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RESEARCH ARTICLE

Anti-inflammatory, anti-oxidant and hepatoprotective effects of lactoferrin in rats

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ABSTRACT

Carbon tetrachloride (CCl_4) is a strong hepatotoxic agent. The ability of the anti-inflammatory agent, lactoferrin (LF), to alleviate hepatic inflammation in a Wistar rat model administered with carbon tetrachloride (CCl_4) was examined. Thirty male Wistar rats were segregated into 5 groups (6 rats per group): Control group, LF group (300 mg LF/kg b. wt daily for three weeks), CCl_4 group (1 ml CCl_4/kg b. wt once orally), LF-protected group (300 mg LF/kg b. wt daily for 3 weeks followed by 1 mL CCl_4/kg b. wt once orally), and LF-treated group (1 mL CCl_4/kg b.wt once orally followed by 300 mg LF/kg b. wt orally every day for three weeks). Erythrogram, leukogram, activity of oxidative stress markers (Superoxide dismutase [SOD], Glutathione peroxidase [GPx], and Malondialdehyde [MDA]), and expression of hepatic paraoxonase-1 (PON1), interleukin (IL)-1β, and IL-10 mRNA were determined. Histopathological examination of the hepatic tissue was carried out. CCl_4 caused liver injury, loss of liver antioxidant activity of SOD and GPx, and a significant increase in the level of malondialdehyde in the serum. Moreover, CCl_4 induced up-regulation of hepatic pro-inflammatory (IL-1β) factors, and down-regulation of anti-inflammatory (IL-10 and PON1) factors. Based on histopathological examination, the hepatic tissues had severe inflammation and were damaged. However, LF mitigated the liver damage, oxidative stress, and hepatotoxicity caused by CCl_4. Overall, these results suggest that LF-mediated immunological mechanisms alleviate CCl4-induced hepatic toxicity and provide a novel perspective on the potential use of LF for prophylactic and therapeutic applications in treating liver diseases.

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Introduction

Liver is a multifunctional and highly regenerative organ. It performs detoxification of exogenous xenobiotics, drugs, viral infections, and alcohol (Guerra *et al.* 2016). Despite its high recovery capability, prolonged liver damage leads to serious inflammation resulting in fibrosis, followed by cirrhosis and ultimately liver failure. In both the chronic and acute conditions, liver damage has serious outcomes (Szkolnicka *et al.* 2016).

Carbon tetrachloride (CCl_4) is a strong hepatotoxic agent, which has been broadly utilized in animal models to induce liver injury that is comparable with human hepatic toxicity and causes the aggravation of many inflammatory processes along with the recruitment of inflammatory cells. The mechanism of CCl_4 toxicity involves the excessive production of reactive oxygen species (ROS). In the liver, the increased ROS may cause hepatic damage or fibrosis (Perazzoli *et al.* 2017). Although the mechanism underlying the pathogenesis of liver fibrosis is known, there is currently no established pharmacological treatment for this disorder (Wynn and Ramalingam 2012). Therefore, it is important to thoroughly analyze the pathologic mechanism of liver fibrosis to identify new targets for antifibrotic therapies.

Lactoferrin (LF), an element of the transferrin family, is an iron-binding glycoprotein present in exocrine secretion and serum. LF is produced by the glandular cells, and is present in the saliva, milk, tears, and mucous secretions. It is also found in neutrophils that are released in the blood, and the inflamed infected tissues (Farnaud and Evans 2003). LF plays a major role in immune responses and defends against various infections. It has antimicrobial activities against bacterial and parasitic infections. It also has an anticancer activity (Farnaud and Evans 2003, Actor *et al.* 2009). Moreover, LF has different physiological properties and bioactivities, for example, it is anti-oxidative (Kanyshkova *et al.* 2001, Legrand *et al.* 2005). These activities rely not only on its capacity to bind iron, but also to interact with molecular and cellular components of both the host and pathogens (Latorre *et al.* 2010).

The use of LF in the treatment of hepatitis C virus infections (Berlutti *et al.* 2011), osteoporosis (Bharadwaj *et al.* 2009), and cancer (Gibbons *et al.* 2010) has been reported.

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The main objective of this study was to investigate the possible protective or therapeutic effects of LF against CCl_4-induced hepatitis in the male rat.

Materials and methods

Experimental animals

Eight-week-old Male Wistar rats (120–150 g) were purchased from the Experimental Animal Unit, Faculty of Veterinary Medicine, Benha University, Egypt. Animals were kept in cages and given a standard diet and clean water *ad libitum*. The environment was adjusted. All protocols for animal experiments were approved by the institutional review board of Benha University.

