Possible Protective Effects of high- versus low- dose of lutein in combination with irinotecan on Liver of Rats: Role of Oxidative Stress and Apoptosis

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Abstract

Objectives: The current study was designed to describe roles of oxidative stress via the measurement of malondialdehyde and total antioxidant markers, and apoptosis through the measurement of caspase 3 marker, as mechanisms of liver toxicity induced by irinotecan; and to explore the possible protective effects of high- and low- doses of lutein against irinotecan induced toxicity in the liver of rats.

Methods: Thirty six (36) Sprague-Dawley rats were randomly divided into six groups: **Groups I**, rats received single oral daily dose of dimethyl sulfoxide (4 ml/kg); **Group II (irinotecan-treated)**, received single oral daily dose of dimethyl sulfoxide (4ml/kg) for 25 days by oral gavage and subsequently received irinotecan (50mg/kg) on days: 5, 10, 15 (total dose=150 mg/kg) by intraperitoneal injection; **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** (**lutein+ irinotecan**), received oral dose of lutein (6mg/kg/day) and (24mg /kg/day), respectively by oral gavage for 25 successive days, and subsequently received irinotecan (50mg /kg body weight) on days: 5, 10, 15 (total dose=150 mg/kg) by intraperitoneal injection.

Results: Orally-administered lutein with total cumulative dose of irinotecan (**Groups V** and **VI**), resulted in significant reduction ($P<0.05$) of serum aspartate aminotransferase and alanine aminotransferase, and significant reduction ($P<0.05$) of malondialdehyde; but, significant elevation ($P<0.05$) of serum total antioxidant capacity; and there was significant reduction in caspase 3in liver tissues homogenates compared to the corresponding levels in the group of rats administered irinotecan (**Group II**).

Conclusion: Results of the current finding suggested that administration of lutein may be a useful compound that alleviated irinotecan induced toxicity to the liver.

Keywords: *Lutein, Irinotecan, AST, ALT, Malondialdehyde (MDA), TAOC, Caspase 3 (Casp-3).*

Introduction

The DNA topoisomerase enzymes are fundamental to cell function and abundantly found in all fields of life; and different topoisomerase enzymes precede a vast range of functions regarding to the maintenance of DNA topology within DNA transcription and replication; and are targets of a broad range of anti-microbial and cancer chemotherapeutic agents [(1)].

Irinotecan (CPT-11), a semisynthetic drug that was derived from the natural alkaloid plant camptothecin which was clinically introduced in the late of 1980s [(2)]. Such drug is vastly used in the treatment of pancreatic, colorectal, and lung cancer; furthermore, irinotecan is a prodrug for the active metabolite SN-38, which is 100- to 1000-fold further cytotoxic more than irinotecan [(3)].

Authors reported that, the mechanism of the antitumor action of irinotecan is through the inhibition of DNA topoisomerase I that it can cause DNA damage

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which consequently may lead to cell death in fast-proliferating natural cells such as intestinal basal cells and BM cells [4]; the interaction of irinotecan with topoisomerase type I (Topo I)–DNA cellular complexes has cytotoxicity in S-phase-specific. Irinotecan also kill the non-S-phase cells at greater concentrations through mechanism related to DNA damage by transcriptionally induced and other mechanism of apoptosis [5]; thus, treatment with irinotecan can be associated with gastrointestinal (GI) and hematologic toxicities; where, the main dose-limiting toxicities are sever delayed diarrhea and neutropenia, respectively [4].

Lutein is a carotenoid pigment; and it is a compound of xanthophyll group that found abundantly in kale, corn, animal fat, spinach, etc. [6]. It cannot be synthesized by human body but can be provided only by diet or supplementation [7]. Moreover, lutein can selectively be taken up into the macula of the eye where it thought to protect against the development of age-related macular degeneration [8]. Also, It has been reported that lutein exhibited antioxidant, anti-genotoxic property, reduces inflammation and immunosuppression induced by ultraviolet radiation in mouse models and may have protective activity against macular degeneration due to age effect [6].

Objectives

The current study was designed to describe roles of oxidative stress and apoptosis as mechanisms of liver toxicity induced by irinotecan; and to explore the possible protective effects of high- and low- dose of lutein against irinotecan induced toxicity in the liver of rats.

Materials and Methods

Reagents: Rat's kits Standards were obtained from SUNLONG BIOTECH CO., LTD, China.

