Life Sciences 231 (2019) 116573

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

# Hepatoprotective effect of celecoxib against tamoxifen-induced liver injury via inhibiting ASK-1/JNK pathway in female rats

Dalia H. El-Kashef*, Ahmed R. El-Sheakh

*Department of Pharmacology and Toxicology, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt*

ARTICLE INFO

*Keywords:*
ASK-1
Bax
Caspase3
JNK
Tamoxifen
TNF-α

ABSTRACT

Hepatotoxicity is a common side effect encountered with tamoxifen (TAM) administration. Due to the great value of TAM in breast cancer treatment, hepato-protection is seriously recommended.

*Aims:* The present study investigated the hepato-protective effect of celecoxib (CX) against TAM-induced hepatotoxicity in rats.

*Main methods:* Female rats were injected with TAM (45 mg/kg, i.p.) for 7 days and given CX (15 mg/kg, orally) 7 days before TAM injection, then continued for the following 7 days.

*Key findings:* Administration of CX for 14 days conferred significant hepatoprotection against TAM-induced hepatotoxicity indexed by decreased liver/body weight ratio, boosted cytoprotection and substantial reduction in serum LDH activity besides functional hepatic improvement; marked decrease in ALT, AST and ALP with significant elevation in serum albumin. Oxidant/antioxidants hemostasis was improved upon CX treatment with profound decrease in hepatic MDA content and elevation of GSH and SOD levels. Furthermore, hepatic content of NO decreased along with significant decrease in ASK-1, JNK and Bax levels as well as TNFα and caspase3 expression. Finally, CX administration resulted in obvious diminution of TAM-induced necrotic and apoptotic alterations.

*Significance:* Celecoxib might be used in combination with TAM in treatment protocol of breast to prevent liver injury induced by TAM and further clinical studies might be needed to approve this notion.

## 1. Introduction

Breast cancer is a major health problem in women all over the world. It is one of the most common causes of cancer death in both developing and developed countries [1]. Tamoxifen (TAM) is a non-steroidal estrogen antagonist that is used in treating breast carcinoma as well as being a chemopreventive agent in women vulnerable to breast cancer [2]. TAM exerts its anti-estrogenic activity via competition with estrogen for estrogen receptor (ER) in tumor tissue. This mechanism helps in decreasing the risk of recurrence after primary resection of ER-positive breast tumor [3].

Hepatotoxicity is a critical side effect regularly associated with TAM administration. However, it is not banned due to the encouraging benefit-risk ratio. TAM undergoes metabolism in liver via CYP enzymes, namely CYP3A4 and CYP2D6 and generates 4-hydroxytamoxifen and endoxifen respectively, which account for the anticancer effect of TAM. TAM hepatotoxicity is mainly mediated by overproduction of reactive oxygen species during TAM metabolism [4].

Celecoxib, is a selective COX2 inhibitor, used as analgesic, antipyretic and anti-inflammatory drug. It is also used in alleviating acute pain, arthritis and menstrual pain [5]. Previous studies reported the hepatoprotective effect of celecoxib against autoimmune hepatitis [6], ischemia-reperfusion-induced hepatic injury in rats [7] and non-alcoholic fatty liver disease [8]. This hepatoprotective effect of celecoxib might be attributed to its anti-inflammatory effect.

This study was executed to probe the impact of celecoxib against TAM-induced liver injury and highlight the probable mechanisms by appraising different biomarkers of liver toxicity, inflammation and apoptosis.

## 2. Materials and methods

### 2.1. Animal

Adult female Sprague-Dawley rats weighing (180–240) g were housed in plastic cages on a 12 h light/dark cycle and allowed food and water freely under constant temperature and humidity throughout the experiment. The experimental procedures were adopted by the

* Corresponding author.
*E-mail address:* Dalia_elkashef@mans.edu.eg (D.H. El-Kashef).

https://doi.org/10.1016/j.lfs.2019.116573
Received 20 April 2019; Received in revised form 5 June 2019; Accepted 13 June 2019
Available online 14 June 2019

Research Ethical Committee of Faculty of Pharmacy, Mansoura University, Egypt (2019-49).

### 2.2. Drug and chemical

Tamoxifen citrate (Nolvadex®) was obtained from AstraZeneca UK Limited (Macclesfield, Cheshire, UK). Celecoxib was purchased as commercial product (Celebrex capsule contain 100 mg celecoxib) from Pfizer pharmaceutical company (New York City, USA).