Chemicals

CCl_4 (99.8%) (NICE Co., India) was given once orally to each model animal at a dose of 1 mL/kg b. wt. Lactoferrin was purchased from Jarrow Formulas (Superior Nutrition and Formulation, Los Angeles) and administered orally at a dose of 100 mg/kg b. wt once in 3 days for 3 weeks.

Preparation of the liver homogenate

Liver tissue (1 g) was collected from each rat belonging to each group at the end of the first and second week of the experiment. The hepatic tissue was washed in ice-cold normal saline and homogenized in an ice-cold solution of 1.15% potassium chloride in a 50 mmol potassium phosphate buffer solution (pH 7.4), to obtain a 10% liver homogenate [w/v; weight of liver tissue (g) per volume of the buffer (mL)]. The tissue was homogenized using a sonicator (4710 Ultrasonics Homogenizer, Cole-Parmer Instrument Co., USA). It was then centrifuged at 4000 rpm for 5 min at 4 °C. The obtained supernatant was used to determine the activity of glutathione peroxidase (GPx) and superoxide dismutase (SOD), as well as the concentrations of the by-products, obtained post lipid peroxidation.

Assay methods

Hematological parameters measurement

Hematological variables including erythrogram and leukogram were determined using an electronic particle hematology analyzer (HA-22/Vet Hematology Analyzer, Clindiag, Belgium).

Determination of total SOD activity

The total SOD activity in the hepatic homogenate was determined according to Misra and Fridovich (1972). The enzyme activity was determined by its ability to prevent the autoxidation of epinephrine in an alkaline medium. The total SOD activity was expressed in unit/mg of protein.

Determination of GPx

The activity of GPx in the homogenate was determined according to Paglia and Valentine (1967).

Determination of the by-products of lipid peroxidation

The by-products of lipid peroxidation were estimated in accordance with the protocol described by Ohkawa *et al.* (1979), based on the reaction between thiobarbituric acid and malondialdehyde in an acidic medium at 95 °C for 45 min. Absorbance was determined at 535 nm. Concentrations of the by-products of lipid peroxidation were expressed as nmol/mg of protein.

Determination of total protein in liver homogenate

A colorimetric method was used to determine the total proteins according to Titz (1995).

The mRNA expression analysis of hepatic PON1, IL-1, and IL-10 genes using real time-PCR

For further understanding, the effect of LF administration on hepatic inflammation, the expression of hepatic PON1 and various cytokines was analyzed using real time-PCR using previously described sense and anti-sense primers (Farid *et al.* 2010). The following primers sets were used: PON1 (GenBank ID: NM_032077.1), sense (5′-AAG TAT GTC TAT ATC GCT GAA TTG C-3′) and antisense (5′-CAC AGG ATC CAC AGA GAT GTT ATC-3′); IL-1β (GenBank ID: M98820.1), sense (5′-CAC CTC TCA AGC AGA GCA CAG-3′) and antisense (5′-GGG TTC CAT GGT GAA GTC AAC-3′); IL-6 (NM_012589.2), sense (5′-TCC TAC CCC AAC TTC CAA TGC TC-3′) and antisense (5′-TTG GAT GGT CTT GGT CCT TAG CC-3′); and 18S rRNA (GenBank ID: NR_046237.1) housekeeping gene, sense (5′-GAG GTG AAA TTC TTG GAC CGG-3′) and antisense (5′-CGA ACC TCC GAC TTT CGT TCT-3′).

Fluorescence detection and thermal cycling were performed using a 7300 real-time-PCR system (Applied Biosystems, Foster City, CA).

Change in the expression of the genes was calculated from the obtained cycle threshold values (C_t) provided by the real-time PCR using the comparative CT method using the housekeeping gene, 18S rRNA, as a reference (Schmittgen and Livak 2008).

Histopathological examination

The liver and spleen tissue of rats from all groups were taken and immersed in neutral buffered formalin (10%) for fixation. It was then washed under tap water followed by washing with serial dilutions of alcohol (methyl, ethyl, and absolute ethyl alcohol) for dehydration. Xylene was used to clear the specimens, which were then embedded in paraffin and incubated in a hot air oven at 56 °C for 24 h. Blocks were used to prepare 4 μm sections of samples with the help of a sledge microtome. The prepared tissue sections were placed on glass slides, deparaffinized, and stained with hematoxylin and

eosin (Bancroft and Cook 1994) for histopathological examination under a light microscopic.

