST, in particular, is associated with hepatocellular degeneration and necrosis[28]. The hyperbilirubinemia can occur after bacteremia and endotoxemia is the reason for septic jaundice[29, 30]. The results of the present study indicated that *E.coli* infection resulted in hepatotoxicity detected by remarkable elevation of serum liver function biomarkers. This in accordance with Unger et al. [31]who reported that *E.coli* bacteremia caused a redistribution of hepatic microvascular blood flow within the liver lobule leads to perfusion of certain microvascular segments and decrease perfusion of others, which produced an elevation in liver enzymes. Moreover, *E.coli* infection also induced obstructive jaundice in the animal [32]. Our data can be confirmed by the histopathological results, which revealed necrosis of hepatic tissue and dysplastic alteration of hepatocytes.

Our data showed some improvement upon CFPM treatment in the *E.coli* + CFPM treated group that detected by the significant reduction in the elevated liver function biomarkers induced by *E.coli* infection nearly to the control levels. This may be due to the bactericidal effect of cefepime against infection with gram-negative bacteria [5] Additionally, cefepime documented to have free radical scavenging property which may contribute to reduce hepatotoxicity [9] as a consequence of a direct drug scavenging capacity towards HOCl [33]. The same results reported by Oter et al. [34] in Wistar rats injected by *E. coli* ($2.1x10^9$ CFU by I/P) and treated with CFPM.

In our work, no statically changed was detected in the serum TP, albumin and globulin levels as well as A/G ratio in all investigated groups. These results agree with Risha [35]who observed that serum level of TP, albumin and globulin levels insignificantly changed in Guinea pig I/P injection with *E.coli* ($1-2\times10^8$) compared with the control group. Additionally, Abou-Samra [25] found no noticeable alterations in TP and albumin in rats intramuscular treated with CFPM at different doses (50 and 100 mg/kg bw for 10 consecutive days) compared to the control group.

Bacterial endotoxin can have a bad impact on the cellular functions of the liver, which plays an important role in glucose homeostasis [34]. In our study, *E.coli* infected rats showed a significant increase in blood glucose levels all over the experiment compared to the control group, which may be attributed to severe hepatic dysfunction and the elevated ratio between corticosteroids and insulin-induced by *E.coli* that affect hepatic glucose metabolism and altered glucose homeostasis [36, 37].

In our work CFPM treatment in the CFPM group insignificantly changed the serum glucose level all over the experiment compared to control. Our results were in agreement with what was previously stated by Elsayed et al. [11]. Our data further suggest that treatment with CFPM in the *E.coli* +CFPM group improved the elevated serum level of glucose-induced by *E.coli* in rats. This may be due to the bactericidal effect of cefepime, which caused changes in the enzymatic activities in the metabolism of hepatic glucose and glucose homeostasis[38].

Our data indicated that total cholesterol and triglycerides were significantly increased in *E.coli* infected group. This is referred to metabolic dysregulation as *E.coli*infection accelerated free radicals release that enhances cellular cholesterol accumulation by increasing cholesterol biosynthesis and its esterification and decreasing its utilization by inhibition of cholesteryl ester hydrolysis and reducing cholesterol efflux, consequently caused hyperlipidemic effect[39]. In the existing study, cholesterol and triglyceride levels were significantly reduced upon administration of CFPM in *the E.coli* +CFPM group, which may be referred to the bactericidal effect of the antibiotics, which caused amelioration in lipid metabolism. These data agree with Ozbudak et al.[40]who mentioned that I/M injection of cefepime significantly reduced the elevated total serum cholesterol caused by *Pseudomonas aeruginosa* strain *ATCC 1942*.

Malondialdehyde (MDA) is the most important component among reactive aldehydes originating from lipid peroxidation. Consequently, it is commonly considered as an

index for oxidative stress severity [41]. In this work, *E.coli* induced an elevation in the hepatic MDA level that was related to excessive free radicals production, mediated oxidative stress and lipid peroxidation as well as cellular toxicity [42]. In contrast, our data showed that hepatic activities of SOD and CAT enzymes, as well as hepatic GSH, were significantly reduced in *E.coli* infected group. This could have occurred a consequence of interference with hepatic intracellular oxidant/antioxidant balance and accumulation of reactive oxygen species (ROS)upon *E.coli* infection [43].

