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The Effects of Vitamins D3 and B12 in Rats with Tamoxifen Induced Non-alcoholic Fatty Liver Disease

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Tamoxifen (TMX) is a synthetic drug utilized for breast cancer treatment, but it has severe adverse impacts such as hepatic steatosis. The goal of this research was to discover the molecular prophylactic mechanisms of vitamins D_3 and/ or vitamin B12 versus pathogenesis of hepatic steatosis induced by TMX treatment in female rats. The results showed that co-administration of vitamins D_3 (0.5µg/kg, i.p.) and/ or vitamin B_{12} (15 µg/Kg, i.p.) to TMX (40 mg/Kg, orally)-treated rats, significantly ameliorated the alterations in the serum total lipid, hepatic triglycerides (TG), fatty acid translocase (CD36), peroxisome proliferator-activated receptor-γ (PPAR–γ), malondialdehyde (MDA), nitric oxide (NO), reduced glutathione (GSH), interleukin-6 (IL-6), tumor necrosis factor –α (TNF – α), transforming growth factor-β (TGF-β), C-reactive protein (CRP), caspase-3, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and albumin versus the normal rats. The present biochemical studies were confirmed by histopathological investigations. Conclusion, the present results may suggest that prophylactic treatment with vitamins D_3 and/ or vitamin B_{12} may consider a promising protective therapy versus TMX induced hepatic steatosis and steatohepatitis in breast cancer patients as well as in subject at risk of incidence of liver steatosis.

Keywords: Tamoxifen, vitamin B_{12}, triglycerides, peroxisome proliferator-activated receptor-γ, liver steatosis

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is considered a serious disease worldwide due to great incidence and progression to extreme hepatic illness (Adams et al., 2006). It comprises a wide array of pathology extending from moderate steatosis to steatohepatitis (NASH; in 12 to 40 %). Steatosis is a mild state that can be treated by pharmaceutical agents, however NASH is an irretrievable condition of the disease, in which inflammatory hepatocyte damage is dominant and can advance to hepatic cirrhosis (in 15 to 25 %) and hepatic carcinoma (in 7 %) through 10 years (Lobaom and Sanyal, 2013). Abnormal accumulation of fats in hepatocytes can be induced by the following disorders: (i) elevated uptake of free fatty acids (FFA) by hepatocytes from lipolyzed TG in adipose tissue or dietary lipids; (ii) increased de novo lipogenesis (DNL); (iii) block of mitochondrial fatty acid oxidation; and (iv) reduced lipid release from hepatocytes as VLDL (Postic and Girard, 2008;Nagle et al., 2009).

Fatty acid transport protein, known as fatty acid translocase (CD36), is a plasma membrane protein that has a key role in hepatic fat accumulation via increasing hepatic FFA uptake from circulation (Miquilena-Colina et al., 2011).

Elevated hepatic expression of CD36 and a decrease in its expression by adipose tissue have been reported in NAFLD and appear to mediate increased FA uptake by hepatocytes, suggesting that this membrane FA transport protein has an important role in steatosis pathogenesis and fat building up via switching blood FFA uptake from the adipocytes into the hepatocytes (Miquilena-Colina et al. 2011).

Peroxisome proliferator-activated receptor – γ (PPAR-γ) is a nuclear transcription factors that has a fundamental role in the incidence of steatosis via the expression of genes implicated in lipid uptake and lipogensis (Sfeir et al., 1997; Morán-Salvador et al., 2011). Thus, CD36 and PPAR-γ are considered important molecular targets for therapeutic agents against hepatic steatosis.

Tamoxifen (TMX), 2-[4-[(Z)-1,2-diphenylbut-1-enyl phenoxy] - N,N-dimethylethanamine, is a non-steroidal anti-estrogen agent, utilized broadly for treating breast cancer (El-Beshbishy, 2005). Although the beneficial impacts of TMX, treatment with this agent has shown many side impacts in patients with breast cancer (Pan et al. 2016). NAFLD is one of the side effects of TMX (Pan et al. 2016). About 43% of breast cancer patients who are used TMX as a chemotherapy, have developed fatty liver (Pan et al., 2016). Hepatic deleterious impacts of TMX, include increase in hepatic function indices, jaundice, steatohepatitis, hepatic necrotic cell death (Bruno et al., 2005) and even cirrhosis (Oien et al., 1999). The mechanisms by which TMX induces hepatic steatosis have been addressed by many investigations. It has reported that TMX stimulates liver lipid accumulation by promoting TG biosynthesis (Cole et al., 2010), suppressing fatty acid oxidation and decreasing hepatic TG secretion (Larosche et al., 2007). It has reported that the alteration in lipid metabolism of TMX-mediated steatosis is greatly related to the dosage (0.5- 200 mg/kg/day) and exposure time (5-28 days) (Larosche et al., 2007). Oxidative stress and inflammation are the major mechanisms of TMX that lead to NASH (El-Beshbishy et al., 2010; Koek et al., 2011).

Attempts to discover effective therapeutic agents for mitigating liver steatosis and steatohepatitis induced by drugs are considered very urgent in clinical conditions. Without effective treatments of liver steatosis at an early stage, irreversible liver damage may be caused by steatohepatitis.