Experimental design

Thirty male Wistar rats were randomly segregated into 5 groups (6 animals per group). To the first group (control) and the second group, 300 mg/kg b. wt of LF was administered every day for 3 weeks. The third group was administered with CCl_4 (1 mL/kg b. wt) once, orally. The fourth group was administered with LF (300 mg/kg b. wt) daily for three weeks and CCl_4 (1 mL/kg b. wt) once orally at the end of 3 weeks. The fifth group was administered with CCl_4 (1 mL/kg b. wt) once orally followed by LF (300 mg/kg b. wt) daily for three weeks. Clinical examinations were recorded for all experimental groups during the period of the experiment. Blood was collected from the retro-orbital venous plexus and liver specimens from all the six rats from each group 24 h after CCl_4 administration for hematological examination, testing oxidative parameters, gene expression, and studying the histopathological changes.

Statistical analysis

Statistical analysis was performed using the statistical software package SPSS for Windows (Version 16.0; SPSS Inc., Chicago, IL). The significance of difference between more than two groups was evaluated using ANOVA. If one-way ANOVA showed a significant difference between two groups, the difference was estimated using the Duncan test. Results were examined as the mean ± standard error of mean (SEM). A p value of less than 0.05 was considered significant (Kinnear and Gray 2006).

Results

No mortality was observed in any of the experimental groups during the period of the experiment.

The mean and standard error values of the blood parameters obtained from various groups are mentioned in Table 1. A comparison between the CCl_4 group and the control group, showed a significant decrease ($p < 0.05$) in the RBC count, volume of RBCs, and hemoglobin content. It was also accompanied by normal MCV and MCHC resulting in a state of normocytic normochromic anemia. When LF, LF-protected, and LF-treated groups were compared to the control group, a non-significant change in the hematological parameters was observed. With respect to the LF and LF-protected groups, there was a significant increase ($p < 0.05$) in the RBC count, the volume of RBCs, and hemoglobin content when compared with the CCl_4 group. The LF-treated group showed a non-significant change in the volume of RBCs and hemoglobin content in comparison with the CCL_4 group.

With respect to the leukogram, a significant decrease ($p < 0.05$) in the total leukocyte, monocyte, and lymphocyte counts was reported in the CCl_4 and LF-treated groups when compared with the control group. However, there was no significant change in the total leukocyte, monocyte, and lymphocyte counts in the LF group when compared with the control group. A comparison between the LF-protected group and the control group showed a significant increase ($p < 0.05$) in the total leukocyte and lymphocyte counts, and a normal monocyte count (Table 2).

A comparison between the LF group and CCl_4 group, showed a significant increase ($p < 0.05$) in the total leukocytic, lymphocyte, and monocyte count. The LF-protected group showed a significant increase ($p < 0.05$) in the total leukocyte and lymphocyte count in comparison with the CCl_4 group. However, a non-significant change in the total leukocyte, lymphocyte, and monocyte count was observed in the LF-treated group compared to the CCL_4 group (Table 2).

A significant down-regulation ($p < 0.05$) in PON1 synthesis was observed when the CCl_4 group was compared with the control group. However, a non-significant change in the synthesis of PON1 was observed in the LF, LF-protected, and LF-treated groups when compared with the control group. Moreover, there was no significant change in the synthesis of PON1 in the LF, LF-protected, and LF-treated groups when compared with the CCl_4 group. The LF-protected group showed a significant over-expression of PON1 compared to the CCl_4 group (Table 3).

Table 1. Hemogram in Control, CCL_4, Lactoferrin, L + CCL_4, and CCL_4 + L groups (Mean ± SE).

Parameters	Control	CCL_4	LF	LF + CCL_4	CCL_4 + LF
RBCs (10^9/L)	8.29 ± 0.12^{b}	7.34 ± 0.38^{a}	8.24 ± 0.33^{b}	8.41 ± 0.09^{b}	$8.05 \pm 0.16^{a,b}$
PCV%	$45.96 \pm 0.43^{b,c}$	43.86 ± 0.19^{a}	$45.50 \pm 0.27^{b,c}$	45.93 ± 0.33^{c}	$44.86 \pm 0.14^{a,b}$
Hb (g/L)	16.66 ± 0.21^{c}	15.03 ± 0.47^{a}	$16.43 \pm 0.40^{b,c}$	17.20 ± 0.05^{c}	15.70 ± 0.20^{b}
MCV (fL)	55.45 ± 1.30^{a}	$60.1l \pm 1.30^{a}$	55.40 ± 1.90^{a}	54.61 ± 0.92^{a}	55.78 ± 1.20^{a}
MCHC (g/L)	$36.26 \pm 0.13^{a,b}$	$34.37 \pm 0.98^{a,b}$	$36.10 \pm 0.68^{a,b}$	37.44 ± 0.16^{b}	32.41 ± 0.19^{a}

Means with different superscripts (a,b,c) within the same row were significantly different ($p < 0.05$).

