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RESEARCH ARTICLE

Ameliorative effects of *Moringa oleifera* leaf extract on levofloxacin-induced hepatic toxicity in rats

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ABSTRACT

Fluoroquinolones are some of the most common antibiotics used by clinicians all over the world. Levofloxacin, a fluoroquinolone, is used therapeutically in numerous countries; however, it can cause an increase in liver function tests and liver dysfunction. The current study was designed to determine the effect of Levofloxacin (40 mg/kg body weight (b.wt.) daily for 2 weeks) on rat liver function and oxidative stress markers as well as to evaluate the potential hepatoprotective effects of *Moringa oleifera* leaf extract as a known antioxidant herb. *M. oleifera* leaf extract was found to improve the hepatic dysfunction induced by Levofloxacin by recovering liver enzymatic activities (alanine aminotransferase [ALT], aspartate aminotransferase [AST] and gamma-glutamyl transferase [GGT]) to normal levels. The extract also reversed the antioxidant imbalance as measured by catalase and superoxide dismutase activities as well as by reduced glutathione and malondialdehyde levels. Moreover, *M. oleifera* leaf extract induced anti-inflammation by improving the production of interleukin (IL)-10. Additionally, its presence attenuated the downregulation of IL-1 induced by Levofloxacin alone from hepatic tissue. It can be concluded that *M. oleifera* extract can help reduce the side effects caused by Levofloxacin administration.

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KEYWORDS
Moringa oleifera; levofloxacin; hepatic enzymes; antioxidants

Introduction

Levofloxacin is a third-generation fluoroquinolone antibiotic. It has broad-spectrum activity against Gram-positive and Gram-negative bacteria as well as certain other pathogens such as *Mycobacteria* spp., *Mycoplasma, Chlamydia,* and *Legionella* (Fish and Chow 1997). The effectiveness of Levofloxacin against infections of the respiratory tract, skin and genitourinary tract has been demonstrated (Croom and Goa 2003). Levofloxacin triple therapy containing Levofloxacin, a proton pump inhibitor, and amoxicillin has been effective for the treatment of *Helicobacter pylori* infection for more than 10 years (Malfertheiner *et al.* 2012, Nista *et al.* 2003). Levofloxacin and other fluoroquinolones produce serious adverse effects more frequently than other broad-spectrum antibacterial drugs (Stahlmann and Lode 2013). The most common side effects are abdominal pain, diarrhea, vomiting, nausea, dizziness, and rash (Ceydilek *et al.* 2005). Hepatotoxicity, phototoxicity, cardiotoxicity, and central nervous system side effects are less frequent. Increases in liver function tests and liver failure (Ceydilek *et al.* 2005, Gulen *et al.* 2015, Yagawa 2001) are mentioned.

The hepatotoxicity of Levofloxacin could be induced by membrane lipid peroxidation, free radical formation, impairment of mitochondria (Hıncal and Taskın 1995) or alterations in glutathione (GSH) redox status (Gürbay *et al.* 2001). Oxidative stress has been demonstrated to be an important mechanism in hepatotoxicity (Rampal *et al.* 2008). Oxidative stress results from an imbalance between the cellular antioxidant system and the production of reactive oxygen species (ROS) (Poli 2000).

Moringa oleifera, Lam (*M. oleifera*), also known as *Moringa pterygosperma* Gaertn, is a member of the Moringaceae family of perennial angiosperm plants, which includes 12 other species (Olson 2002). It has been used as a potential antioxidant, anticancer, anti-inflammatory, antidiabetic and antimicrobial agent (Gopalakrishnan *et al.* 2016). Furthermore, *Moringa* flowers contain flavonoid (quercetin), which might be responsible for its potent hepatoprotective activity (Ruckmani *et al.* 1998, Selvakumar and Natarajan 2008). Therefore, the present study explores markers of the hepatotoxic potential of Levofloxacin and the possible protective effects of *M. oleifera* extract against this toxicity.

This study is the first *in vivo* report of the potential protective effects of *Moringa oleifera* extract in Levofloxacin-induced hepatotoxicity in rats.