The treatment with CFPM alone insignificantly affected the hepatic MDA level, SOD, CAT activities and GSH level, although treatment with cefepime in *E.coli* +CFPM group induced a significant reduction in the hepatic MDA, whilst hepatic SOD, CAT, and GSH were significantly increased PT. According to Soejima et al.[44] cephalosporins as cefepime are thioether had free radical scavenging potential and effective in preventing the free radical-mediated oxidation of sulfhydryl group resulted in reduce hepatic oxidative damage.

Lysozyme is an enzyme lytic the cell walls of certain bacteria, it is a protein in nature existing in the body fluids, cells and tissue of many living organisms where it appears to have a digestive and defense function[45]. The serum bactericidal activity is an important host defense mechanism, which plays a role in preventing the initiation of *E. coli* infection [46]. In our study, *E.coli* resulted in significantly increased the serum lysozyme activity, as the result of increased lysozyme gene transcription [47, 48] or increased lysozyme release during infection from the lysosomal compartment because of phagocytosis-induced degranulation[49].

Nitric oxide is a short-lived, highly reactive free radical, synthesized from L-arginine by the enzyme nitric oxide synthase[50]. Herein, the serum NO level was remarkably elevated in *E.coli* infected group. This could be explained by *E.coli* infection increases the expression of nuclear factor kappa B (NF-κB) which led to over-expression of inflammatory mediators such as cytokines and inducible NO synthase (iNOs)which is responsible for the excessive formation of NO [51]. Furthermore, the increase of TNF-α led to increasing NO which cause tissue damage by inducing iNOs [52]. the treatment by CFPM in E.coli +CFPM group associated with a significant reduction in the NO level that may be correlated with the ability of CFPM to inhibit TNF-α induced by E.coli endotoxin consequently led to decrease NO[53].

Macrophages in response to many Gram-positive and Gram-negative bacteria produce TNF-α which is important for fighting bacterial infection. Also, IL-10 that is produced by macrophages and T-cells is a potent inhibitor of macrophage-derived inflammatory cytokines synthesis [54]. As pathogen activates the innate immune response through activation of the NF-κB signaling pathways which is essential to the generations of inflammatory cytokines as TNF-αand interleukins which played a crucial role in fighting infection [55].In the current study, *E.coli* infected group showed a significant increase in serum level of TNF-α and IL-10. That may be also attributed to the essential role of TNF-α in the pathogenesis of liver damage caused by *E. Coli* derived endotoxin, subsequently increased IL-10 production as a direct protective effect against *E.coli* induced liver injury to suppress the host defense [56]. Furthermore, Xie et al. [57] reported a positive correlation with Gram -ve bacterial infection and increased IL-10 in serum, thus, IL-10 could serve as potential biomarkers for distinguishing Gram+ve infection from Gram -ve one.

Our data further suggested that treatment with CFPM improved the changes in the TNF-α and IL-10 levels induced by *E.coli* infection. This is in parallel with Arhoumah [58] who revealed that CFPM at dose 45 mg/kg bw significantly ameliorated the alterations in TNF-α and IL-10 levels induced by I/P injection with *E. coli* LPS which attributed to the immunomodulatory effect of CFPM.

Conclusion

Overall our results, using of broad-spectrum antibiotics as cefepime in a fixed-dose once daily for 5 consecutive days effective, safe and possess antioxidant , free radical scavenging potential and reduce inflammatory response that contribute in improving its efficacy against adverse effect induced *by E.coli* infection. Therefore, our results suggest that cefepime is a good choice for the treatment of *E.coli* infection, but still further studies are needed for evaluating the impact of higher doses of cefepime.

Acknowledgment

Conflict of interest

There is no conflict of interest in the current research work.

Reasearch Ethics Committee Permission

The study was approved by the Research Ethical Committee of the Faculty of Veterinary Medicine, Mansoura.University.

Authors´ contribution

All authors contributed in planning the research, data analysis and manuscript writing and review of the final manuscript. Huda Elbaz was responsible for conducted the experimental study and all laboratory measurements. All authors approved the final version of the manuscript.

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