### 2.3. Experimental design

The animals were allocated at random into five groups (n = 6). Group 1: Control group, received 0.5% carboxymethyl cellulose (CMC) orally for 14 days; Group 2: TAM group: rats were injected with TAM (45 mg/kg/day in 0.1% dimethylsulfoxide (DMSO), i.p., for 7 successive days [9]; Group 3: TAM + CX: rats received celecoxib (15 mg/kg/day in 0.5% CMC orally) for 14 successive days and TAM was injected starting from day 8 to day 14; Group 4: CX: celecoxib-treated rats; they were orally administered celecoxib only for 14 successive days); Group 5: DMSO: rats received only 0.1% DMSO, i.p., for 14 days.

On day 15, animals were weighed, anaesthetized with secobarbital (50 mg/kg) then blood samples were withdrawn via the retro-orbital sinus and centrifuged to obtain serum. These samples were then used for estimation of liver function. Afterwards, rats were euthanized by cervical dislocation and the liver was isolated instantly by dissection. Liver was rinsed with chilled saline then divided into 2 portions. The first one was preserved in 10% w/v formalin for histopathological analysis and the other portion was used to obtain liver homogenates (10% w/v) in phosphate buffer (pH 7.5) and kept in −80 °C for further analysis.

### 2.4. Evaluation of liver biomarkers

Serum was used to determine alanine aminotransferase (ALT), aspartate aminotransferase (AST) levels, alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and albumin. Procedures were performed in accordance to the guidelines provided with the kits (BioMed-Diagnostics, Cairo, Egypt). Also, lactate dehydrogenase (LDH) activity was assessed in serum using a kit (Human, Wiesbaden, Germany) depending on the manufacturer procedures. In brief, samples were mixed with NADH, sodium pyruvate and TRIS buffer. The change in absorbance was detected at 340 nm. Ratio of liver weight to body weight (liver/body weight) was also calculated. Tissue homogenates were used to measure oxidative stress, Apoptosis signal-regulating kinase 1 (ASK-1), c-Jun N-terminal kinase (JNK) and BCL2 Associated X protein (Bax).

### 2.5. Assessment of antioxidant profile

Lipid peroxidation was evaluated as malondialdehyde (MDA) following the method of [10]. The principle depends on the reaction of MDA with thiobarbituric acid to form a pink chromogen which was measured spectrophotometrically at 532 nm. The level of GSH in the liver was evaluated depending on its reaction with Ellman's reagent in accordance to the method defined by [11]. The formed yellow color was determined colorimetrically at 412 nm. The activity of SOD was determined according to [12]. The principle depends on calculating the quantity of the enzyme that cause 50% inhibition of pyrogallol autoxidation. The change in absorbance was recorded for 3 min at 1-min interval at 420 nm.

### 2.6. Measurement of nitric oxide (NOx) content

NOx content was determined in liver homogenates following the method of [13]. The produced color was measured colorimetrically at 540 nm.

### 2.7. Estimation of ASK-1 level in liver homogenate

ASK-1 level was evaluated using commercial ELISA kit Catalogue number MBS260107 from MyBioSource (San Diego, California, USA).

### 2.8. Determination of phosphorylated JNK level in liver homogenate

Phosphorylated JNK level was assessed using RayBio® Phospho-JNK (Thr183/Tyr185) ELISA kit Catalogue number PEL-JNK-T183 from RayBio tech 3607 Parkway Lane, Suite 100 Norcross, GA.

### 2.9. Assessment of Bax level in liver homogenate

Bax level was determined using commercial ELISA kit Catalogue number LS-F5064 from Lifespan Bioscience.

### 2.10. Histological examination

Samples of liver were kept in 10% buffered formalin and specimens were entrenched in paraffin wax. These paraffinized tissues were sectioned using microtome at 5 µm then stained with hematoxylin and eosin. Tissues were randomly examined using a microscope and the degree of liver injury was scored semiquantitively as previously described [14]. Briefly, score (_) was considered normal while scores +, ++, and +++ are mild, moderate, and severe levels, reflecting < 25, 50, and 75% histopathological lesions of total fields examined, respectively.

### 2.11. Immunohistochemical evaluation of TNF-α and caspase 3

Sections were dewaxed and immersed in a solution of 0.05 M citrate buffer, pH 6.8 for antigen retrieval. These sections were then treated with 0.3% $H_2O_2$ and protein block. After that, they were incubated with polyclonal anti-TNF-α and anti-caspase 3 antibodies (Thermo Fisher Scientific, dilution 1/100). After rinsing with PBS, they were incubated with a goat anti-rabbit secondary antibody (cat. no. K4003, EnVision +™ System Horseradish Peroxidase Labelled Polymer; Dako) for 30 min at room temperature. Slides were visualized with DAB kit (Liquid DAB +Substrate Chromogen System; Dako) and eventually stained with Mayer's hematoxylin as a counterstain. The staining intensity was assessed using image J analysis software and presented as a percentage of positive cells in about 5 to 8 high power fields.