Drugs: Irinotecan 100mg vials obtained from Fresenius Kabi, India and the pure powder of lutein was obtained from Xi`an Rongsheng Biotechnology Co., Ltd. China.

Animals and experimental design

Thirty six (36) adult Sprague-Dawley rats, each weighing 150-200gm 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, of temperature (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. The animals were divided into six groups of six rats each as follows:

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

- **Group II** (**irinotecan-treated**): received single daily oral dose of dimethyl sulfoxide (DMSO) (4 ml/kg body weight/day) for 25 days, and subsequently received irinotecan (50mg per kg body weight) on days: 5, 10, 15 (total dose=150 mg/kg) by IP injection.

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

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

- **Group V** (**6mg lutein /kg/day + irinotecan**): received oral dose of lutein (6mg/kg/day) daily by oral gavage for 25 consecutive days, and subsequently received irinotecan (50mg per kg body weight) on days: 5, 10, 15 (total dose=150 mg/kg) by IP injection.

- **Group VI** (**24mg lutein /kg/day+ irinotecan**): received oral dose of lutein (24mg/kg/day) daily by oral gavage for 25 consecutive days, and subsequently received irinotecan (50mg per kg body weight) on days: 5, 10, 15 (total dose=150 mg/kg) by intraperitoneal injection.

Twenty-four (24) hours after the end of treatment, all rats were euthanized by diethyl ether anesthesia and from each rat blood sample were withdrawn from the carotid artery at the neck and collected into the labeled centrifuging tubes and leaved to clot for 20 min at room temperature to obtain serum, which was separated by centrifugation at 3000 rpm for 20 min, and is utilized for the assessments of biochemical parameters [Aspartate

aminotransferase (AST), alanine aminotransferase (ALT), and total antioxidant capacity (T-AOC), levels].

Furthermore, rats' liver 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 the liver, then the 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 (-180C) unless worked immediately for the measurement of MDA and Casp-3 in liver tissue homogenates.

Statistical Analysis

Data was expressed as the values of mean±standard deviation (SD). Data were analyzed by utilizing computerized Statistical Package for the Social Sciences (SPSS) 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

Irinotecan (**Group II**) cause significant (P<0.05) elevations in serum levels of AST (Fig. 1), and ALT (Fig. 2) each compared to the corresponding levels in control (**Group I**) rats; and, there were significant (P<0.05) reduction in serum levels of TAOC (Fig. 3); furthermore, there were significant (P<0.05) elevations in MDA contents (Fig. 4) in liver tissue homogenates compared to control (**Group I**) rats; similarly, there were significant (P<0.05) elevations in Casp-3 level in liver tissues homogenates of rats (Fig 5) 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 serum AST, ALT, and TAOC; also, there were non-significant differences (P>0.05) in MDA and Casp-3 in liver tissues homogenates with respect to corresponding levels in **Group I** rats (Figures 1-5, respectively).

Administration of lutein at a dose of 6mg/kg body weight, and 24mg/kg each in association with irinotecan (**Groups V** and **VI** respectively) significantly (P<0.05) reduced serum enzymes AST (Fig. 1), ALT (Fig. 2) and significantly (P<0.05) elevated serum TAOC (Fig. 3) levels with respect to **Group II**; Moreover, significant (P<0.05) reduction in MDA contents in liver tissues homogenates (Fig. 4) and significantly (P<0.05) reduced Casp-3 in liver tissues homogenates (Fig. 5) with respect to **Group II**.

By comparing various markers measured in groups of rats that received low versus high doses of lutein in association with irinotecan (**Groups V** and **VI**), there were significant (P<0.05) reduction in serum levels of AST (Fig 1), and ALT (Fig 2) in **Group VI** rats compared to the corresponding serum level in **Group V** rats. Furthermore, there were non-significant elevation (P<0.05) in serum TAOC (Fig. 3), non-significant (P<0.05) differences in MDA contents (Fig. 4) and in Casp-3 (Fig 5) in liver tissue homogenate of **Group VI** rats compared to the corresponding levels in **Group V** rats.

Additionally, by comparing various markers measured in groups of rats that received low and high dose of lutein in association with irinotecan (**Groups V** and **VI**) compared to control (**Group I**) rats, there were non-significant (P>0.05) differences in serum level of AST and ALT (Fig. 1 and 2) of **Group VI** compared to the corresponding levels in **Group I** rats; there were non-significant (P>0.05) differences in serum level of TAOC (Fig. 3); MDA contents (Fig. 4) and in Casp-3 (Fig. 5) in liver tissue homogenate of **each of Group V and Group VI** rats compared to the corresponding levels in **Group I** rats.

