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Possible Protective Effects of Lutein against Ciprofloxacin Induced Bone Marrow Toxicity in Rats

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Abstract

Ciprofloxacin, which is a second generation of fluoroquinolone and one of the most effective and widely used drugs within fluoroquinolone. Unfamiliar adverse effects of ciprofloxacin such as bone marrow (BM) suppression, thrombocytopenia, anemia, agranulocytosis, renal failure, and others observed. Lutein, is a xanthophyll (an oxygenated carotenoid), was focused by most studies as it has a strong antioxidant activity *in vitro*; and also, it has been associated with reducing the risk of the age-related disorders. The current study was designed to describe the role of apoptosis through the measurement of Bcl-2 associated X protein (Bax) marker, as mechanisms of bone marrow toxicity induced by ciprofloxacin and to find whether lutein may have protective effects on ciprofloxacin-induced toxicity in bone marrow of rats.

Thirty six Sprague-Dawley rats were randomly divided into six groups (six animal each): **Groups I** (control), rats received single oral daily dose of liquid paraffin (4ml/kg) for 25 successive days by oral gavage; **Group II**, **(ciprofloxacin-treated)**, received single oral daily dose of liquid paraffin (4ml/kg body weight/day) for 25 days, and subsequently received 500 mg/kg ciprofloxacin by oral gavage for the last 5 days; **Groups III** and **IV**, received oral dose of lutein (6mg/kg/day) and (24mg/kg/day), respectively by oral gavage for 25 successive days (**lutein-treated**); **Groups V** and **VI**, received oral dose of lutein (6mg and 24mg /kg/day), respectively by oral gavage for 25 successive days, and subsequently received 500 mg/kg ciprofloxacin orally for the last 5 days (**lutein+ ciprofloxacin**).

Ciprofloxacin (**Group II**) caused significant (P<0.05) reduction in total RBCs counts and -WBCs, and significantly elevations (P<0.05) Bcl-2 associated X protein (Bax) in bone marrow (BM) tissues homogenates compared to control (**Group I**) rats. Rats that orally received lutein (**Groups III** and **IV**), each produced non-significant differences (P>0.05) in total -RBCs and -WBCs and also produced non-significant differences (P>0.05) in Bax levels in BM tissues homogenates with respect to corresponding levels in **Group I** rats. Orally-administered lutein with ciprofloxacin (**Groups V** and **VI**), resulted in significant elevation (P<0.05) of total -RBCs and -WBCs, and significantly reduced (P<0.05) Bcl-2 associated X protein (Bax) in bone marrow (BM) tissues homogenates caused by ciprofloxacin compared to the corresponding levels in group of rats administered ciprofloxacin (**Group II**).

Results of the current research suggested that lutein may be a useful compound that alleviated ciprofloxacin-induced toxicity on bone marrow.

Keywords: Lutein, Ciprofloxacin, Total RBCs count and total WBCs count.

التأثيرات الوقائية المحتملة للوتين ضد سمية نخاع العظم التي يسببها السيبروفلوكساسين في الجرذان

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الخلاصة

سيبروفلوكساسين ، وهو الجيل الثاني من الفلوروكينولون وواحد من الأدوية الأكثر فعالية والأكثر استخدامًا في الفلوروكينولون. الآثار الضارة غير المألوفة للسيبروفلوكساسين مثل تثبيط نخاع العظام ، قلة الصفيحات ، فقر الدم ، ندرة المحببات ، الفشل الكلوي ، وغيرها. اللوتين ، هو زانثوفيل (كاروتينويد مؤكسج) ، ركزت عليه معظم الدراسات لأنه يحتوي على نشاط قوي مضاد للأكسدة في المختبر ؛ وأيضًا ، فقد ارتبط بتقليل مخاطر الاضطرابات المرتبطة بالعمر .صممت الدراسة الحالية لوصف دور موت الخلايا المبرمج من خلال قياس مؤشر Bcl-2 (Bax)المرتبط بالبروتين X ، كآليات لتسمم نخاع العظم الناجم عن سيبروفلوكساسين ولإيجاد ما إذا كان اللوتين قد يكون له تأثيرات وقائية على السمية التي يسببها السيبروفلوكساسين في نخاع عظم الجرذان.