### 2.12. Statistical analysis

Values were presented as means ± SEM, and comparisons between means were performed using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test. Statistical analysis was carried out using Instat software III and Graphpad prism V, $p < 0.05$ was fixed as a level of significance in all statistical tests.

## 3. Results

### 3.1. Celecoxib ameliorated TAM-induced liver injury

Repeated injection of TAM for 7 days resulted in a progressive hepatic injury as demonstrated by significant rise in serum levels of ALT, AST, ALP, GGT and liver/body weight concomitantly with reduced albumin level when compared to control rats (Table 1). These biochemical results were confirmed by the histopathological results of liver tissue which presented marked hepatic damage in the form of focal hepatic inflammation, necrosis and fatty changes in TAM group in comparison with normal rats that revealed normal hepatic architecture (Fig. 1& Table 2). Administration of celecoxib significantly reversed these changes induced by TAM, however there was slight edema observed in liver of rats treated with celecoxib only.

**Table 1**
Effect of tamoxifen (TAM, 45 mg/kg) and/or celecoxib (CX) (15 mg/kg) on liver functions.

| | Control | TAM | TAM + CX | CX | DMSO |
|---|---|---|---|---|---|
| ALT (U/L) | 34 ± 0.7 | 105 ± 1.8$^{\$}$ | 48.3 ± 1.2$^{\$\#}$ | 39.1 ± 1.1$^{\#\&}$ | 35 ± 1.1$^{\#\&}$ |
| AST(U/L) | 114 ± 1.4 | 262 ± 7.3$^{\$}$ | 151.6 ± 12.4$^{\$\#}$ | 109.5 ± 3.2$^{\#\&}$ | 146 ± 1.7$^{\$\#*}$ |
| ALP (U/L) | 1. 2 ± 0.07 | 6.3 ± 0.15$^{\$}$ | 3.4 ± 0.07$^{\$\#}$ | 1.5 ± 0.18$^{\#\&}$ | 1.70 ± 0.14 $^{\#\&}$ |
| GGT (U/L) | 118 ± 1 | 885 ± 10.9 $^{\$}$ | 270 ± 7.3$^{\$\#}$ | 120 ± 1.8 $^{\#\&}$ | 130 ± 3.6 $^{\#\&}$ |
| Albumin (g/dl) | 3.8 ± 0.07 | 3.1 ± 0.03$^{\$}$ | 3.4 ± 0.07$^{\$\#}$ | 3.9 ± 0.07$^{\#\&}$ | 3.4 ± 0.03$^{\$\#*}$ |
| Liver/body weight ratio | 0.023 ± 0.0008 | 0.045 ± 0.0007$^{\$}$ | 0.038 ± 0.002$^{\$\#}$ | 0.032 ± 0.0008$^{\$\#\&}$ | 0.022 ± 0.001$^{\#\&*}$ |

Data expressed as means ± SEM (n = 6). Analyses performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test. \$ $p < 0.05$ vs. control; # $p < 0.05$ vs. TAM; & $p < 0.0.05$ vs. TAM + CX; * $p < 0.05$ vs. CX.

### 3.2. Celecoxib attenuated oxidative and nitrosative stress

Fig. 2 illustrated that injection of TAM produced significant elevation in MDA and NOx content in hepatic tissues simultaneously with significant reduction in levels of the antioxidants; GSH and SOD compared to control rats. However, celecoxib concurrent administration significantly reversed TAM-induced alterations.

### 3.3. Celecoxib decreased inflammatory biomarkers

As presented in Fig. 3, TAM injection resulted in marked elevation in LDH (Fig. 3I) and tumor necrosis factor-alpha (TNF-α) (Fig. 3II) levels when compared with the control group. Celecoxib administration significantly reduced these elevated levels.

### 3.4. Effect on ASK-1/JNK signaling pathway

TAM injection produced significant elevation in levels of ASK-1, JNK and Bax in tissue homogenate on comparison with the control group (Fig. 4). Celecoxib significantly reduced these abnormal alterations compared to TAM group.

**Table 2**
Effect of tamoxifen (TAM, 45 mg/kg) and/or celecoxib (CX) (15 mg/kg) on liver histopathology.

| | Control | TAM | TAM + CX | CX | DMSO |
|---|---|---|---|---|---|
| Steatosis | − | + + + | + | − | − |
| Lobular inflammation | − | + + + | + | − | − |
| Portal inflammation | − | + + + | + | − | − |
| Congestion | − | + + + | + | − | − |

Histopathological scoring of liver lesions severity. Score (−) was considered normal while scores (+, + +, and + + +) are mild, moderate and severe, reflecting < 25, 50, and 75% histopathological alterations of total fields examined, respectively.