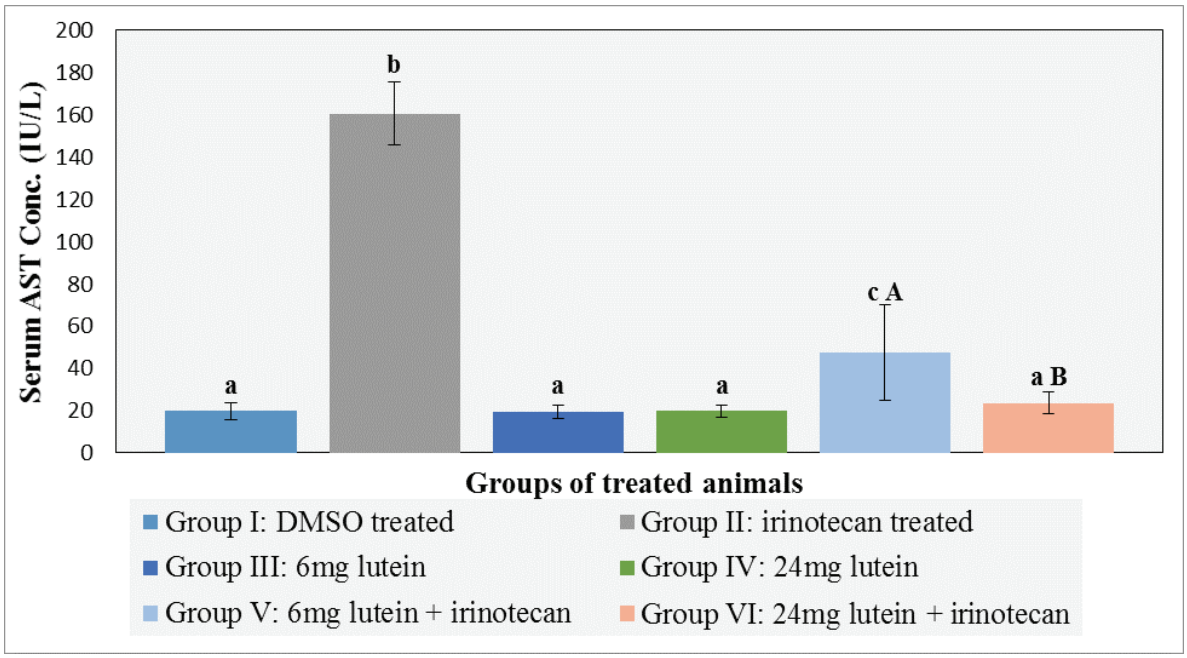

Fig. 1: Effects of lutein (6mg and 24mg) on irinotecan induced liver toxicity on serum aspartate aminotransferase (AST) in rats.

Data are expressed as Mean±SD, n =6.

Values with non-identical small letters (a, b, and c) are significantly different (P< 0.05).

Values with non-identical capital letters (A and B) are significantly different (P< 0.05).