Vitamin D_3 (calcitriol) is a fat-soluble vitamin (1α-25-dihydroxy vitamin D_3 [1, 25(OH) 2D3]). The correlation between vitamin D_3 and fatty liver has been confirmed first by Targher et al (2007), who found that the depletion in blood calcitriol level is related to the development of NAFLD. Subjects with fatty liver have low serum level of 25-hydroxy vitamin D, indicating that vitamin D deficiency has a principle role in fat accumulation in liver. Vitamin D_3 has many pharmacological properties, including hypolipidemic, antioxidant, anti-inflammatory, antifibrinogenic and antiapoptosis activities (Gren, 2013; Beilfuss et al. 2015 ; Yüksek et al., 2017).

Vitamin B_{12} (cyanocobalamin) is a water dissolvable vitamin that has a key function in lipid metabolism. It functions as a cofactor for the mitochondrial methyl malonyl CoA mutase, which controls the delivery of long-chain fatty acyl-CoA into the mitochondria, thus affecting metabolic lipid mechanisms (O'Learyand Samman, 2010). Studies have illustrated that a deficiency in B12 has deleterious impacts on lipid metabolism in animal models (Deshmukh et al., 2013). Koplay et al (2011) reported that depletion in blood B_{12} in subject with NAFLD was correlated to liver dysfunction. Cobalamine has many therapeutic activities for the treatment of various pathological situations related to hyperlipidemia, inflammation, apoptosis, oxidative stress, fibrosis and cellular damage (Veber et al., 2008 ; Majumdar et al., 2012; Ahmad et al., 2014; Abdulmajeed et al., 2015; Hajihashemi et al., 2017).

This research was undertaken to explore the efficacy of TMX on the expressions of PPAR γ and CD36 as molecular mechanisms of hepatic steatosis induced by TMX. Also the potential roles of both vitamins D_3 and B_{12} in counteracting hepatic fat accumulation and its complications, including oxidative stress, inflammation, apoptosis and fibrosis under the effect of TMX treatment in female rats were investigated. To the extent of our information, no in vivo studies have demonstrated the adverse impact of TMX on the expression of hepatic CD36 and PPAR γ, which are responsible for building up of hepatic fats. In addition, the current research for the first time investigated the prophylactic mechanisms of vitamin D3 and/or vitamin B12 versus hepatic steatosis and steatohepatitis induced by TMX.

MATERIALS AND METHODS

Chemicals

Tamoxifen (TMX), 2-[4-[(Z) -1, 2-diphenylbut-1-enylphenoxy] -N, N imethylethanamine tablets

(Astrazeneca UK Ltd, UK), with the National Agency for Food and Drug Administration and Control [NAFDAC] registration no 83/7/25. Vitamin D3 (1,25 OH cholecalciferol; C27H44O; ID IUPAC: (3β, 5Z, 7E)-9,10-secocholesta-5,7,10 (19)-trien-3-ol) injection (Memphis Co. for Pharm. & Chem. Ind, Cairo , Egypt) with NAFDAC registration no 2010/26505.Vitamin B_{12} (cobalamin) injection (Eisai Co, Ltd. , Japan) with NAFDAC registration no 98/50/27.

Animals and treatment

Fifty female albino rats (eight weeks age), weighing 150-170 g, were utilized for this work. The rats were bought from Laboratory Animal Production, King Fahd Research Centre, King Abdulaziz University. Animals were housed under controlled conditions (23-25 ºC, humidity 50-65%, 12 h dark/light cycles). Animals were given a diet with standard composition and water *adlibitum*. Rat handling was performed in accordance to the roles of the King Abdulaziz University, Faculty of Science (Approval number 25-18). The animals were left for seven days for adaptation and then distributed into five groups, each of 10 rats.

Group I: Normal animals treated orally with normal saline only.

Group II: Rats treated orally with a suspension of TMX (40 mg/Kg) (Gudbrandsen et al., 2006) for 28 consecutive days.

Group III: Rats treated with TMX concurrently with vitamin D_3 injection (0.5 µg/Kg, i.p.) (Elattar et al., 2017) for 28 consecutive days.

Group IV: Rats treated with TMX concurrently with vitamin B_{12} injection (15 µg/Kg, i.p.) (Ekaidem et al. 2006) daily for 28 days.

Group V: Rats treated with TMX concurrently with the combination of vitamin D_3 and B_{12}.

After the experimental period, rats were fasted overnight (12-14 hours). Blood specimens were taken for clotting and serum separation by centrifugation at 2000 g for 15 min and kept at -20 ºC till use. The animals were then scarified and the livers were taken, washed with normal saline and weighed for biochemical analysis and histopathological examination.

Biochemical analysis

Serum analysis

Serum total lipid as well as ALT, AST and albumin were measured as a biomarkers of liver injury utilizing an automated analyzer.

Liver tissue analysis

TG in liver tissue was measured using commercial kits (Sigma-Aldrich). Nitrite level (as an indicator of NO production) and malondialdehyde (MDA, marker of lipid oxidation) were assayed as markers of oxidative stress (Buege and Aust, 1978; Moshage et al. 1995). GSH was estimated as antioxidant marker (Bentler et al. 1963). Rat ELISA kits were used for estimation of PPAR-γ (MyBioSource, Inc, USA), CD36 (BosterBio 3942 B Valley Ave,Pleasanton, CA, USA), TNF – α (ABCAM, UK) , TGF- β (ABCAM, UK), CRP (ABCAM, UK) and caspase-3 (LifeSpan BioSciences, Inc) following the instructions supplied by the manufacturer.