### 3.5. Celecoxib ameliorated caspase-3 expression

As shown in Fig. 5, TAM administration produced a profound increase in the immuno-expression of caspase-3 in the hepatic tissue compared to normal animals. However, celecoxib treatment significantly decreased the protein expression of caspase-3 compared to TAM group.

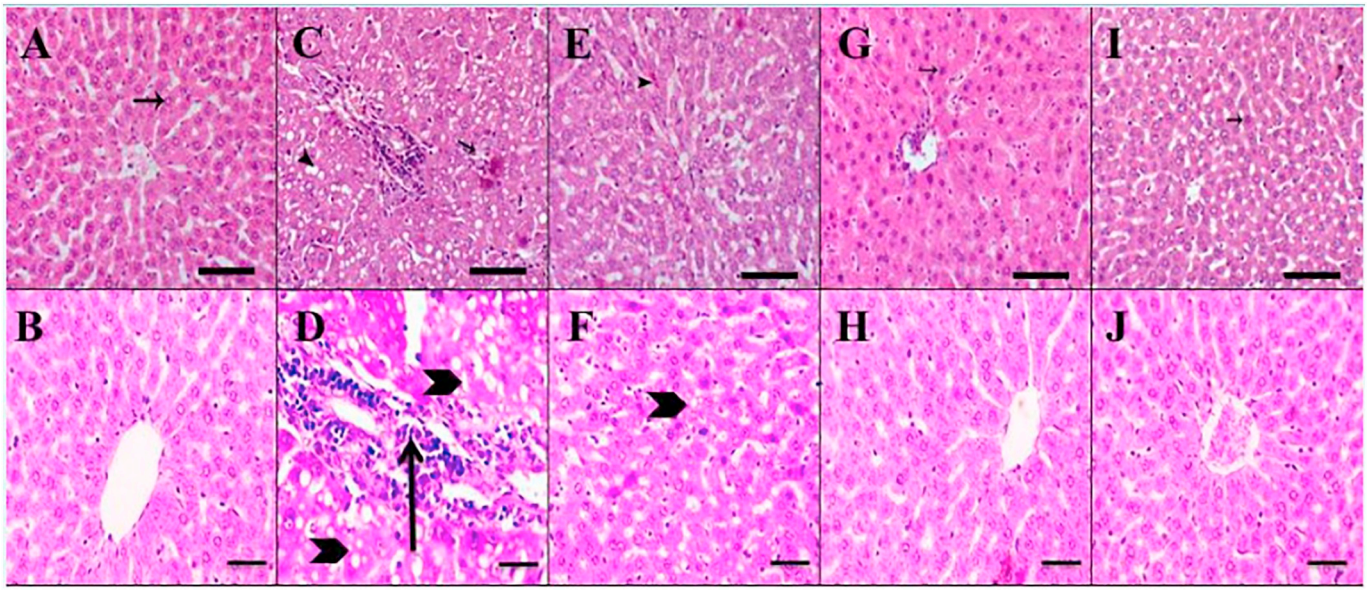


**Fig. 1.** Effect of celecoxib (CX) on tamoxifen (TAM)-induced histopathological damage.
Liver specimen of different groups stained with H&E. Control: A) Normal hepatocytes arranged in cords around the central vein (arrow) (X 200) and B) Normal radial arrangement of hepatic cords around central vein with normal sinusoids (X 400). TAM-injected rats C) Fatty changes (arrowhead) and focal hepatic necrosis (arrow) (X 200) and D) Portal inflammation (arrow) with marked macrovesicular steatosis (arrowhead) (X 400). TAM + CX group E) Normal hepatocytes with single cell apoptosis (arrowhead) (X 200) and F) Very mild microvesicular steatosis (arrowhead) (X 400). CX only group G) Liver presented normal hepatocytes with mild granular cell swelling (arrow) and activated sinusoidal cell (X 200) and H) Normal radial arrangement of hepatic cords around central vein with normal sinusoids (X 400). DMSO group I) Liver exhibited normal hepatocytes arranged in cords around the central vein (arrow) (X 200) and J) Normal radial arrangement of hepatic cords around central vein with normal sinusoids (X 400), H&E; A,C,E,G &I (bar = 100 μm) and B,D,F, H &J (bar = 50 μm).
Control group, rats received 0.5% carboxymethyl cellulose (CMC) orally for 14 days; TAM group, rats were injected with TAM (45 mg/kg/day in 0.1% dimethylsulfoxide (DMSO), i.p., for 7 successive days, TAM + CX: rats received celecoxib (15 mg/kg/day in 0.5% CMC orally) for 14 successive days and TAM was injected daily from day 8 to day 14; CX, rats were orally administered celecoxib only for 14 successive days; DMSO, rats received only 0.1% DMSO, i.p., for 14 days.