ستة وثلاثون جرذان Sprague-Dawley قسمت عشوائيا إلى ست مجموعات (ستة حيوانات لكل مجموعة): المجموعة I (مجموعة التحكم) ، تلقت الجرذان جرعة يومية واحدة عن طريق الفم من البارافين السائل (٤ مل / كجم) لمدة ٢٥ يومًا متتاليًا عن طريق الحقن الفموي. المجموعة II، (المعالجة بالسيبروفلوكساسين) ، تلقت جرعة يومية واحدة عن طريق الفم من البارافين السائل (٤ مل / كجم من وزن الجسم / يوم) لمدة ٢٥ يومًا ، ثم تلقت بعد ذلك ٥٠٠ مجم / كجم من سيبروفلوكساسين عن طريق الحقن عن طريق الفم خلال آخر ٥ أيام ؛ المجموعات III و IV ، تلقت جرعة فموية من اللوتين (٦ مجم / كجم / يوم) و (٢٤ مجم / كجم / يوم) ، على التوالي عن طريق الحقن الفموي لمدة ٢٥ يومًا متتاليًا (المعالجة باللوتين) ؛ المجموعتان V و VI ، تلقيا جرعة فموية من اللوتين (٦ مجم و ٢٤ مجم / كجم / يوم) ، على التوالي عن طريق الحقن الفموي لمدة ٢٥ يومًا متتاليًا ، وبعد ذلك تم تلقي ٥٠٠ مجم / كجم من سيبروفلوكساسين عن طريق الفم خلال آخر ٥ أيام (لوتين + سيبروفلوكساسين).

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تسبب سيبروفلوكساسين (المجموعة الثانية) في انخفاض معنوي (P <0.05) في إجمالي عدد كرات الدم الحمراء و عدد كرات الدم البيضاء ، وارتفاع ملحوظ (P <0.05) في Bcl-2 (Bax) المرتبط بالبروتين X في متجانسات أنسجة نخاع العظام (BM) مقارنةً بالسيطرة (المجموعة الأولى) للفئران. الفئران التي تلقت لوتين فمويا (المجموعات III و IV) ، أنتجت كل منها فروقًا غير معنوية (P> 0.05) في إجمالي كرات الدم الحمراء و كرات الدم البيضاء وأنتجت أيضًا اختلافات غير معنوية (P> 0.05) في مستويات Bax في متجانسات أنسجة نخاع العظام BM مع ما يتعلق بالمستويات المقابلة في مجموعة السيطرة للجرذان. أدى تناول اللوتين الفموي مع سيبروفلوكساسين (المجموعتان V و VI) إلى ارتفاع كبير (P <0.05) في إجمالي كرات الدم الحمراء و عدد كرات الدم البيضاء ، وانخفاض ملحوظ (P <0.05) بروتين X المرتبط بـ Bcl-2 (Bax)) في نخاع العظام (BM) المتجانسة للأنسجة التي يسببها السيبروفلوكساسين مقارنة بالمستويات المقابلة في مجموعة الفئران المعطاة للسيبروفلوكساسين (المجموعة الثانية).

الكلمات المفتاحية :لوتين ، سيبروفلوكساسين ، إجمالي عدد كرات الدم الحمراء وإجمالي عدد كرات الدم البيضاء

Introduction

The carboxylic acid derivation of fluoroquinolones is Ciprofloxacin, which is a second generation of fluoroquinolone and one of the most effective and widely used drugs within fluoroquinolone [(1)]. Its mode of action is brought about by inhibiting topoisomerase II (DNA-gyrase) and topoisomerase IV enzymes that are essential for bacterial DNA transcription, replication, repair, recombination, and strand supercoiling repair [(2)].

Treatment with fluoroquinolones, including ciprofloxacin was reported to cause severe liver failure, elevated intracranial pressure, central and peripheral neuropathy [(3)]. Furthermore, unfamiliar adverse effects of ciprofloxacin such as bone marrow (BM) suppression, thrombocytopenia, anemia, agranulocytosis [(4)].