Histopathological studies

Liver tissues were examined to evaluate the histomorphological changes in different experimental TMX groups. Liver specimens were collected and fixed in 4% formaldehyde for 24 hours, then embedded into paraffin, sectioned for 5–6-µm thick, and mounted on the microscope slides. Some sections were stained with hematoxylin and eosin (H&E) and others were stained with Masson's trachoma (MT) to show the deposition of collagen fibers.

Statistical analysis

Data were analyzed by comparing the mean values for different TMX groups with the mean values of controls. Results are expressed as mean ± SD. Significant differences among values were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni's test post-ANOVA. Values were considered statistically significant at $p \leq 0.05$. Correlations between the studied parameters were analyzed utilizing Pearson's correlation analysis.

RESULTS

Figure 1 demonstrates the influence of vitamins D_3 and /or B_{12} on fatty liver biomarkers, including serum total lipid, hepatic triglycerides (TG), hepatic fatty acid translocase (CD36) and hepatic peroxisome proliferator-activated receptor-γ (PPAR–γ) in TMX –treated rat groups. The data revealed that treatment of female rats with TMX, significantly boosted the levels of these markers versus the normal ones ($P \leq 0.001$. Co-injection of vitamins D_3 and /or B_{12} to TMX - treated rats, markedly reduced the increases in these indices versus TMX-untreated ones ($P \leq 0.001$).

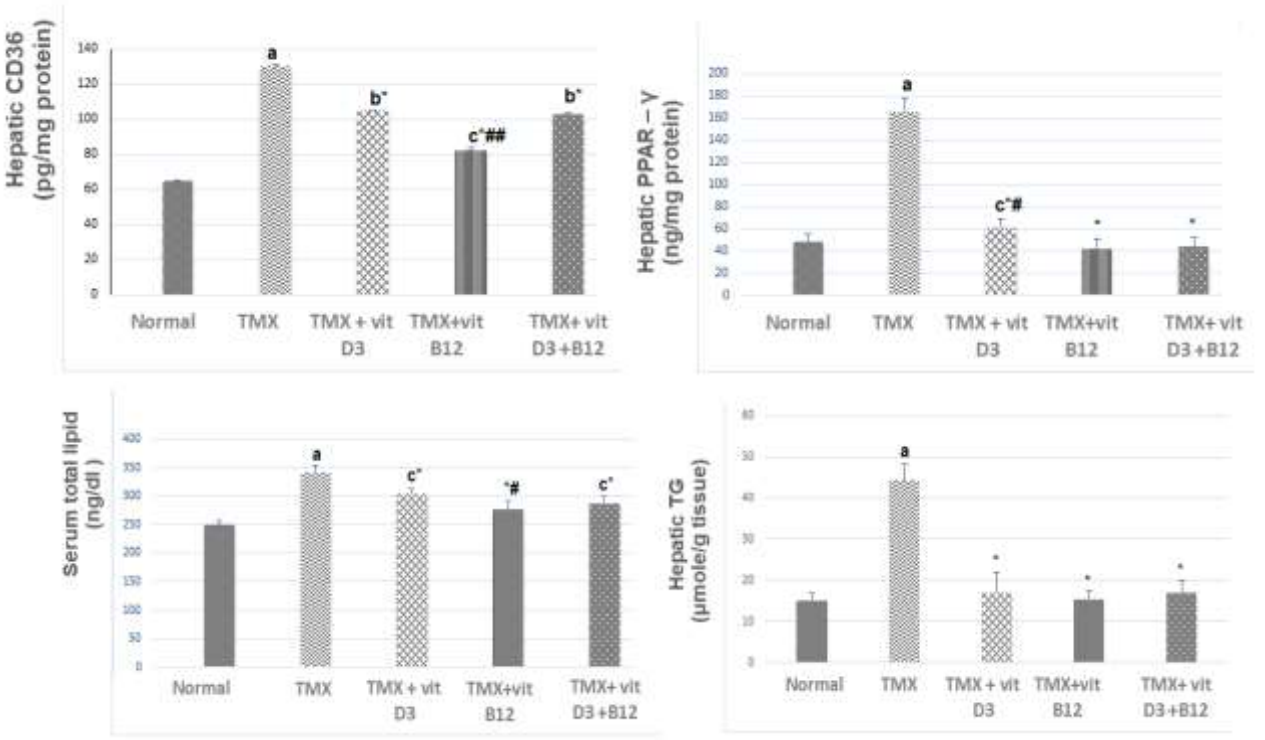

Figure 1: Effect of vitamins D3 and/or B12 on fatty liver markers in TMX induced fatty liver rats. Values are expressed as mean ± SD of 10 rats. [a]P≤0.001, [c]P≤0.05, compared with normal group; [*]P≤0.001compared with TMX treated group; [#]P≤ 0.05, [##]P≤ 0.01 compared with the combination group (TMX+D3+B12).