Materials and methods

Chemicals

Levofloxacin (Levoflox®) tablets (500 mg/tablet) were purchased from Memphis Co., Egypt. They were dissolved in

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distilled water then given orally to individual animals at a dose of 40 mg/kg b.wt. daily for two weeks.

Plant extract

Two hundred grams of dried, fresh *M. oleifera* leaves (obtained from El-Shekh Zoed Station, Desert Research Center, (North Sinai, Egypt) were blended with 1 liter of 70% ethanol, and the suspension was stirred using a magnetic stirrer at 4 °C overnight. The decoction solution was filtered through filter paper. The filtrates were pooled then lyophilized. The dried ethanol extract of fresh *M. oleifera* leaves (yield 10% (Wight/Wight)) was kept in a freezer at −40 °C.

Experimental animals

Adult male Albino rats (120–140 g), 5 weeks of age, were purchased from the Animal House of the National Cancer Institute (Cairo, Egypt). The rats were maintained in clean cages and given a standard diet and clean water *ad libitum*. All animals were subjected to a normal light/dark cycle (12-h light-dark cycle starting at 8:00 AM) and room temperature (23 ± 3 °C) and allowed free access to chow and water. All protocols were approved by the Institutional Review Board for Animal Experiments of Benha University, Egypt (Approval No. 2015–134).

Preparation of liver homogenates

Preparation of liver tissue homogenates was performed according to Hegazy *et al.,* (2018). Liver homogenate (prepared from 1 g of hepatic tissue) was obtained from all rats at the end of the first and second weeks of the experiment then washed and homogenized in ice-cold 1.15% solution of potassium chloride in 50 mmol in buffer solution of potassium phosphate (pH 7.4) to obtain a liver homogenate of 10% (W/V; Weight of liver tissue, g per Volume of the buffer, mL). Homogenization of the liver tissue was performed by using a sonicator (4710 Ultrasonics Homogenizer, Cole-Parmer Instrument Co., USA). The homogenate was then centrifuged (Sigma 30 K refrigerated centrifuge; Sigma-Aldrich Co., St Louis, MO, USA) at 4000 rpm for 5 min at 4 °C. The collected supernatant was used for determination of the concentration of reduced glutathione (GSH) and lipid peroxidation by-products and determination of the activities of superoxide dismutase (SOD) and catalase (CAT).

Assay methods

Serum liver enzyme activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyl transferase (γ-GT):

Determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities was performed according to (Reitman and Frankel 1957). The activity of gamma glutamyl transferase (GGT) was estimated by the method of (Rosalki and Rau 1972).

Oxidant/antioxidant markers in liver homogenates

CAT activity was measured by the method of Luck (1963), in which the enzyme activity was determined on decomposing of H_2O_2 and expressed as unit/mg protein (Model, JASCO 7800, UV/VIS, Japan).

SOD activity was measured according to Misra and Fridovich (1972). The enzyme activity was determined by considering the degree of inhibition of the autoxidation of epinephrine at alkaline medium. The activity of SOD was expressed as unit/mg protein.

The reduced glutathione (GSH) content was measured according to Ellman (1959). The reduced chromogen is directly proportional to the GSH concentration, and its absorbance was measured at 405 nm using a spectrophotometer. Concentrations of GSH are expressed as μmol/mg protein.

Lipid peroxidation by-products in liver tissue homogenates were determined according to Ohkawa *et al.* (1979) which depends on the formation of MDA as an end product of lipid peroxidation that reacts with thiobarbituric acid producing thiobarbituric acid reactive substance (TBARS), a pink chromogen, which can be measured spectrophotometrically at 535 nm, an MDA standard was used to construct a standard curve against which readings of the samples were plotted. Concentrations of lipid peroxides are expressed as nmol/mg protein.

The total protein content of the homogenates was determined by the method of Lowry *et al.* (1951).