A possible molecular mechanism provided for adverse effects of the ciprofloxacin was mentioned by researchers to be provoked by the inhibition of mitochondrial topoisomerase II (DNA-gyrase) that can lead to impairment of mitochondrial DNA transcription and replication, thus has an impact on the cellular differentiation and proliferation [(5)].

Hematopoiesis which is the biological process occurs in the bone marrow in which the hematopoietic stem cells (HSC) proliferated into blood cells [(6)]. Development of multicellular organism relies on the balance between the cell proliferation and the cell death (that apoptosis). However, the scenario of increasing apoptosis, in long term number of the proliferating cells is decreased and then unable to reach to the normal levels [(7)].

Bax is a pro-apoptotic protein of mitochondria, in the intrinsic pathway of apoptosis, that cause release of the cytochrome c, the endoribonuclease G, and other proteins of mitochondria through opening of the mitochondrial outer membrane [(8)]; this release is mediated by the permeability transition pore of the mitochondria [(9)]. This cytochrome release lead to the activation of the initiator caspase 9 and finally caspase 3 that cause apoptosis [(8)].

The nutrients carotenoids are widely distributed in foods, especially in vegetables and fruit [(10)]. Carotenoids appear to have antioxidant activity [(11)]. The beneficial effects of carotenoids were associated in many systemic diseases when consumed in large dietary intake [(12)] and with retinal protection against eye disorders and damage caused by phototoxic light [(13)].

Lutein was focused by most studies as it has a strong antioxidant activity *in vitro*; and also, it has been associated with reducing the risk of the age-related disorders [(12)]. It is a xanthophyll (an oxygenated carotenoid), which in all mammalians including humans, that are unable to synthesize it but deriving it through their diet [(14)]

By absorbing blue light, lutein appears yellow in low concentrations; while in high concentrations, it appears orange-red; moreover, in green vegetables, lutein accounts for about 48% of total xanthophylls [(15)].

Furthermore, lutein is absorbed through the gastrointestinal (GI) with fat and it can be transported by lipoproteins; where lutein is transported via apolipoprotein E, and its transference facilitated mostly (52%) via high density lipoproteins (HDLs) and (22%) via low density lipoproteins (LDLs) [(16).]

Objectives

This study was designed to describe the role of apoptosis as mechanisms of BM toxicity induced by ciprofloxacin; and to explore the possible protective effects of high- and low- doses of lutein against ciprofloxacin-induced toxicity in BM of rats.

Materials and Methods

Animals

Thirty-six adult Sprague-Dawley rats weighing 150-200g were used; the animals were taken from The Animal House of the College of Pharmacy / University of Baghdad, under controlled and conventional laboratory conditions. Rats were housed in cages of stainless steel, at (25°C), relative humidity and natural light/dark cycle. Standard laboratory rodent tap water and chow were supplied *ad libitum*, and the animals adapted for a one-week period prior of the experiment. All animal procedures were approved by Ethical Committee of College of Pharmacy/ University of Baghdad.

Materials

The pure powders of ciprofloxacin obtained from Shaanxi Yuantai Biological Technology Co., Ltd. China; and the pure powder of lutein was obtained from Xi`an Rongsheng Biotechnology Co., Ltd. China. Rat Bcl-2 associated X protein, Bax ELISA Kit was obtained from Sunlong Biotech CO., LTD, China.

Animals grouping and tested doses

Rats were allocated into six groups of six rats each as follows:

•**Group I** (Control): received single daily oral dose of liquid paraffin (4 ml/kg) for 25 consecutive days by oral gavage. This group served as control.

•**Group II** (**ciprofloxacin-treated**): received single oral daily dose of liquid paraffin (4ml/kg body weight/day) for 25 days, and subsequently received 500 mg/kg ciprofloxacin by oral gavage for the last 5 days.

•**Group III** (**lutein-treated**): received oral dose of lutein (6mg/kg/day) daily by oral gavage for 25 consecutive days.

•**Group IV** (**lutein-treated**): received oral dose of lutein (24mg/kg/day) daily by oral gavage for 25 consecutive days.