Table 1: Effects of vitamins D3 and /or B12 on the body and the liver weights in TMX treated rats

Parameters	Normal	TMX	TMX +D3	TMX +B12	TMX +D3 +B12
Final body weight (g)	188.33±7.50	186.66±7.09	182.66±3.21	184.33±7.50	183.33±10.01
Liver weight /100g body weight (g)	3.03±0.21	4.66±0.20[a]	3.03±0.15[*]	2.70±0.26[*]	2.86±0.20[*]

Values are expressed as mean ± SD of 10 rats. [a]P≤0.001, versus the normal group; [*]P≤0.001, versus the TMX treated group.

The obtained results showed also non-significant differences in the final body weights of different TMX treated rat groups versus the normal group, however, liver hypertrophy was seen in TMX treated rats as shown by pronounced increases in their liver weights with respect to normal ones (P≤0.001) (Table 1). Injection of TMX treated rats with D_3 and /or B_{12}, effectively reduced their liver weights close to the normal values.

The impacts of vitamins D_3 and /or B_{12} on hepatic oxidative stress (MDA and NO) and antioxidant (GSH) markers in TMX -treated rats are illustrated in Figure 2. The data showed that TMX significantly increased the levels of hepatic MDA (an index of membrane lipid peroxidation) and NO (an index of nitrosative stress) and decreased the concentration of hepatic GSH versus the normal ones (P≤0.001). Injection of vitamins D_3 and /or B_{12} simultaneously with TMX administration effectively ameliorated the alterations in these indicators close to their normal levels.

Figure 3 shows elevation in hepatic inflammatory molecules, namely TNF-α, IL-6, TGF-β and CRP as well as in hepatic apoptotic enzyme, caspase-3, in TMX treated rats with relation to normal animals (P≤0.001). Co-injection of D_3 and /or B_{12} to TMX- treated rats markedly reduced the level of these markers with respect to

TMX untreated rats.

Table 2 reveals increases in the activity levels of serum ALT and AST and a depletion in albumin concentration in TMX treated rats versus the normal animals (P≤0.001). Injection of D3 and /or B 12 concurrently with TMX administration markedly diminished the serum levels of ALT and AST in comparison to TMX untreated ones (P≤0.001) and restore the albumin close to the normal level. A positive correlation was found between the studied parameters (1 ≥ r ≥0.984).

Histopathological observation of liver tissue

The histological sections of normal and TMX treated rat groups were examined by H&E staining (Figure 4, a–f) and MT (Figure 5, a-f) staining. H& E liver sections of normal animals exhibited normal liver architecture (Figure 4a). Rats treated with TMX (Figure 4b) exhibited variable histopathological changes and marked injury as evidenced by a disruption of tissue architecture, vesicular steatosis (appeared as scattered fat droplets with different sizes), apoptotic degenerative changes of many hepatocytes with condensed/ karyopyknotic nuclei and/or with devoid nuclei, congestion and accumulation of red blood cell in the sinusoidal spaces as well as in the central vein. Infiltration of inflammatory immune cells was also evident (Figure 4c). Liver sections of rats injected with vitamin D_3, B_{12} or their combination concurrently with TMX administration (Figure 4d, 4e &4f respectively) showed more or less normal hepatocytes with no vesicular steatosis. MT liver sections of normal rats showed normal collagen deposition (Figure 5a). However, liver sections of TMX treated rats showed large area of fibrosis observed by excessive deposition of collagen as wavelike fibrils, either singly or united in bundles around the hepatic lobules (Figure 5b) with excrescent deposition of collagen in the portal tract (Figure 5c). Prophylactic injection of vitamins D_3 (Figure 5d) or B_{12} (Figure 5e) or their combination (Figure 5f) simultaneously with TMX administration, effectively inhibited the excessive deposition of collagen fibers as shown by more or less normal collagen deposition .