Analysis of mRNA expression of hepatic IL-1 and IL-10 genes using real time-PCR. To better understand the effect of Levofloxacin and/or *M. oleifera* extract administration on hepatic inflammation, expression of hepatic IL-1 and IL-10 cytokines was analyzed by real time-PCR using sense and antisense primers throughout the experiment as previously described (Farid *et al.* 2010) using the following primer sets: 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-10 (GenBank ID: L02926.1), sense (5′-AGA AGC TGA AGA CCC TCT GGA TAC-3′) and antisense (5′-GCT CCA CTG CCT TGC TTT TAT T-3′); and 18S rRNA (GenBank ID: NR_046237.1) as a housekeeping gene, sense (5′-GAG GTG AAA TTC TTG GAC CGG-3′) and antisense (5′-CGA ACC TCC GAC TTT CGT TCT-3′).

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

Changes in gene expression were calculated from the obtained cycle threshold (C_t) values provided by real-time PCR instrumentation using the comparative CT method to a reference (housekeeping) gene (18S rRNA) (Schmittgen and Livak 2008).

Histopathological examination

Specimens were taken from liver of all groups, fixed in 10% buffered neutral formalin for 24 hours. After proper fixation, the specimens were washed in running tape water, dehydrated in different grades of ethyl alcohol, cleared in xylol and embedded in paraffin, then blocked and sectioned as

Table 1. Effect of *Moringa oleifera* extract against Levofloxacin on liver function tests after the first and second weeks of the experiment.

Test	Week	Control	*M. olifera*	Levofloxacin	*M. olifera* and Levofloxacin
ALT(U/L)	1st	22.701 ± 1.880[b]	20.888 ± 1.973[b]	30.275 ± 2.602[a]	28.318 ± 1.591[a]
	2nd	22.693 ± 2.758[b]	22.938 ± 1.253[b]	42.750 ± 2.624[a]	24.183 ± 3.708[b]
AST(U/L)	1st	60.448 ± 3.640[b]	67.673 ± 3.203[b]	116.525 ± 4.543[a]	107.020 ± 6.126[a]
	2nd	67.473 ± 3.348[b]	72.943 ± 6.389[b]	135.175 ± 7.040[a]	88.190 ± 7.579[b]
γGT(U/L)	1st	4.733 ± 0.990[b]	4.248 ± 0.496[b]	9.075 ± 0.860[a]	8.828 ± 0.464[a]
	2nd	5.200 ± 0.506[b]	6.198 ± 0.305[b]	9.578 ± 0.886[a]	5.455 ± 0.403[b]

Data are expressed as the mean ± SE. Means with different superscript within the same row are significant ($p < 0.05$). ALT: Alanine transaminase; AST: Aspartate aminotransferase; γGT: gamma-glutamyl transferase.

5 μm thickness. Then stained by hematoxylin and eosin after (Bancroft and Cook 1994) and examined microscopically.

Experimental design

Forty male Wistar rats were randomly allocated into four groups (10 animals per group): a control group and second group were given *M. oleifera* leaf extract orally 300 mg/kg b.wt. (Saalu *et al.* 2012) in distilled water (D.W.) three times a week for 2 weeks. The third group was given Levofloxacin orally 40 mg/kg b.wt. (Rawi *et al.* 2011) in 1 mL D.W. daily for 2 weeks. The fourth group was given *M. oleifera* leaf extracts (300 mg/kg b.wt. three times a week) and Levofloxacin (40 mg/kg b.wt. daily) orally for 2 weeks. Clinical signs were recorded for all groups during the period of the experiment. Blood for serum samples was taken from the retroorbital venous plexus and hepatic samples were collected from the rats of all groups at the end of the 1st week and the 2nd week of the experiment for estimation of the function of liver, the oxidative parameters, gene expression and the histopathological changes.

Statistical analysis

Statistical analysis was performed using the statistical software package SPSS for Windows (Version 20.0; SPSS Inc., Chicago, Ill). The significance of differences between more than two groups was evaluated by one-way analysis of variance (ANOVA). If one-way ANOVA indicated a significant difference, then differences between individual groups were estimated using Duncan's *post hoc* test. The results are expressed 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 experiment.

Serum enzymatic activities relative to liver dysfunctions:

The mean and standard error values of liver ALT, AST and GGT activities of the various groups are depicted in Table 1. ALT, AST and GGT activities were significantly increased in the Levofloaxicin-treated groups when compared with control and moringa-treated groups after the first and second weeks of treatment. On the other hand, rats cotreated with Levofloxacin and moringa showed significant increases in the activities of ALT, AST and GGT after the first week and non-significant changes after the second week of the experiment when compared with control and moringa groups alone; this indicates that *M. oleifera* extract could not prevent hepatic damage after the 1st week of the experiment.