•**Group V**: received oral dose of lutein (6mg/kg/day) daily by oral gavage for 25 consecutive days, and subsequently received 500 mg/kg ciprofloxacin orally by oral gavage for the last 5 days.

•**Group VI**: received oral dose of lutein (24mg/kg/day) daily by oral gavage for 25 consecutive days, and subsequently received 500 mg/kg ciprofloxacin by oral route for the last 5 days.

Twenty-four hours after the end of treatment, all animals were euthanized by diethyl ether anesthesia and from each rat, blood sample were withdrawn from the carotid artery at the neck and collected for the hematological assessments [total -RBCs and -WBCs counts].

Furthermore, BM were quickly excised, placed in chilled phosphate buffer solution (PBS) (pH 7.4) at 4^0 C, blotted with filter paper and weighed. For the preparation of 10% tissues homogenates, 9ml of PBS (pH 7.4) was added to 1gram of BM, then each tissue was homogenized by tissue homogenizer that set at 3 for 1 minute at 4 ^{0}C. All preparations were freshly prepared and kept frozen at (-18 C0) unless worked immediately for the measurement of Bcl-2 associated X protein (Bax) marker in BM tissue homogenates.

Bax determination in the BM tissue homogenate

The principle of Rat Bcl-2 associated X protein, Bax determination in this ELISA kit (Rat Bcl-2 associated X protein, Bax ELISA Kit was obtained from Sunlong Biotech CO., LTD.) is that: this ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to Bax. Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP) - conjugated antibody specific for Bax is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain Bax and HRP conjugated Bax antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of Bax. You can calculate the concentration of Bax in the samples by comparing the OD of the samples to the standard curve.

Statistical analysis

Data was expressed as the values of mean standard deviation (SD). The data were analyzed by utilizing computerized IBM SPSS statistics 23.0 program. The statistical significance of the differences among various groups is determined by one-way analysis of variance (ANOVA). The statistically significant differences were considered when *P* value less than 0.05 ($P<0.05$).

Results and Discussion

Ciprofloxacin (**Group II**) caused significant ($P<0.05$) reduction in total RBCs counts (Figure. 1), total WBCs counts (Figure. 2) each compared to the corresponding levels in control (**Group I**) rats; furthermore, there were significant ($P<0.05$) elevations in Bax contents (Figure. 3) in BM tissue homogenates compared to control (**Group I**) rats.

Groups III and **IV** rats that orally received lutein 6mg/kg and 24mg/kg, respectively each produced non-significant differences ($P>0.05$) in total -RBCs and -WBCs (Fig 1 and 2, respectively) and also produced non-significant differences ($P>0.05$) in Bax levels in BM tissues homogenates with respect to corresponding levels in **Group I** rats (Figures 3).

Administration of lutein at a dose of 6mg/kg body weight, and 24mg/kg each in association with ciprofloxacin (**Groups V** and **VI**) produced significant ($P<0.05$) elevation in total RBCs counts (Figure. 1), total WBCs counts (Figure. 2) each compared to blood counts in **Group II** rats; moreover, significant ($P<0.05$) reduction in Bax contents in BM tissues homogenates (Figure. 3) was shown compared to the corresponding contents to **Group II** (ciprofloxacin-treated) rats.