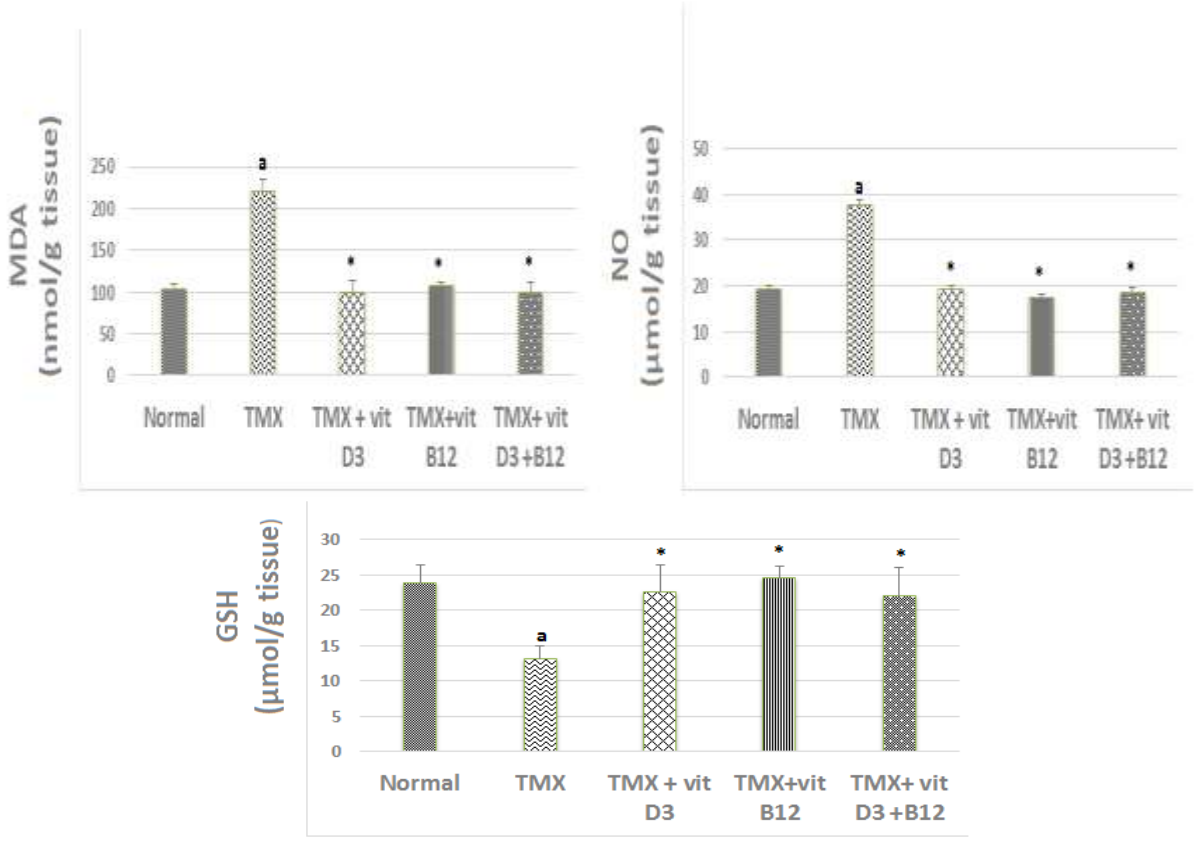

Figure 2: Effect of vitamins D3 and /or B12 on oxidative and antioxidant markers in TMX treated rats. Values are expressed as mean ± SD of 10 rats. [a]P≤0.001 versus the normal group; *P≤0.001 versus the TMX treated group.

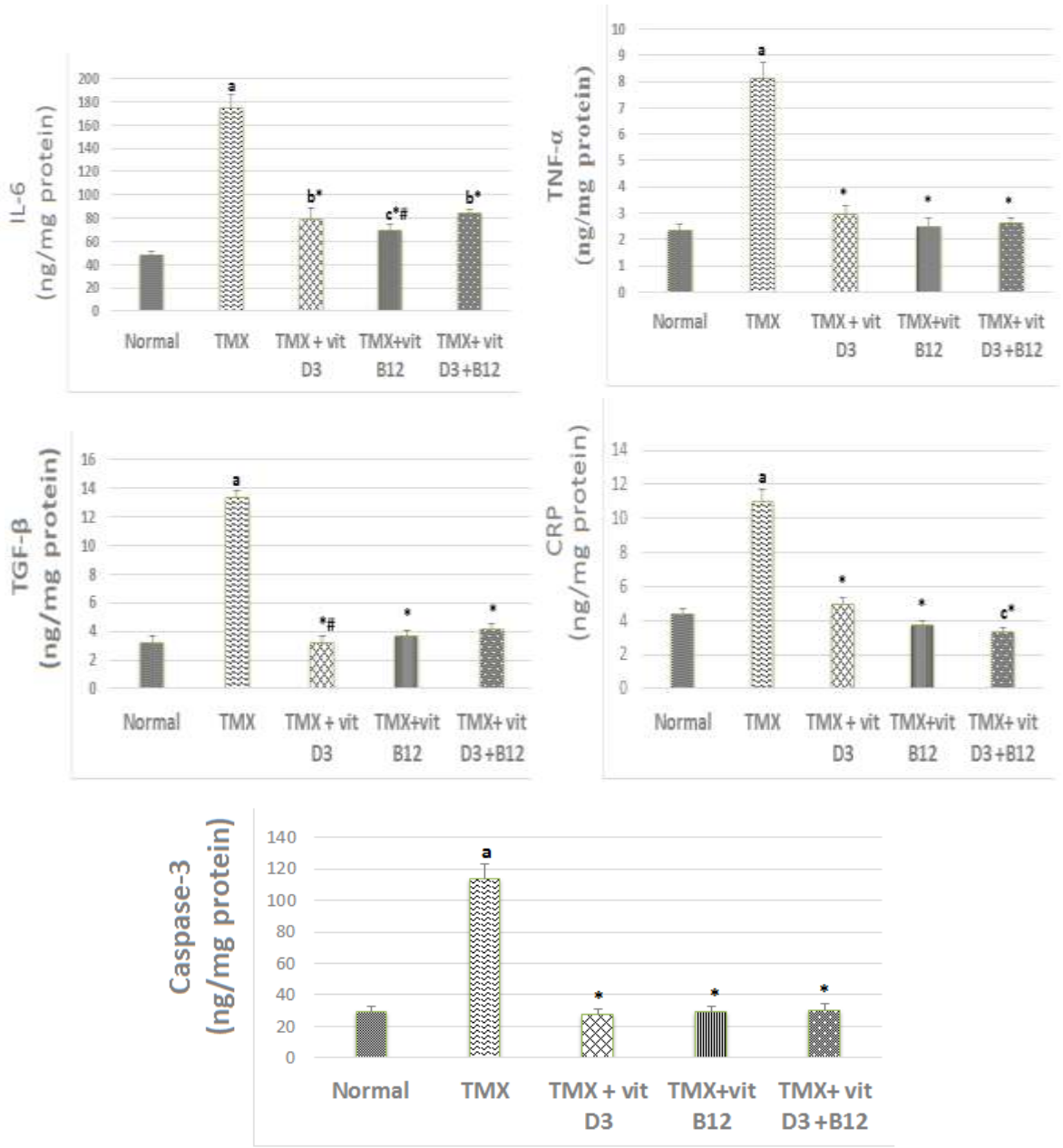

Figure 3: Effect of vitamins D3 and /or B12 on hepatic inflammatory, fibronogenic (IL-6, TNF-α, TGF-β and CRP) and apoptosis (caspase-3) markers in TMX treated rats. Values are expressed as mean ± SD of 10 rats.