Changes in the hepatic antioxidant parameters:

The activities of CAT, SOD and GSH in the liver tissue of the Levofloxacin-treated group decreased significantly when compared with control and moringa-treated groups, while cotreatment of the Levofloxacin group with moringa resulted in recovery of the enzymatic activities to normal levels after both the first and second weeks of the experiment. Likewise, the levels of MDA, a marker of lipid peroxidation in liver tissue, were significantly increased in the Levofloxacin-treated group when compared with control and moringa-treated groups, while cotreatment of the Levofloxacin group with moringa resulted in recovery of MDA levels to normal values after both first and second weeks of the experiment (Table 2).

Changes in hepatic IL-1 and IL-10 mRNA expression.

As shown in Figure 1, the intensity of hepatic IL-1 and IL-10 mRNA normalized to 18 s rRNA in the Levofloxacin-, *M. oleifera*- and *M. oleifera*+Levofloxacin-treated rats in comparison with controls after 2 weeks of the experiment.

Administration of *M. oleifera* extract to rats induced a significant upregulation in hepatic IL-1 and IL-10 mRNA while Levofloxacin induced significant downregulations to both genes. The presence of *M. oleifera* extract together with Levofloxacin treatment attenuated the downregulation of IL-1 induced by Levofloxacin alone and induced upregulation in IL-10 mRNA.

Histopathological examination of hepatic tissue

To identify the correlation between alterations in hepatic function manifested by reduced changes in liver enzymatic activities and the hepatic innate immune response, we checked the liver sections stained with HE. The examined sections showed that rats in the group that received Levofloxacin showed congestion in the portal vein (after the first and second weeks) and newly formed bile ducts in the portal area as well as focal hemorrhage in the hepatic parenchyma after the first week of the experiment (Figure 2).

Table 2. Effect of *Moringa oleifera* extract against Levofloxacin on antioxidant parameters after the first and second weeks of the experiment.

	Week	Control	*M. olifera*	Levofloxacin	*M. olifera* and Levofloxacin
CAT (unit/mg protein)	1st	3.551 ± 0.161[a]	3.066 ± 0.123[a]	1.168 ± 0.251[b]	3.104 ± 0.386[a]
	2nd	3.440 ± 0.069[a]	2.907 ± 0.201[a]	0.697 ± 0.248[b]	3.498 ± 0.601[a]
SOD (unit/mg protein)	1st	0.566 ± 0.053[a]	0.565 ± 0.033[a]	0.054 ± 0.013[b]	0.479 ± 0.081[a]
	2nd	0.449 ± 0.057[a]	0.562 ± 0.031[a]	0.065 ± 0.019[c]	0.368 ± 0.071[b]
GSH (μmol/mg protein)	1st	0.365 ± 0.031[a]	0.355 ± 0.019[a]	0.173 ± 0.033[b]	0.357 ± 0.024[a]
	2nd	0.387 ± 0.032[a]	0.378 ± 0.038[a]	0.183 ± 0.016[b]	0.408 ± 0.021[a]
MDA (nmol/mg protein)	1st	0.285 ± 0.066[b]	0.270 ± 0.057[b]	0.942 ± 0.112[a]	0.323 ± 0.033[b]
	2nd	0.357 ± 0.062[b]	0.222 ± 0.035[b]	0.977 ± 0.124[a]	0.384 ± 0.044[b]

Data are expressed as the mean ± SE. Means with different superscript within the same row are significant ($p < 0.05$). CAT: catalase; SOD: superoxide dismutase; GSH: reduced glutathione; MDA: malondialdehyde.