Table 2. Leukogram in Control, CCL_4, Lactoferrin, L + CCL_4, and CCL_4 + L groups (Mean ± SE).

Parameters	Control	CCL_4	LF	LF + CCL_4	CCL_4 + LF
WBCs (10^9/L)	9.50 ± 0.20^{b}	6.73 ± 0.46^{a}	10.4 ± 0.18^{b}	11.56 ± 0.46^{c}	6.90 ± 0.20^{a}
Granulocytes (10^9/L)	0.87 ± 0.05^{a}	0.83 ± 0.02^{a}	1.49 ± 0.18^{b}	1.12 ± 0.06^{a}	0.88 ± 0.05^{a}
Monocyte (10^9/L)	1.35 ± 0.21^{b}	0.67 ± 0.16^{a}	$1.2 \pm 0.18^{a,b}$	$1.11 \pm 0.14^{a,b}$	0.68 ± 0.06^{a}
Lymphocyte (10^9/L)	7.37 ± 0.16^{b}	5.21 ± 0.16^{a}	7.89 ± 0.24^{b}	9.63 ± 0.48^{c}	5.05 ± 0.06^{a}

Means with different superscripts (a,b,c) within the same row were significantly different ($p < 0.05$).

Table 3. mRNA expression of hepatic PON1, IL-1β, and IL-10 genes.

Groups	PON1	IL-1β	IL-10
Control	0.97 ± 0.06^{a}	1.07 ± 0.15^{b}	1.00 ± 0.08^{c}
CCl_4	0.02 ± 0.01^{b}	3.52 ± 0.56^{a}	0.15 ± 0.07^{a}
LF	$0.63 \pm 0.51^{a,b}$	$1.79 \pm 0.88^{a,b}$	1.16 ± 0.04^{c}
LF + CCl_4	0.47 ± 0.04^{a}	$1.53 \pm 0.36^{a,b}$	0.50 ± 0.16^{b}
CCl_4 + LF	$0.28 \pm 0.14^{a,b}$	$2.23 \pm 0.59^{a,b}$	$0.36 \pm 0.09^{a,b}$

Total RNA was prepared from hepatic tissues of different experimental groups. The expression levels were evaluated by real-time PCR (Mean ± SE). Means with different superscripts (a,b,c) within the same column were significantly different ($p < 0.05$).

With respect to the pro-inflammatory cytokine, IL-1β, a significant increase ($p < 0.05$) in the synthesis of IL-1β in the CCl_4 group was observed compared to the control group. However, a non-significant change was observed in the LF, LF-protected, and LF-treated groups in comparison with the control group. There was a significant reduction ($p < 0.05$) in the synthesis of IL-1β in the LF, LF-protected, and LF-treated groups in comparison with the CCl_4 group. There was a non-significant change in the synthesis of IL-1β in the LF-treated group when compared with the CCl_4 group (Table 3).

With respect to the anti-inflammatory cytokine, IL-10, our results reported a significant decrease ($p < 0.05$) in the synthesis of IL-10 in the CCl_4, LF-protected, and LF-treated groups in comparison with the control group. However, a non-significant change in IL-10 was observed in the LF group when compared with the control group. Moreover, there was a significant increase ($p < 0.05$) in the synthesis of IL-10 in the LF and LF-protected groups when compared with the CCl_4 group. There was a non-significant change in IL-10 in the LF-treated group when compared with the CCl_4 group (Table 3).

Analysis of antioxidants revealed that a significant ($p < 0.05$) reduction in the level of SOD and GPx, and a non-significant increase in the level of MDA was observed in the CCl_4 group when compared with the control group. The LF group showed a significant increase in the level of SOD, and a non-significant difference in the level of GPx and MDA when compared to the control group. Furthermore, the level of SOD increased significantly ($p < 0.05$) in the LF-protected group in comparison with the CCl_4 group. The level of GPx differed non-significantly in the LF-protected group compared to the CCl_4 group. However, the level of MDA decreased significantly in LF-protected group when compared to the CCl_4 group. The LF-protected group showed a non-significant difference in the level of SOD, GPx, and MDA in comparison with the control group. In the LF-treated group, there was a significant decline in the level of SOD, and a non-significant change in the level of GPx and MDA when compared to the control group. Moreover, the level of GPx and SOD showed a non-significant difference in the LF-treated group in comparison with the CCl_4 group. However, the level of MDA significantly decreased in the LF-treated group when compared to the CCl_4 group (Figure 1).