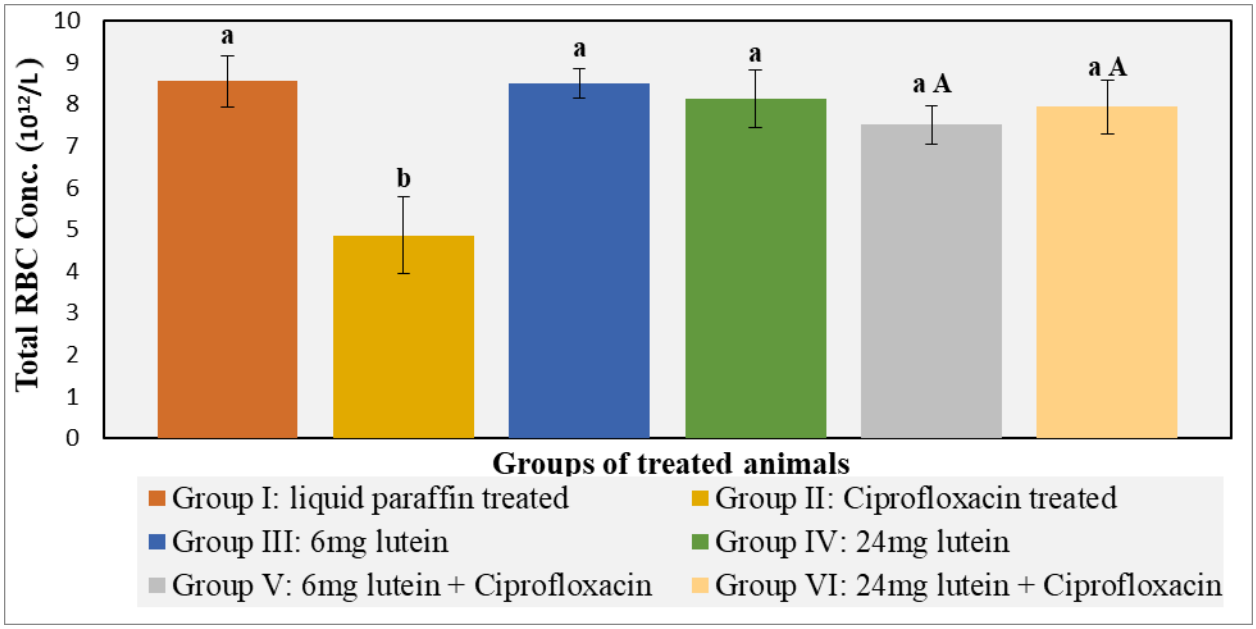

Figure 1. Effects of various treatments on Total RBC counts in rats.
Data are expressed as Mean ± SD, n =6.
Values with non-identical small letters (a, and b) are significantly different ($P< 0.05$).
Values with an identical capital letter (A) are non-significantly different ($P> 0.05$).