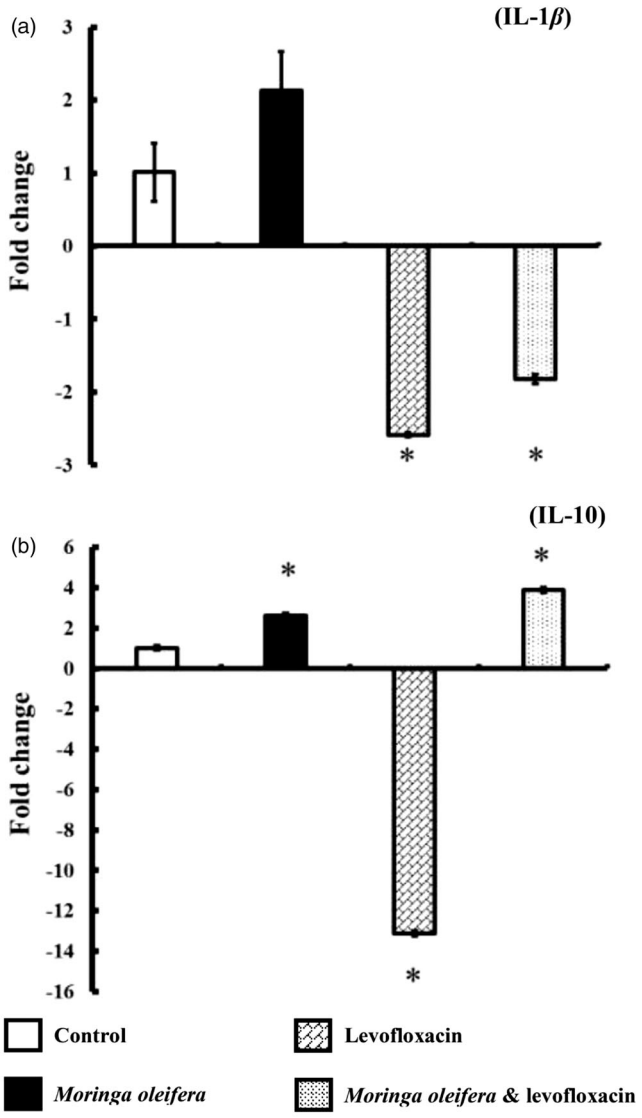

Figure 1. mRNA expression of hepatic IL-1 and IL-10 genes. Total RNA was prepared from hepatic tissues of rats treated with *M. oleifera* extract, levofloxacin, *M. oleifera* extract against Levofloxacin and controls on week 2 after treatments. The expression levels were evaluated by real-time PCR. $^*p < 0.05$ compared with control values. Bars represent means ± S.E.M. ($n = 3$).

The examined sections of this group showed inflammatory cell infiltrations (lymphocytes and neutrophils), especially around the portal vein and in the septa between hepatic lobules, as well as diffuse fatty changes in the hepatocytes that were evident by weeks 1 and 2 of the experiment (Figure 2). On the other hand, the liver tissues of rats that received both Levofloxacin and *M. oleifera* showed mild dilatation and congestion in the central vein after the 1st and 2nd week of the experiment. The liver tissues of rats that received *M. oleifera* appeared to be normal (Figure 2).

Discussion

The liver is classically perceived as a nonimmunological organ required for metabolic activities, nutrient storage and detoxification (Robinson *et al.* 2016). Numerous metabolic processes including metabolism of both endobiotics and xenobiotics take place in the liver. While the majority of these metabolic processes result in largely nontoxic quantities of metabolites with favorable excretion profiles, some of these reactions result in toxic compounds that can directly elicit liver damage (Woolbright and Jaeschke 2015). Hepatic innate immune responses are involved in and contribute to the systemic response to local inflammation, clearance of particles and soluble molecules from the circulation, and killing of invading cells such as neoplastic cells (Parker and Picut 2005).

In our study, there were no apparent clinical signs of toxicity at the dose level of Levofloxacin tested. Also, there was no significant effect on the body weight of the Levofloxacin-treated rats, giving no indication of stress on the rats due to being given doses of Levofloxacin.