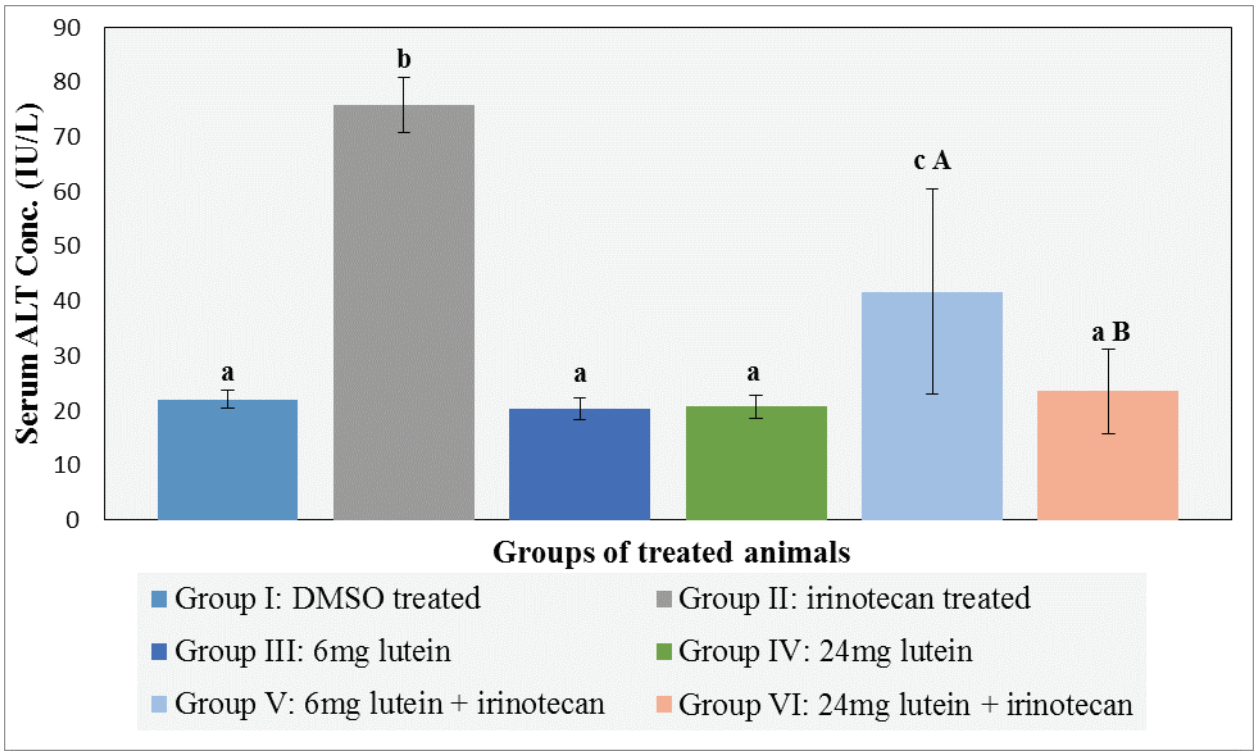

Fig. 2: Effects of lutein (6mg and 24mg) on irinotecan induced liver toxicity on Serum Alanine Aminotransferase (ALT) levels in rats.

Data are expressed as Mean±SD, n =6.

Values with non-identical small letters (a, b and c) are significantly different (P< 0.05).

Values with non-identical capital letters (A and B) are significantly different (P< 0.05).