With respect to the histopathological examination of the hepatic tissue, the CCl_4 group showed a diffused ballooning degeneration of the hepatocytes of the parenchyma and it was associated with congestion in the portal vein (Figure 2(C)). However, in the LF-protected group, there was

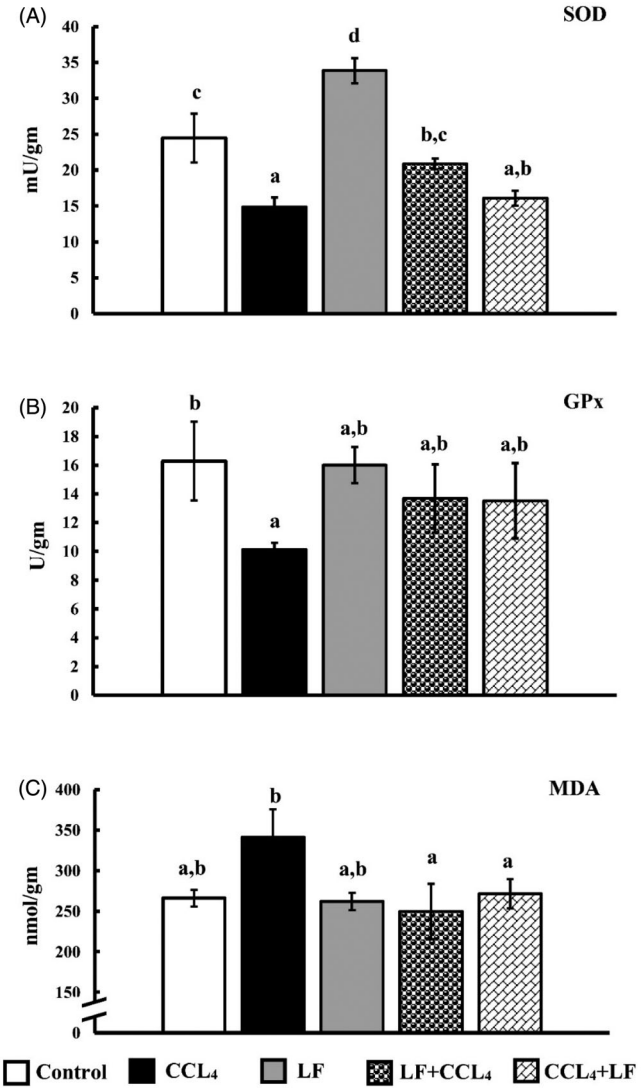

Figure 1. Antioxidant markers in Control, CCl_4, Lactoferrin, L + CCL_4, and CCl_4 + L groups (Mean ± SE). Means with different superscripts (a,b,c,d) were significantly different ($p < 0.05$).

no histological alteration as mentioned in Figure 2(D). Moreover, in the LF-treated group, the hepatocytes showed a ballooning degeneration of the parenchyma at a lower degree than the CCl_4 group (Figure 2(E)). The parenchyma of the control (Figure 2(A)) and LF group (Figure 2(B)) had a normal histological structure of the central vein, and surrounding hepatocytes, with no histopathological changes. It was found that the white pulps of the spleen belonging to the rat that received CCl_4 showed lymphoid cells depletion associated with congestion in the red one and blood vessels (Figure 3(C)), while the LF-protected group showed no histological alterations (Figure 3(D)). No histological alterations were observed in the control group (Figure 3(A)), LF group (Figure 3(B)), and LF-treated group (Figure 3(E)).

Discussion

Liver is the main target organ for chemicals and drugs. Therefore, hepatotoxicity is an important endpoint in the assessment of the effect(s) of xenobiotics (Malaguarnera *et al.*