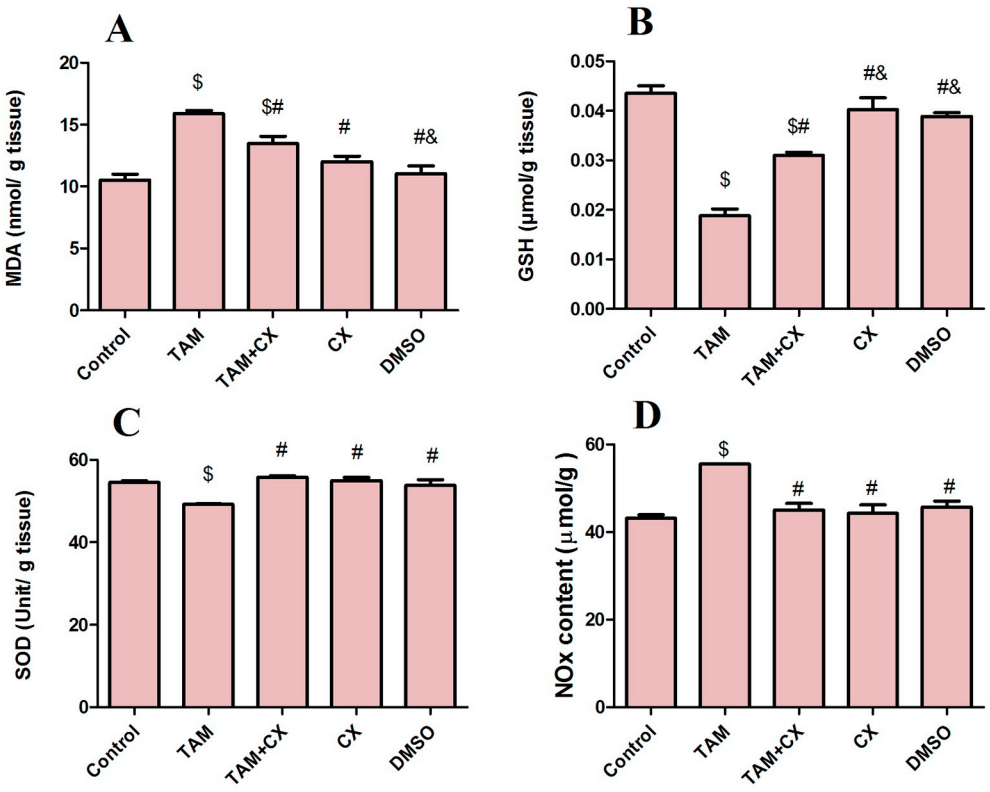


**Fig. 2.** Effect of celecoxib (CX) on tamoxifen (TAM)-induced oxidative and nitrosative stress.
A) Malondialdehyde (MDA); B) Superoxide dismutase (SOD); C) Reduced glutathione (GSH); D) Total nitrite/nitrate (NOx).
Control group, rats received 0.5% carboxymethyl cellulose (CMC) orally for 14 days; TAM group, rats were injected with TAM (45 mg/kg/day in 0.1% dimethylsulfoxide (DMSO), i.p., for 7 successive days, TAM + CX: rats received celecoxib (15 mg/kg/day in 0.5% CMC orally) for 14 successive days and TAM was injected daily from day 8 to day 14; CX, rats were orally administered celecoxib only for 14 successive days; DMSO, rats received only 0.1% DMSO, i.p., for 14 days.
Data expressed as means ± SEM (n = 6). Analyses performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test. $ $p < 0.05$ vs. control; # $p < 0.05$ vs. TAM; & $p < 0.0.05$ vs. TAM + CX; * $p < 0.05$ vs. CX.

## 4. Discussion

Drug-induced liver injury (DILI) is regarded as a main cause for withdrawal of drug and development of new treatments. Concerning the withdrawal, restriction or termination of existing drugs always happens. DILI can be aggravated by the drug itself and/or its metabolites, it may be unpredictable [15].

This study showed that TAM intoxication elicited liver injury in hepatocytes as indicated by elevation in serum levels of ALT, AST, ALP, GGT and LDH with concomitant reduction in albumin level. These observations were in accordance with [16]. Also, TAM significantly increased liver to body weight ratio, which might reflect increased edema and inflammation in the liver tissue. Co administration of celecoxib with TAM efficiently reversed these alterations in liver enzymes caused by TAM. Although a slight edema was observed in the CX group, edema is identified as a side effect of celecoxib, but it didn't affect liver function (no changes on biochemical markers). Co administration of celecoxib and TAM significantly decreased elevated LDH caused by TAM alone, which confirmed anti-inflammatory and cytoprotective effect of celecoxib alone. These finding agreed with the study of [17], and suggested that celecoxib not only exerted hepatoprotective effect against TAM but it restored liver normal function [17]. Also it has been reported that LDH production was increased in hepatocytes of patients with early stage acute liver failure [18].