[a]P≤0.001, [b]P≤0.01, [c]P≤0.05 versus the normal group; *P≤0.001 versus the TMX treated group; [#]P≤ 0.05 versus the combination group (TMX+D3+B12).

Table 2: Effect of vitamins D3 and /or B12 on serum hepatic damage markers in TMX treated rats

Parameters	Normal	TMX	TMX+D3	TMX+B12	TMX+D3+B12
ALT (U/L)	17.40±1.62	78.88±4.26[a]	24.71±2.65[c*#]	19.18±1.06[*]	19.18±2.13[*]
AST (U/L)	33.39±4.03	170.22±20.98[a]	34.41±1.69[*]	40.48±2.11[*]	33.75±3.42[*]
Albumin (g/dl)	4.92±0.535	2.75±0.495[a]	3.99±0.572[**c]	4.49±0.149[*]	3.99±0.253[**c]

Values are expressed as mean ± SD of 10 rats.[a]P≤0.001, [c]P≤0.05 versus the normal group. [*]P≤0.001, [**]P≤0.01 versus TMX treated group. [#]P≤ 0.05 versus the combination group (TMX+D3+B12).

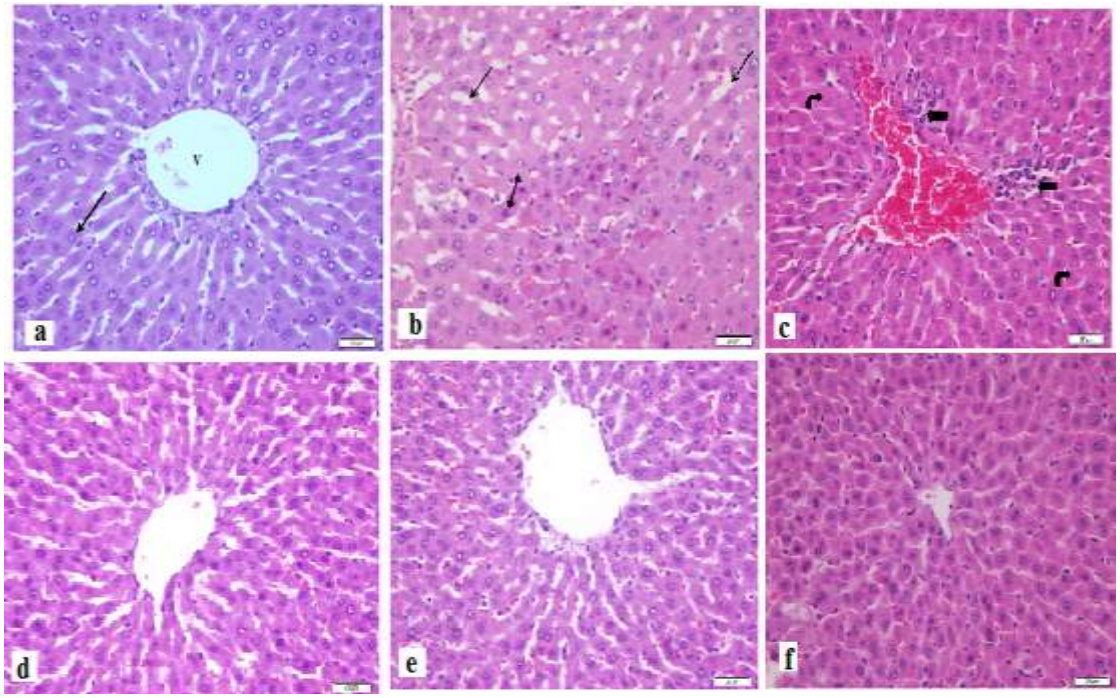

Figure 4: Light micrograph of rat liver sections of TMX treatment groups stained with H&E

(a) liver section of normal rat, showing normal hepatocytes around the central vein (V). (b) liver section of TMX treated rats showing , vesicular steatosis appeared as scattered fat droplets with different sizes (arrows), many hepatocytes with apoptotic degenerative changes and condensed/ pyknotic nuclei (double arrows) and other cells appear with devoid nuclei. The section also showing congestion and accumulation of red blood cell in the sinusoidal spaces. (c) Another rat liver section treated with TMX , showing congestion of central vein , infiltration of inflammatory immune cells (arrows) and hepatocytes with devoid nuclei (bent arrows). (d, e & f) liver sections of TMX treated rats concurrently with vitamins D3 (d), B12 (e) or vitamins D3 + B12 (f) , showing more or less normal liver architecture with complete absence of vesicular steatosis (H&E, X400).