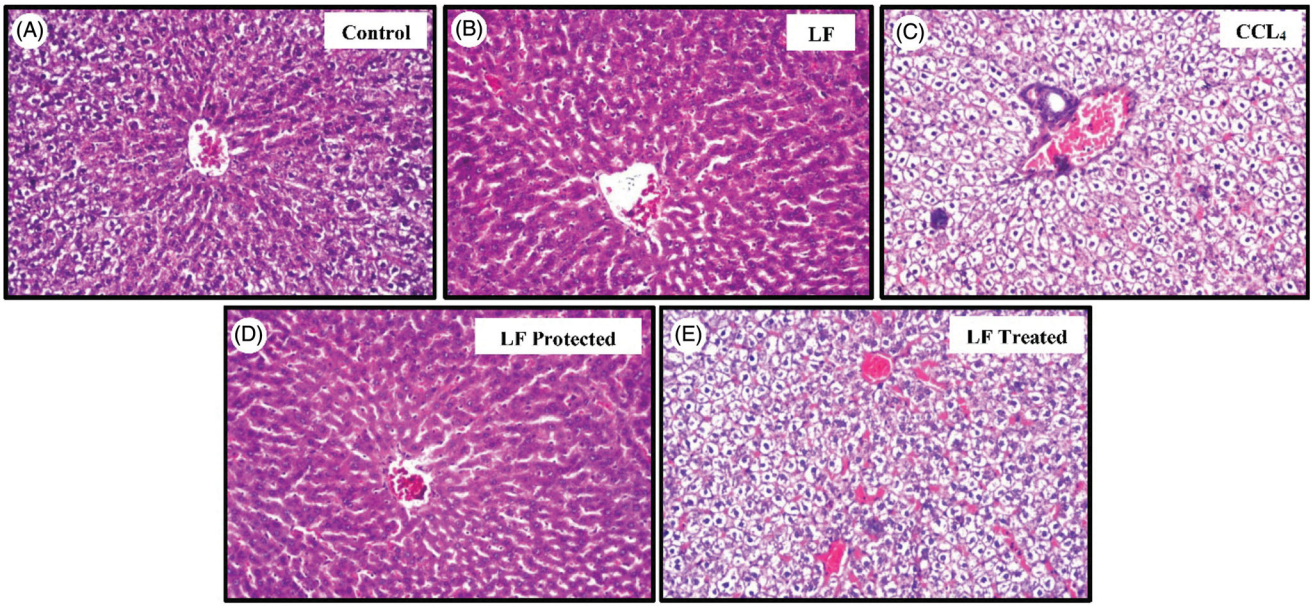

Figure 2. Histopathological examination of rat liver sections of different experimental groups. Liver sections of rat tissue were deparaffinized, stained with hematoxylin-eosin (HE), and examined under light microscopy. There were no histopathological alterations with normal histological structure of the central vein and surrounding hepatocytes in the parenchyma in control (A) and lactoferrin group (B). The examined sections of liver in CCL4 group showed diffuse ballooning degeneration all over the hepatocytes of the parenchyma associated with congestion in the portal vein (C). While, LF-protected group showed no histological alterations (D). Moreover, in the LF-treated group, the hepatocytes showed mild ballooning degeneration the parenchyma (E). 40× magnification.