Intoxication of TAM significantly increased hepatic contents of MDA and NOx and decreased levels of hepatic GSH and SOD, these findings were supported by previous study [2]. Co administration of celecoxib with TAM reduced the elevated MDA and decreased ROS activation pathway which in turn might help in liver tissue survival. In addition, celecoxib markedly increased levels of hepatic GSH and SOD when compared to TAM group, suggesting that celecoxib has antioxidant properties. Our results agreed with a previous study that demonstrated that celecoxib decreased the oxidative stress by reducing lipid peroxidation and increasing the GSH levels in cigarette smoking-inhaled mice [19].

Apoptosis signal-regulating kinase 1 (ASK-1) is a family member of the MAP3K which responds to cell damage induced by stress. Activation of ASK-1 can regulate both MKK4/MKK7-JNK and MKK3/MKK6-p38 MAPK signaling pathways [20]. ASK-1 might be activated by oxidative stress as well as inflammatory cytokines such as TNFα [21]. Upon resting, ASK-1 forms with reduced thioredoxin (Trx) an inactive complex. Under stressful conditions due to TNFα or ROS, ASK-1 is liberated from Trx and turns active [22]. Activated ASK-1 then stimulates phosphorylation of the downstream MAPKK, MKK4/MKK7 which can activate JNK and MKK3/MKK6 which in turn may activate p38 MAPK [23], thus contribute to the liver injury.

Our results showed that injection of TAM markedly increased hepatic levels of ASK and JNK in addition to increased expression of TNF-α in corresponding to normal rats. In contrast, oral administration of celecoxib significantly decreased hepatic levels of ASK and JNK, and TNF-α expression when compared to TAM group. This result matched with Farag et al. who reported that celecoxib significantly reduced TNF-α production in a model of renal ischemia/reperfusion [24]. Concerning JNK, our results were supported by a recent study that demonstrated that celecoxib inhibited JNK in radiation-resistant lung cancer cells [25]. These observations proposed that celecoxib might act against liver injury via its anti-inflammatory properties.

TNF-α could bind to cell surface receptors then stimulate the activation and degradation of caspase 8 and caspase 10; initiator caspases. Upon activation, caspase 8 could galvanize two diverse apoptotic pathways. The first one is cleavage and activation of effector caspases like caspases 3 and 7, which in turn cleave many target proteins which have essential roles in mediating apoptosis. The other one, caspase 8 could stimulate a mitochondrial pathway which is umpired by the caspase 8 substrate Bid. After Bid activation, it translocates to the mitochondria, where it cooperates with Bcl2 family members to stimulate cytochrome C release which in turn results in activation of caspase 9 followed by cleavage and activation of caspase 3, resulting in apoptosis [26].

Bcl-2 proteins are essential in controlling apoptosis. These proteins include anti-apoptotic proteins such as Bcl-2 and pro-apoptotic proteins, such as Bax. Any factor disrupts the balance between these two