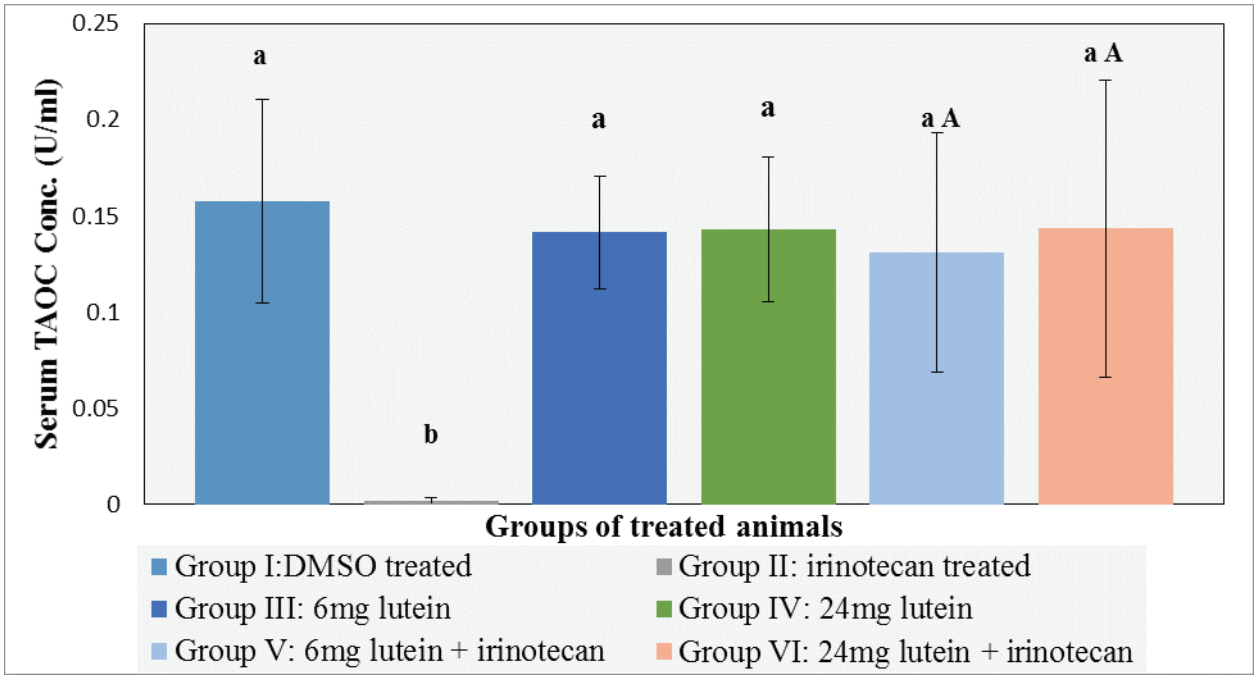

Fig. 3: Effects of lutein (6mg and 24mg) on irinotecan induced liver toxicity on serum Total antioxidant capacity (T-AOC) in rats.

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 non-identical capital letters (A) are significantly different (P< 0.05).

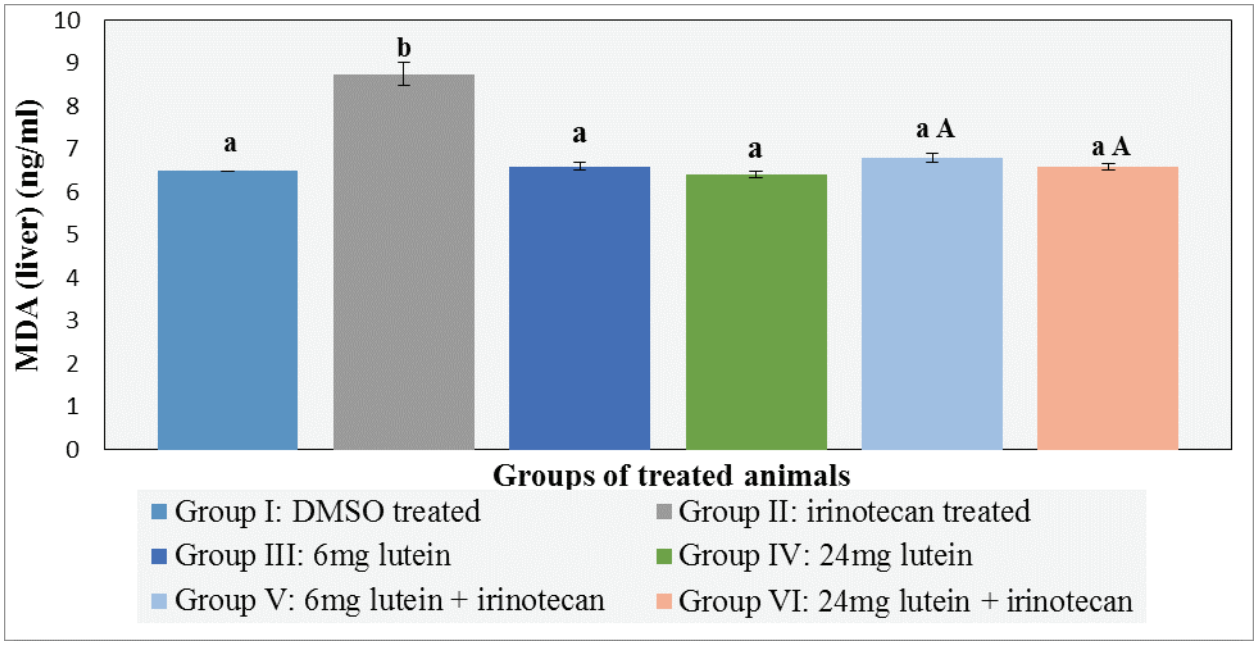

Fig. 4: Effects of lutein (6mg and 24mg) on irinotecan induced toxicity on MDA in liver tissues homogenates of rats.

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).