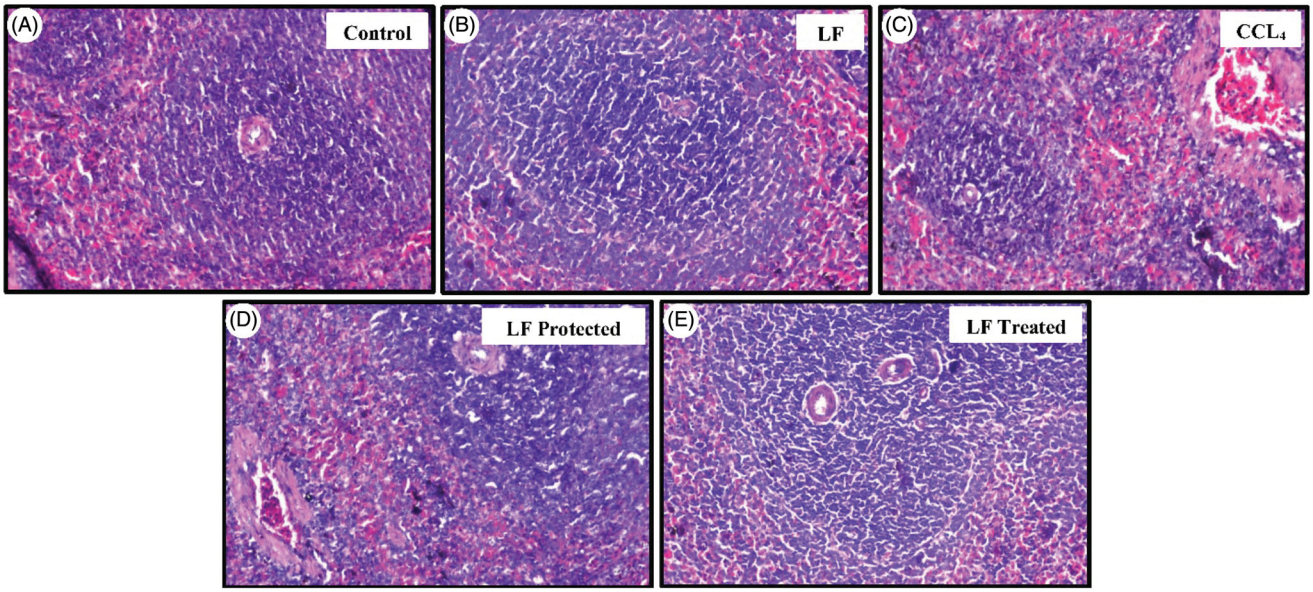

Figure 3. Histopathological examination of rat spleen sections of different experimental groups. Spleen sections of rat tissue were deparaffinized, stained with hematoxylin-eosin (HE), and examined under light microscopy. No histological alteration was recorded in the control group (A), lactoferrin group (B) and LF-protected or treated group (D,E), while CCL_4 given group showed lymphoid cells depletion in the white pulps associated with congestion in the red one and blood vessels (C). 40× magnification.

2012). Hepatotoxicity has previously been associated with oxidative stress and redox imbalance (McGill *et al.* 2012). CCl_4 is a well-known liver toxicant. CCl_4-induced hepatotoxicity is the most commonly used model to screen the hepatoprotective activity of many compounds including plant extracts (Knockaert *et al.* 2012). There is growing evidence of the role of oxidative stress in CCl_4-induced hepatotoxicity (Hafez *et al.* 2014). LF is a member of the transferrin family. It binds strongly with two ferric ions. It is found in exocrine secretions, such as tears and colostrum. Moreover, it is also found in the secondary granules of neutrophils (El-Loly and Mahfouz 2011). Among many biologically beneficial effects of LF, its anti-inflammatory and antioxidant effects have been reported (Håversen *et al.* 2002, Ward *et al.* 2005).

In this study, CCL_4 administration induced a marked decrease in the RBC count, hemoglobin concentration, and PCV without a significant difference in MCV and normocytic normochromic anemia (MCHC). This anemia might be due to the hepatic injury induced by CCl_4 and reduction in the RBC formation rate (Essawy *et al.* 2010). These results match with the results observed by Mandal *et al.* (1998) and Amer *et al.* (2015). On the contrary, LF alone induced non-significant

changes in the number of RBCs. Moreover, the LF-protected group showed alleviated effects of CCl_4 demonstrated by a significant increase in the RBC count and related parameters (hemoglobin and PCV). These observations may be due to the intake of LF, as it increases the absorption and utilization of iron (Koikawa *et al.* 2008). Iron-loaded lactoferrin can bind to the surface receptors of macrophages and deliver its iron to intracellular ferritin stores (Birgens 1984). Moreover, administration of a therapeutic dose of LF after administration of CCl_4, could alleviate the anemic effects of CCl_4.

The mechanism of LF involves many components that regulate the cellular immune responses in *in vivo* models of inflammation. CCl_4 alone induced a significant decrease in the number of total WBCs and lymphocytes. The protective effects of LF administered concurrently with CCl_4 was manifested by a significant increase in the total WBCs and lymphocytes counts when compared with the group administered with CCl_4, and control group. Thus, the leukogram result demonstrated the regulatory role of LF in the immune system, by protecting the leukocytes from CCl_4, as mentioned by Ward and Conneely (2004). They reported the up and down-regulation of the immune system in the presence of LF *in vivo*. This is supported by the depletion of the lymphoid cells associated with the congestion of the red bulb and blood vessels of the spleen in rats that received CCl_4 (Figure 3(C)). This effect was reversed by LF, a protective and therapeutic agent (Figure 3(D,E)). Zimecki *et al.* (2001) showed that the effects of LF in experimental models were variant and dependent on an individual PBMC reactivity, mitogen or alloantigen, and LF concentration, and that results suggest that the differential action of LF might be due to its ability to stimulate the activation of lymphocyte.