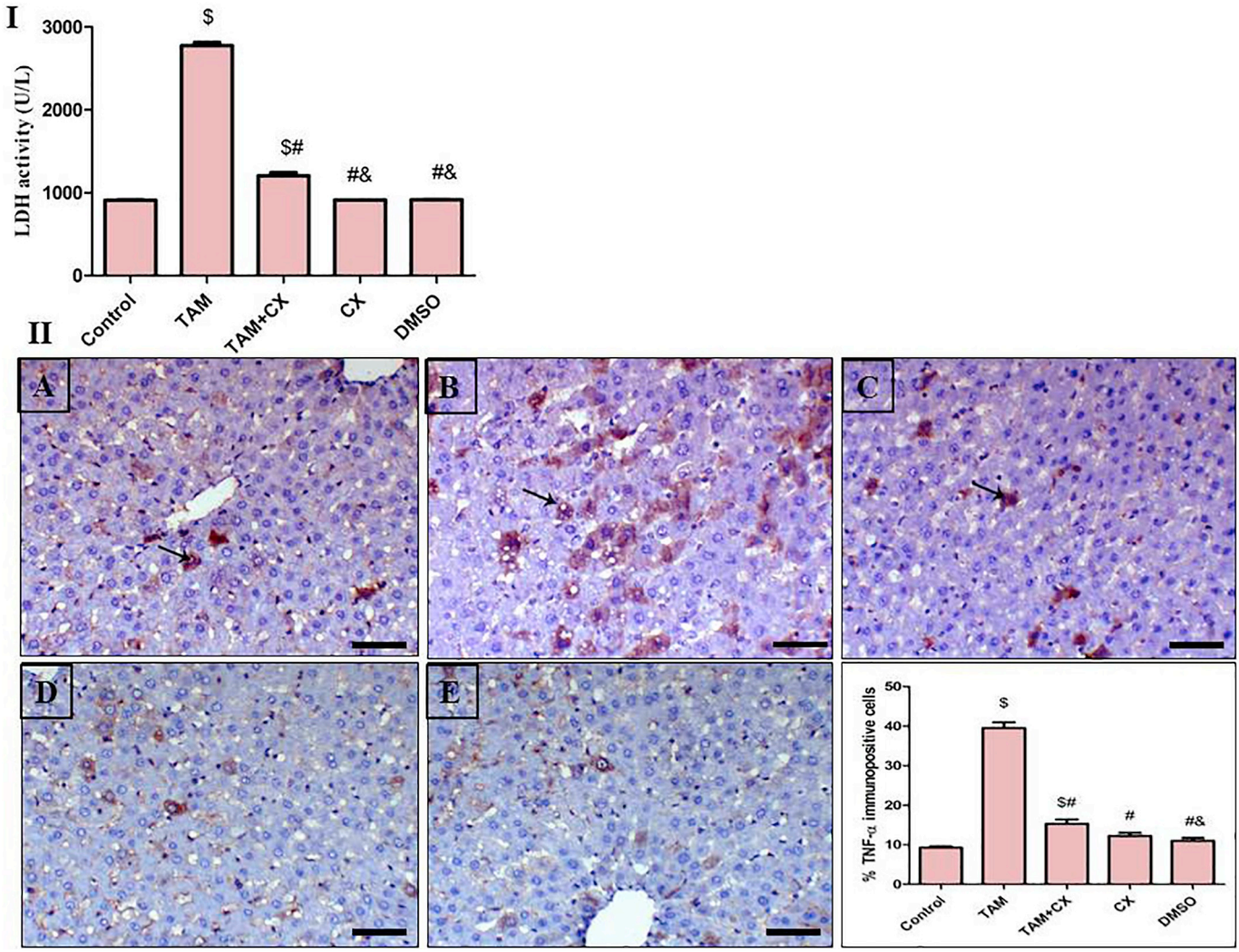


**Fig. 3.** Effect of celecoxib (CX) on tamoxifen (TAM)-induced elevation of inflammatory biomarkers.
I. Lactate dehydrogenase levels in serum. II. Tumor necrosis factor-alpha (TNF-α) protein immuno-expression (X200). A) Liver of control animal exhibited few positive hepatocytes (arrow); B) Liver of TAM group showed marked increase of TNF-α immunostaining within the hepatocytes (arrow); C) Liver of TAM + CX group showed a decrease in the positive-immunostained hepatocytes (arrow); D) & E) Livers of rats from groups CX and DMSO, respectively, showed minimal number of TNF-α immunopositive cells. Semi-quantitative analysis of TNF-α immuno-positive cells. Bar = 100 μm.
Control group, rats received 0.5% carboxymethyl cellulose (CMC) orally for 14 days; TAM group, rats were injected with TAM (45 mg/kg/day in 0.1% dimethylsulfoxide (DMSO), i.p., for 7 successive days, TAM + CX: rats received celecoxib (15 mg/kg/day in 0.5% CMC orally) for 14 successive days and TAM was injected daily from day 8 to day 14; CX, rats were orally administered celecoxib only for 14 successive days; DMSO, rats received only 0.1% DMSO, i.p., for 14 days. Data expressed as means ± SEM (n = 6). Analyses performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test. $ $p < 0.05$ vs. control; # $p < 0.05$ vs. TAM; & $p < 0.0.05$ vs. TAM + CX.

proteins gives rise to apoptosis [27]. Upon activation of Bax the apoptosis pathway is shifted towards the proapoptotic direction resulting in the discharge of pro-apoptotic factors such as cytochrome C from the mitochondria. This cytochrome is liberated by proteolytic cleavage into the cytoplasm leading to activation of procaspase-9 which activates downstream caspases, such as caspase-3, ultimately causing apoptosis in caspase-dependent manner [28]. In this study, the apoptotic effect of TAM was indicated by elevated Bax levels and exacerbated caspase-3 expression and subsequently marked apoptosis of hepatic tissue. Our result agreed with a previous study which reported that an increase in Bax protein expression in luteal cells in pseudopregnant rats following droloxifene treatment [29]. This finding suggested that the ASK-1/JNK signaling pathway might target the mitochondria and cause mitochondrial dysfunction and consequently liver injury. Opposing many studies which demonstrated that celecoxib induced apoptosis [30–32]; our results showed that celecoxib efficiently decreased hepatic levels of of Bax as well as the expression of caspase3 when compared to TAM group. This result proposed that celecoxib has antiapoptotic effect besides exerting antioxidant and anti-inflammatory properties.