The pathophysiology of fluoroquinolone hepatotoxicity is not well understood. There is a higher rate of liver damage in patients receiving molecules that generate reactive intermediates (temafloxacin and trovafloxacin), and this mechanism may be applicable to other fluoroquinolones (Blum *et al.* 1994, Chen *et al.* 2000, Sun *et al.* 2008). In the present study, there were significant increases in ALT, AST and GGT levels, which indicates liver damage in the Levofloxacin-treated group. The elevation in ALT was almost two times that of the value in the control groups, especially in week 2 after administration of Levofloxacin. Similarly, the elevations in ALT and GGT were more than two times the levels of the control groups after the first and second weeks of receiving Levofloxacin (Table 1). Elevated serum levels of ALT and AST may be due to hepatocellular inflammation, which leads to an increase in the permeability of the cell membrane resulting in the release of transaminases in the blood stream (Kaneko *et al.* 2008). A similar increase in ALT, AST and GGT values by Levofloxacin has been reported in humans (Figueira-Coelho et al. 2010, Karim *et al.* 2001). Increases in GGT are associated with all forms of primary and secondary hepatobiliary disorders. Cholestasis due to intrahepatic or

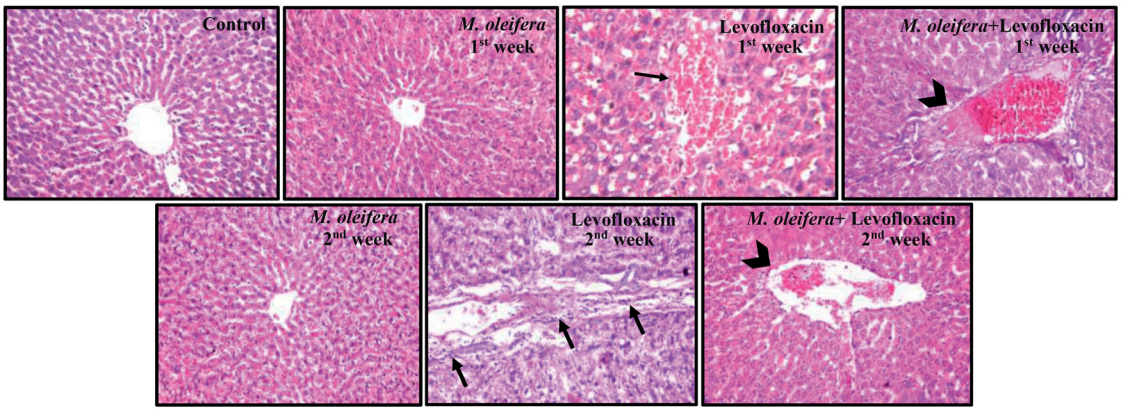

Figure 2. Histopathological examination of rat liver sections on weeks 1 and 2 following treatment with *M. oleifera* extract, Levofloxacin, and *M. oleifera* extract against Levofloxacin. Liver sections of rat tissue were deparaffinized, stained with hematoxylin-eosin (HE), and examined under light microscopy. Narrow arrows indicate focal hemorrhage in hepatic parenchyma; bold arrows indicate inflammatory cell (lymphocyte, neutrophil and eosinophil) accumulations by week; and arrow heads indicate dilatation and congestion in the central vein. Control liver section with no abnormalities.

extrahepatic biliary obstruction causes higher serum levels of GGT (Clark and Kruse 1990). There were significant decreases in ALT, AST, and GGT levels with Levofloxacin and *M. oleifera* cotreatment, and this observation might be due to hepatoprotective activity. The reversal of elevated serum intracellular enzyme levels by *M. oleifera* extract may be attributed to a hepatoprotective property of *M. oleifera* leaf extract. Previous research reported that the hepatoprotective effect was due to the presence of a well-recognized flavonoid (quercetin and kaempferol) (Hamza 2010, Pari and Karthikesan 2007). These results concurred with the improvement in histopathological features of the livers of *M. oleifera* leaf extract-treated rats when compared with the livers of Levofloxacin-treated rats. These findings coincided with Awodele *et al.* (2012) and Sharifudin *et al.* (2013) who found that the *M. oleifera* leaf extract moderately reduced hepatocyte necrosis, indicating the reduction of hepatocellular damage.