The CCl_4-induced hepatic damage in rats is mostly induced by the ROS formed during the CCl_4 biotransformation process. The ROS are more toxic and reactive than the parent compound. CCl_4 biotransformation occurs in the endoplasmic reticulum. CYP2E1 is an isoenzyme that is involved in this process (Rechnagel *et al.* 1973). CCl_4-induced cell damage can result from either covalent binding of the reactive intermediates to cellular components, or from enhanced lipid peroxidation triggered by the interaction of free radical intermediates with oxygen which in turn attacks the unsaturated fatty acids. This causes degradation of lipids, particularly unsaturated phospholipids, resulting in damage to the intracellular and plasma membrane (Boll *et al.* 2001). The results of this study showed a loss in the antioxidant activity of SOD and GPx in rats exposed to CCl_4. Our results were similar to those obtained by Szymonik-Lesiuk *et al.* (2003) who reported that CCl_4 intoxication resulted in a change in the antioxidant enzymes and reactive intermediates involved in the bioactivation of CCl_4. The reactive intermediates may bind to those enzymes to prevent their inactivation. Moreover, our results correspond with Khan and Ahmed (2009), and Khan *et al.* (2009), and are a match to the investigation following CCl_4 intoxication (Manna *et al.* 2006). Moreover, thiobarbituric acid reactive substances (TBARS), the final metabolites of the peroxide form of polyunsaturated fatty acids, are considered as a late oxidative stress biomarker (Cheeseman 1993).

Our study showed that CCl_4 treatment in rats markedly increased the concentration of hepatic MDA due to the markedly changed antioxidant enzymes activity which was reverted by the co-administration of LF. Similarly, a major reduction in lipid peroxidation and consequent reduction in TBARS was obtained by treatment with LF. The LF antioxidant activity has been observed in different chemical and biological environments. LF was reported to lower lipid peroxidation by decreasing the conversion of H_2O_2 to •OH through the Fenton reaction by sequestering iron (Shinmoto *et al.* 1992). Raghuveer *et al.* (2002) reported that including LF in the diet of preterm infants also attenuated iron-induced oxidation products. Britigan *et al.* (1986) and Satué-Gracia *et al.* (2000) also showed that LF had an antioxidant activity because the iron bound to the protein was unable to act as a catalyst for the generation of the hydroxyl radical which acts as an oxidative stress detoxificant.

In the current study, treatment with LF decreased the level of MDA in the hepatic tissue of rats. Although various mechanisms contribute to protection against ROS-mediated cell and tissue injury, intracellular GPx plays a major role, while SOD converts superoxide to H_2O_2. GPx and CAT modulate the conversion of H_2O_2 to H_2O (Ighodaro and Akinloye 2017). Therefore, the rat hepatic tissue antioxidative status was assessed by estimation of the GPx enzyme. Activity of SOD in the hepatic tissue substantially increased in rats administered with LF. Thus, our results indicate that increased levels of oxidative stress were markedly restored by the co-administration of LF with CCl_4 either protective or therapeutic.

The mechanism of CCl_4-induced hepatic damage in rats is mostly due to the up-regulation of the hepatic pro-inflammatory mediators (IL-1β) and down-regulation of the anti-inflammatory (IL-10 and PON1) components associated with hepatic toxicity (Table 3). PON1 exerts anti-atherogenic and anti-inflammatory effects through the hydrolysis of lipid peroxides (Aviram *et al.* 1998). The portal vein of the CCl_4 group showed a diffused ballooning degeneration over the hepatocytes of the parenchyma, and it was associated with congestion (Figure 2(C)).

The protective effects of LF and the damaging effects of the trauma may be due to the mediation of relative amounts of both anti-inflammatory and pro-inflammatory components (Kruzel *et al.* 2002). When LF was administered for protection against CCl_4 it caused generalized deactivation of the monocytes and macrophages as manifested by a significant reduction of both pro- and anti-inflammatory mediators of inflammation (Table 3). Use of a concurrent, protective, or therapeutic administration protocol led to a significant decrease in pro-inflammatory mediators (IL-1β) and restored down-regulation of anti-inflammatory (IL-10 and PON1) mediators when compared to the CCl_4 group. This was demonstrated by an absence of any histological alteration in LF-protected group (Figure 2(D)) while LF-treated group showed ballooning degeneration of the hepatocytes at a lower level (Figure 2(E)) than the CCL_4 group. Collectively, these outcomes demonstrate that LF can maintain physiological homeostasis by regulating the pro-inflammatory response.

The results of this study show the differential regulation of pro-inflammatory and anti-inflammatory mediators. In addition to the antioxidant activity of LF during CCl_4-induced hepatic toxicity, it is observed that LF has a better protective effect than therapeutic effect. These findings are important, especially when considering the clinical importance of hepatic diseases. Further studies will be necessary to determine the mechanism(s) through which LF maintains biological homeostasis.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability

All data generated or analyzed during this study are included in this published article.

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