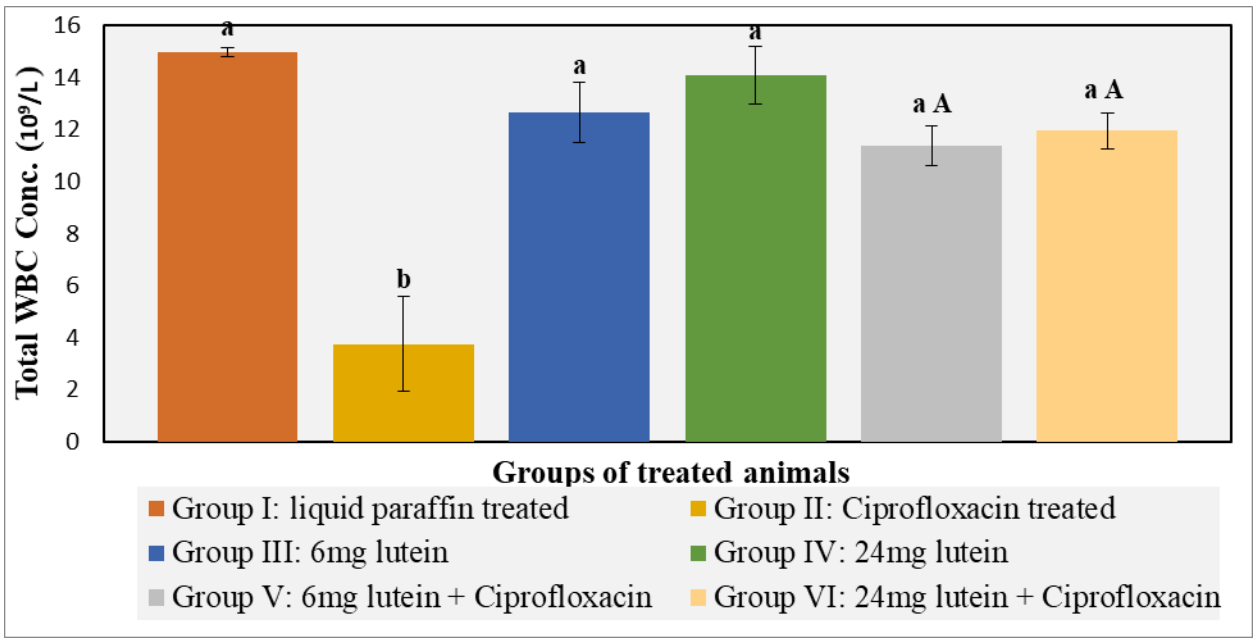

Figure 2. Effects of various treatments on Total WBC counts in rats.
Data are expressed as Mean ± SD, n =6.
Values with non-identical small letters (a, and b) are significantly different ($P< 0.05$).
Values with an identical capital letter (A) are non-significantly different ($P> 0.05$).

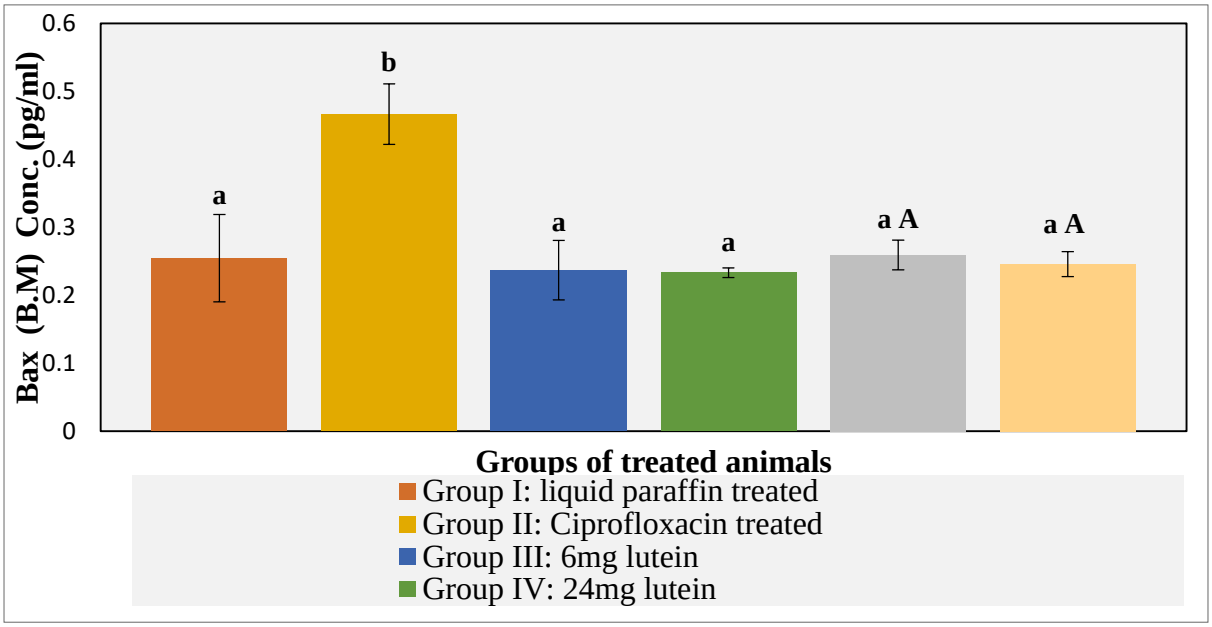

Figure 3. Effects of various treatments on Bax levels in BM tissues homogenates of rats.
Data are expressed as Mean ± SD, n =6.
Values with non-identical small letters (a, and b) are significantly different ($P< 0.05$).
Values with an identical capital letter (A) are non-significantly different ($P> 0.05$).

DNA gyrase which is inhibited by fluoroquinolones, which is an adenosine triphosphate that hydrolyzing topoisomerase II enzyme for keeping safe and state of the supercoiling in the replicating and the non-replicating forms of chromosomes of the bacteria also fluoroquinolones has action on topoisomerase IV [17].