In the present study, there were increases in lipid peroxidation indicated by elevation in MDA level and decreases in CAT, SOD and GSH activities observed in rats treated with Levofloxacin after the 1st and 2nd weeks of treatment (Table 2). This might be due to the peroxidation of membrane lipids and injury to the cellular components (Khan *et al.* 2017). Reduction in lipid peroxidation; increases in CAT, SOD and GSH activities; and reduction in MDA levels were observed with Levofloxacin and *M. oleifera* extract cotreatment (Table 2). *M. oleifera* extract is a rich source of antioxidants (Singh *et al.* 2009). The major bioactive compounds of *M. oleifera* extract are quercetin, kaempferol, vitamin A and ascorbic acid, which are responsible for the antioxidant activities (Bajpai *et al.* 2005, Labbé *et al.* 2009).

IL-10 is a cross-regulation factor of TH_1 and TH_2 responses, is produced by TH_2 subset cells, and is also called cytokine synthesis inhibitory factor (CSIF) (Mosmann and Moore 1991). IL-10 is a cytokine with potent inhibitory effects on TH_1 cells and antigen-presenting cells, such as monocyte/macrophages, causing downregulation of expression of major histocompatibility complex (MHC) class II molecules and attenuated release of pro-inflammatory cytokines, including TNF, TH-1 phenotype cytokines IFN- γ and IL-12 (Oswald *et al.* 1992).

In the present study, we evaluated the hepatic IL-1β expression *in vivo* following Levofloxacin administration in rats and concluded that 2 weeks after administration, Levofloxacin leads to statistically significant downregulation of hepatic IL-1β. Few mechanisms have been described to explain these findings. Fluoroquinolones are known to interfere with NF-κB activation by inhibiting the degradation of IκBα, thus reducing the levels of production of pro-inflammatory cytokines (Choi *et al.* 2003). IL-1 is a potent inflammatory cytokine mainly produced by macrophages (Seki and Schwabe 2014). IL-1, either directly or through induction of other cytokines such as IL-6 and TNF-α, promotes development of liver inflammation by increasing the expression of adhesion molecules and chemotactic factors and by the recruitment of inflammatory cells, thereby inducing liver injury (Kamari *et al.* 2011). The presence of *M. oleifera* leaf extract alone or in combination with Levofloxacin attenuates the effects of Levofloxacin and improves the anti-inflammatory status by upregulation of IL-10 expression. *M. oleifera* flower extract is enriched with major phenolic compounds such as quercetin and kaempferol. Hämäläinen et al. (2007) and García-Mediavilla *et al.* (2007) reported the anti-inflammatory potential of quercetin and kaempferol by inhibition of signal transducer and activator of transcription 1 (STAT-1) and the NF-κB pathway. Consequently, hepatic downregulation of IL-1 and IL-10 after treatment with Levofloxacin and potentiation of the anti-inflammatory effects of *M. oleifera* leaf extract could explain the decreased number of inflammatory cell infiltrations into liver tissue (Table 3 and Figure 2). *M. oleifera* leaf extract, by its effects on the production of Il-1β and IL-10, achieves the balance between the pro- and counter-inflammatory pathways that determine the final outcome of Levofloxacin administration.

In this work, we provide new information about the role of *M. oleifera* leaf extract as an antioxidant and hepatoprotective agent, which could be helpful in reducing the side effects caused by Levofloxacin administration by reducing the ROS production, maintaining the antioxidant potential, and significantly reducing hepatocyte damage. On the other hand, the major limitation of this study was the absence of a comparative assessment with silymarin as a standard drug

Table 3. The severity of histopathological alterations in the hepatic tissue in different experimental groups ($n = 5$).

Groups								
	Control		*M. olifera*		Levofloxacin		*M. olifera* and Levofloxacin	
Findings	1st w	2nd w	1st w	2nd w	1st w	2nd w	1st w	2nd w
Congestion	–	–	–	–	+++	++	+	+
Newly formed bile ducts	–	–	–	–	+++	–	–	–
Fatty change in hepatocytes	–	–	–	–	–	+++	–	–
Inflammatory cells infiltration in portal area	–	–	–	–	–	++	–	–
Focal hemorrhage	–	–	–	–	++	–	–	–

+++ severe ++ moderate + mild – nil.

used for the treatment of impaired liver function. Therefore, future investigations aimed at determining the hepatoprotective power of *M. oleifera* leaf extract compared to standard drugs are warranted.

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Disclosure statement

The authors declare that there is no conflict of interest to disclose for this study.

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