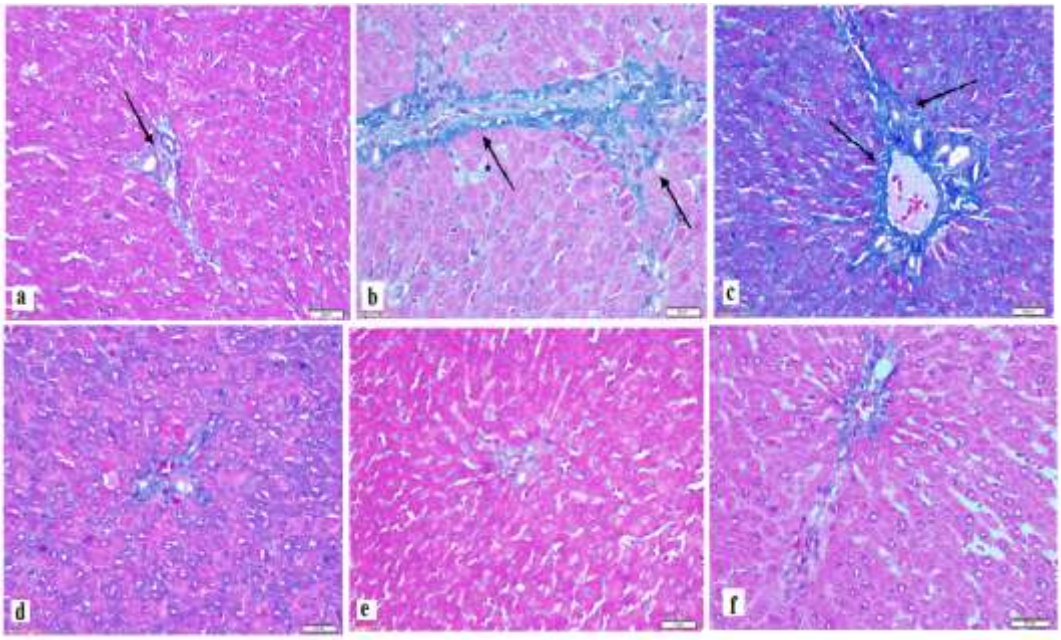

Figure 5: Light photomicrograph of rat liver sections of TMX treatment groups stained with Masson's trichrome.

(a) Liver section of normal rat showing normal distribution of collagen fibres in the portal tract (arrow). (b) Liver section of TMX treated rats showing large area of fibrosis observed by excessive deposition of collagen as wavelike

fibrils, either singly or united in bundles (arrows) surrounding the hepatic lobules. (c) Another rat liver section treated with TMX, showing bile duct proliferation with severe deposition of collagen in the portal tract (arrows). (d, e & f) liver sections of TMX treated rats concurrently with vitamins D3 (d), B12 (e) or vitamins D3 + B12 (f), showing normal collagen deposition in the portal tract (MT X 400).

DISCUSSION

TMX is considered an important innovation in the breast cancer therapy; however, its adverse influence on non-target organs is of main interest in its efficient clinical use (Pan et al., 2016). TMX has reported to have a major role in the development of steatotic liver damage (Pan et al., 2016). The hepatotoxic effect of TMX may be attributed to the greater affinity for hepatic tissue than for other ones (Desai et al., 2002).

In line with previous published data, the current research revealed that oral administration of TMX to female rats, significantly increased the levels of hepatic steatosis markers, namely triglycerides (TG) and serum total lipid, which may ascribe to the imbalance between the synthesis, the uptake, the oxidation, and the export of lipids, resulting in extreme fat aggregation in the liver (Cole et al., 2010; Pan et al., 2017). The decrease in hepatic TG and serum total lipid in rats upon treatment with vitamins D_3 and/or B_{12} concurrent with TMX administration may attributable to the potential hypolipidemic influence of both vitamins (Wang et al., 2012 ; Abdulmajeed et al., 2015) .

The marked increase in the level of hepatic nuclear receptor, PPAR-γ, in TMX treated rats, suggesting that induction of this nuclear receptor may consider one of the important molecular mechanisms of hepatic steatosis associated with TMX therapy (Sfeir et al., 1997; Morán-Salvador et al., 2011). Injection of vitamins D_3 and/or B_{12} to TMX treated rats, simultaneously with TMX administration, significantly reduced the level of hepatic PPARγ compared with TMX-treated animals. This study provides the first evidence that both vitamins could alleviate hepatic steatosis through down-regulation the expression of PPARγ which may be regarded as one of the molecular therapeutic target in treatment of hepatic steatosis.

The present significant elevation in hepatic CD36 level in TMX –treated rats may consider another significant molecular mechanism of TMX induced hepatic steatosis. Some authors have documented that the hepatic expression of CD36 is weakly, but its expression is boosted in animals with fatty liver (Inoue et al. 2005). Studies showed that CD36 is the most target gene of hepatic PPAR-γ which aggravates hepatic steatosis in experimental model (Zhou et al., 2008) and this may potentiate the key adverse influence of this FA transporter in the setup of steatosis (Koonen et al., 2007). Similarly, a clinical investigation supported that the expression CD36 gene is dramatically boosted in NAFLD patients (Miquilena-Colina et al., 2011). Injection of vitamins D_3 and/or B_{12} to TMX treated rats, markedly reduced the increase in hepatic CD36 with respect to TMX-treated animals. This study provide for the first time a confirmation of down modulating effect of both vitamins on CD36 as another important molecular therapeutic target in treatment of hepatic steatosis associated with TMX treatment.