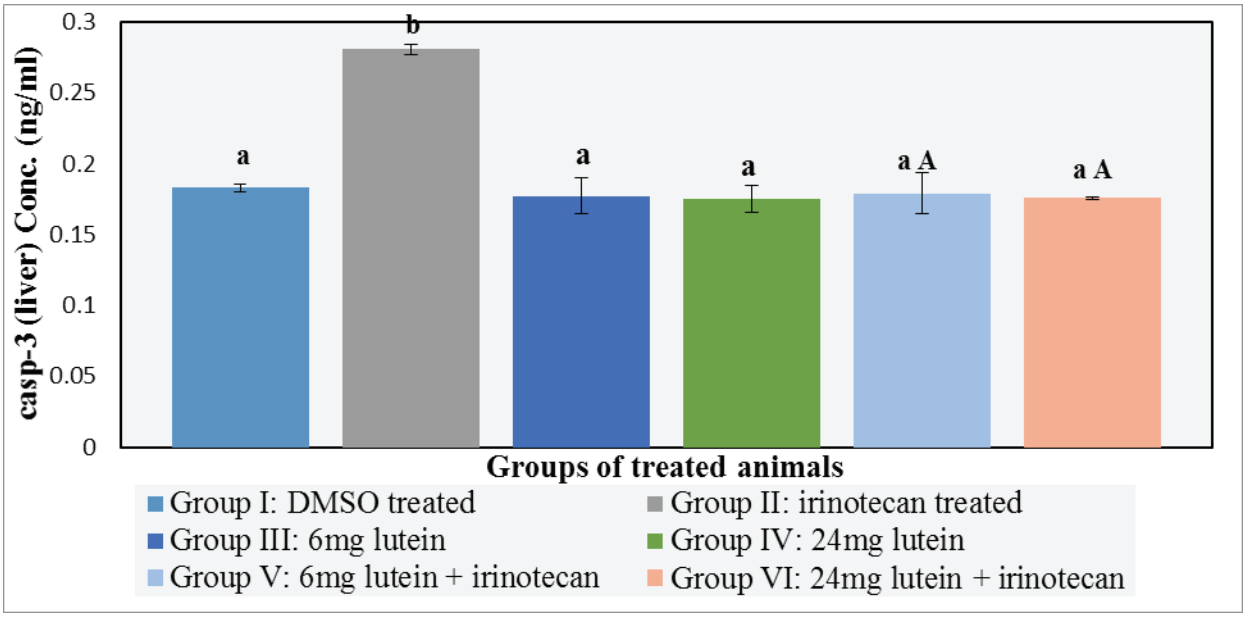

Fig. 5: Effects of lutein (6mg and 24mg) on irinotecan induced toxicity on Casp-3 in liver tissues homogenates of rats.

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).

Discussion

Severe adverse effects of the clinical use of irinotecan including myelosuppression and diarrhea can limit the using of such drug in the aggressive therapy; where, myelosuppression, especially the neutropenia, was frequently-observed in patients received irinotecan chemotherapy [9]. Moreover, authors mentioned that there was a link between irinotecan therapy and the development of steatohepatitis that pathologically characterized by inflammation, accumulation of fat (steatosis), hepatocytes ballooning, and fibrosis [4]. Although exact mechanisms by which irinotecan caused steatohepatitis are not entirely understood, oxidative stress (OS) and mitochondrial dysfunction seems to play a main role [10].

The current study confirms that irinotecan caused liver toxicities, as was evidenced through the significant (P<0.05) elevations in MDA content (figure 4) in liver tissue homogenates; in addition, there were reduction in serum TAOC (figure 3) and elevation in serum AST, and ALT (figures 1 and 2, respectively).

The role of apoptosis as a mechanism provoked by irinotecan on its toxicity on liver was not previously described; but in this study, irinotecan caused significant elevation in Casp-3 level in liver tissue homogenate (Figure 5); and thus, the current study is considered the first that demonstrate the role of apoptosis in liver toxicity-induced by irinotecan. Thus, we did not have a chance to compare the results of this study with other reports concerning this respect.

Lutein was extensively studied for its anti-inflammatory and antioxidant neuro-protectant activity in various disease models like uveitis, diabetic retinopathy, ischemia/reperfusion injury, and light induced retinopathy [11]. Furthermore, it has been reported that lutein exhibited anti-genotoxic property,

and may attenuated the immunosuppression induced by ultraviolet radiation in mouse models and may have protective activity against macular degeneration due to age effect; and it has activity in chemopreventive and can protect from macular degeneration due to age effect [6].

The current study showed that lutein (6mg and 24mg/kg/day) attenuates irinotecan -induced reduction in serum TAOC level and also attenuates irinotecan -induced elevation in serum AST, and ALT levels, and MDA contents and Casp-3 levels in liver tissues homogenates of rats (figures 1, 2, 3, 4 and 5).

Conclusion

Results of this study suggested that oxidative stress and apoptosis have roles in mechanisms of liver toxicity induced by irinotecan; besides, the utilized doses of lutein has protective effect on irinotecan-induced toxicity to the liver during the DNA topoisomerase enzymes inhibitor (irinotecan) chemotherapy.

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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