Oridupa A.O., et al. 2013 reported that ciprofloxacin can cause interstitial nephritis, suppression of BM and haemolytic anaemia in humans [18]. Furthermore, quinolones therapy, including ciprofloxacin can cause severe liver failure, elevated intracranial pressure, central and peripheral neuropathy; also, the occurrence of convulsions after exposure to ciprofloxacin can also be occurred. Mechanisms of toxicity caused by ciprofloxacin were reported to be attributed to various causes, including its binding to glycine, N-methyl-D-aspartate, and GABA-receptors; furthermore, other explanation to the toxic effects of ciprofloxacin was imputed to the toxic effect on the antioxidant's activity and the similarities of quinolone structure to kynurenic acid and similar compounds' structure which are glutamate receptor endogenous ligands [3]. Furthermore, adverse reactions of ciprofloxacin, especially in the CNS, may be due to free radical-formation reactions; where, this hypothesis was supported by the evidence that ciprofloxacin can cause serious alterations of the glutathione redox status in the brain and the liver tissues of rat [19]. Moreover, Lowes, D.A., et al. 2009 suggested that the toxicity of ciprofloxacin to the mitochondria can be due to oxidative stress (OS), topoisomerase inhibition, photosensitization and altered calcium homeostasis [20].

Additionally, ciprofloxacin can have effects on the cellular growth and differentiation. Immediate retardation of the cell division was only present in the cells of functional mitochondria; the retrograde of the signal from the mitochondria to the nucleus; caused either by the impaired of mitochondrial DNA replication or by the oxidative stress [5].

Furthermore, adverse effects of fluoroquinolones including ciprofloxacin can cause tendon rupture, muscle weakness, joint inflammation, epilepsy, peripheral and central neuropathies and psychological symptoms as depression; all these symptoms had been suggested to be due to enhanced the OS [21]; although, the exact molecular mechanism was unclear yet. Altered the topology of mitochondrial DNA (mtDNA) that cause reduction of mitochondrial transcription and mtDNA copy number that may result in serious dysregulation of electron transport chain complexes, as occurring with ciprofloxacin treatment [22], that may lead to respiratory chain dysfunction that may consequently cause the observed increased in the oxidative stress [5].

The current study confirms that ciprofloxacin caused BM toxicities, as was evidenced through the significant ($P<0.05$) reduction in total RBCs counts, and total WBCs

counts and elevation in Bax contents in BM tissues homogenates (Figures 1, 2, 3).

The role of apoptosis as a mechanism of toxicity provoked by ciprofloxacin on BM was not previously described; but in this study, such drug caused significant elevation in Bax level in BM tissue homogenate (Figure 3); as mention previously that (Bax) protein cause activation of the cascade of apoptosis reactions by releasing the mitochondrial cytochrome c that helps in the successive activation of the caspases and leads ultimately to cell death [23]. and thus, the current study is considered the first that demonstrate the role of apoptosis in BM toxicity-induced by ciprofloxacin. Thus, we did not have a chance to compare the results of this study with other reports concerning this respect.

Studies showed that high concentration of lutein, either by diet intake or as supplementation, has beneficial effects on eye disorders, preventing or even ameliorative both the age-related macular degeneration (AMD) [24] and the cataract [12].

Recently, several studies suggested that lutein might indeed has compatible effects via anti-inflammatory effects [25], improving the cognitive functions [26], and reducing the risk of cancers [12], improving cardiovascular diseases [25] and the other systemic conditions [27]. Also, it has been mentioned that lutein has anti-genotoxic property, and it may attenuate the immunosuppression in mouse models induced by ultraviolet radiation [28].

The current study showed that lutein (6mg and 24mg/kg/day) attenuates ciprofloxacin-induced reduction in total -RBCs count, and -WBCs count, and ciprofloxacin-induced elevation in Bax contents in BM tissues homogenates of rats (Figures 1, 2 and 3).

Conclusion

Results of this study suggested that bone marrow suppression and apoptosis have roles in the mechanisms of BM toxicity induced by ciprofloxacin; furthermore, lutein may show some protective effects on ciprofloxacin-induced toxicity and an available as a supplementation to protect the BM during the DNA topoisomerase enzymes inhibitors chemotherapy but this require further studies to support our results.

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