The increase in liver weights of TMX treated rats may confirm the intrahepatic fat aggregation induced by TMX. This result is supported by Nair et al., (2015) who stated an increase of liver weight in a rat model with hepatic steatosis. The marked reduction in the rat liver weights by injection of both vitamins lonely or in a combination may be due to their abilities to mitigate hepatic steatosis via hypolipidemic action as well as down-regulation of PPAR –γ and CD36 expressions.

It has reported that oxidative stress is one of important mechanisms by which TMX can promote hepatic steatosis to NASH (El-Beshbishy et al., 2010; Yarana et al., 2017). The increment in hepatic MDA (an index of lipid peroxidation) and NO (an index of nitrosative stress) with a concomitant decrement in the antioxidant, GSH in TMX- treated rats may ascribe to the excessive production of free radicals which can attack cellular components, including lipids, proteins and DNA, leading to development of steatohepatitis (El-Beshbishy et al., 2010; Koek et al., 2011). Injection of vitamins D_3 and /or B_{12} to TMX treated rats, effectively ameliorated the alterations in hepatic MDA, NO and GSH close to their normal levels, indicating the potential antioxidant effect of both vitamins (Hajihashemi et al., 2017 ; Iqbal et al., 2018).

Inflammation is another mechanism by which TMX can promote hepatic steatosis to steatohepatitis and fibrosis (El-Beshbishy et al., 2010; El-Dessouki et al., 2018). The significant increases in the hepatic proinflammatory

proteins namely IL-6, TNF-α , TGF-β and CRP in association with an increase in hepatic apoptosis enzyme, caspase -3 in rats under the effect of TMX treatment may support the establishment of chronic inflammatory conditions with subsequent apoptotic hepatic cellular death and ultimately the development of NAFLD (Song et al., 2017) . On the other hand, induction of TNF-α , TGF-β has a key role in liver fibrosis via activation of hepatic satellite cells (HSCs) which have the central role in the production of extracellular, matrix proteins including collagen (Conte et al., 2014; Komiya et al., 2017). Increased collagen synthesis as an indicator of liver fibrosis induced by TMX was confirmed in the present study by Masson's Trichrome staining (MT) of liver sections. The result showed excessive deposition of collagen fibrils in dense bundles surrounding the portal area and hepatic lobules in TMX treated rats. This observation was documented by Morsy et al (2010). The present data are compatible with other authors who reported that treatment of rats with TMX caused hepatic inflammatory and apoptotic damage via inducing the generation of inflammatory mediators (TNF-α , IL-1β and others) and caspase -3 activity which contribute to NASH and liver fibrosis (El-Beshbishy et al. 2010; El-Dessouki et al., 2018). Injection of vitamins D_3 and /or B_{12} to TMX treated rats, effectively ameliorated the increases in hepatic TNF-α, IL-6, TGF-β, CRP and caspase-3. Suppressing collagen synthesis with vitamins administration was also evident as seen by TM staining. These results suggest that the protective effect of both vitamins against steatosis induced inflammatory, apoptotic and fibrotic liver damage may be due to their anti-inflammatory, antifbrogenic and antiapoptosis beneficial actions (Nakano et al., 2011; Majumdar et al., 2012; Yousef and Mohamed; 2015, El-Sherbiny et al., 2018).

In parallel with other studies, the pronounced alterations in the serum liver function parameters (ALT, AST and albumin) accompanied with severe histopathological degenerative changes of liver tissue in rats exposed to TMX toxicity may reflect the hepatocellular injury (El-Dessouki et al., 2018). Injection of vitamins D_3 and / or B_{12} to TMX treated rats, effectively ameliorated the alterations in the serum liver function indices as well as the histopathological pictures of liver tissue as shown by normal liver architecture with no vesicular steatosis.

CONCLUSION

The current investigation demonstrated that treatment with TMX caused liver steatosis which can progress to steatohepatitis via inducing oxidative stress, inflammation, apoptosis and fibrosis. Co-treatment with vitamins D_3 and /or B_{12} can protect the liver tissues versus steatosis and steatohepatitis induced by TMX. The prophylactic mechanisms of the used vitamins were via suppressing PPARγ and CD36 expressions which have the key roles in hepatic lipogenesis and uptake of fatty acids by hepatocytes. The vitamins may act differentially. Vitamin D_3 was more effective in reducing hepatic PPARγ, TGF-β and ALT compared to its combination with vitamin B_{12} group, however vitamin B_{12} significantly decreased serum total lipid, hepatic CD36 and IL-6. These results may suggest that prophylactic treatment with these vitamins is regarded as an important protective strategy against TMX induced hepatic steatohepatitis in patients with breast cancer as well as in subjects at risk of incidence of fatty liver. This finding is considered as a promoting and a preventive care for the general population that may reduce the cost for the treatment of fatty liver related diseases.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Weam Khalil Abdali performleed the experiments animal treatments , tissue collection , Elisa experiments, and data analysis. Jehad Mustafa Yousef and Azza Mostafa Mohamed designed the experiment and also wrote the manuscript. Jehad Mustafa Yousef reviewed the manuscript. All authors read and approved the final version.

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