Cell death in liver tissue takes place either by apoptosis or necrosis; they are mediated by different but overlapping pathways implicating cell surface death receptors as well as mitochondria/endoplasmic reticulum [33,34]. The perilous mitochondrial circumstance in apoptosis is mitochondrial outer membrane permeabilization (MOMP), which allows release of cytochrome *c* and other apoptogens resulting in caspase activation. While in primary necrosis, the key mitochondrial event is early opening of the mitochondrial permeability transition pore (mPTP) in the inner membrane, which occurs in the absence of cytochrome *c* release. Unlike primary necrosis, secondary necrosis occurs after apoptosis if the removal of apoptotic bodies is retarded [35].

Bax might be involved in both apoptotic and necrotic responses. Its role in apoptosis has been well discussed, but its role in necrosis is still under investigation. The study of Whelan et al. stated that deletion of

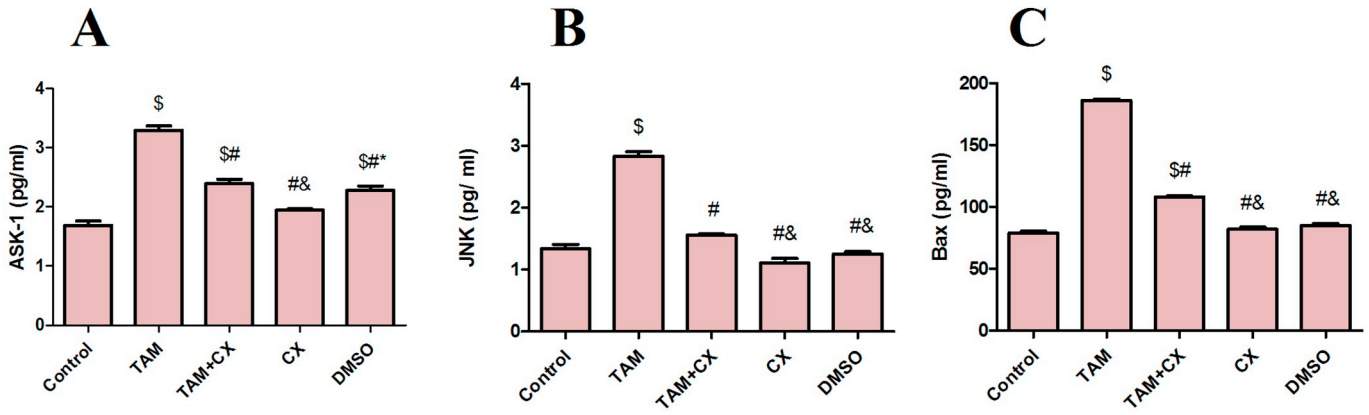


**Fig. 4.** Effect of celecoxib (CX) on ASK-1/ JNK signaling pathway.
A) Apoptosis signal-regulating kinase 1 (ASK); B) C-Jun N-terminal kinase (JNK); C) BCL2 Associated X protein (Bax).
Control group, rats received 0.5% carboxymethyl cellulose (CMC) orally for 14 days; TAM group, rats were injected with TAM (45 mg/kg/day in 0.1% dimethylsulfoxide (DMSO), i.p., for 7 successive days, TAM + CX: rats received celecoxib (15 mg/kg/day in 0.5% CMC orally) for 14 successive days and TAM was injected daily from day 8 to day 14; CX, rats were orally administered celecoxib only for 14 successive days; DMSO, rats received only 0.1% DMSO, ip, for 14 days. Data expressed as means ± SEM (n = 6). Analyses performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test. $ $p < 0.05$ vs. control; # $p < 0.05$ vs. TAM; & $p < 0.0.05$ vs. TAM + CX; * $p < 0.05$ vs. CX.

Bax and Bak dramatically reduces necrotic injury during myocardial infarction in vivo [36].

Collectively, celecoxib exerted its hepatoprotective against TAM by a dual mechanism; decreasing expression of caspase 3 which in turn decreased apoptosis as well as inhibiting JNK /ASK1 signaling pathway and hence Bax which has been also reported to inhibit necrosis. Also, it was previously documented that celecoxib boosted the sensitivity of cancerous cells to anticancer drugs [37]. These results explained why that combination might have a beneficial role in treatment of patients with breast cancer. Despite these benefits, we should be aware of the possible cardiovascular (CV) risks associating this combination. [38] showed that coxibs might increase the chance of a heart attack or stroke in high-risk CV patients, thus precaution as well as reduction of doses must be considered when patients use celecoxib to avoid this risk as well as reduction of doses [38].

All these findings suggest that celecoxib could overcome TAM-induced liver injuries and further clinical are recommended studies to confirm these effects.

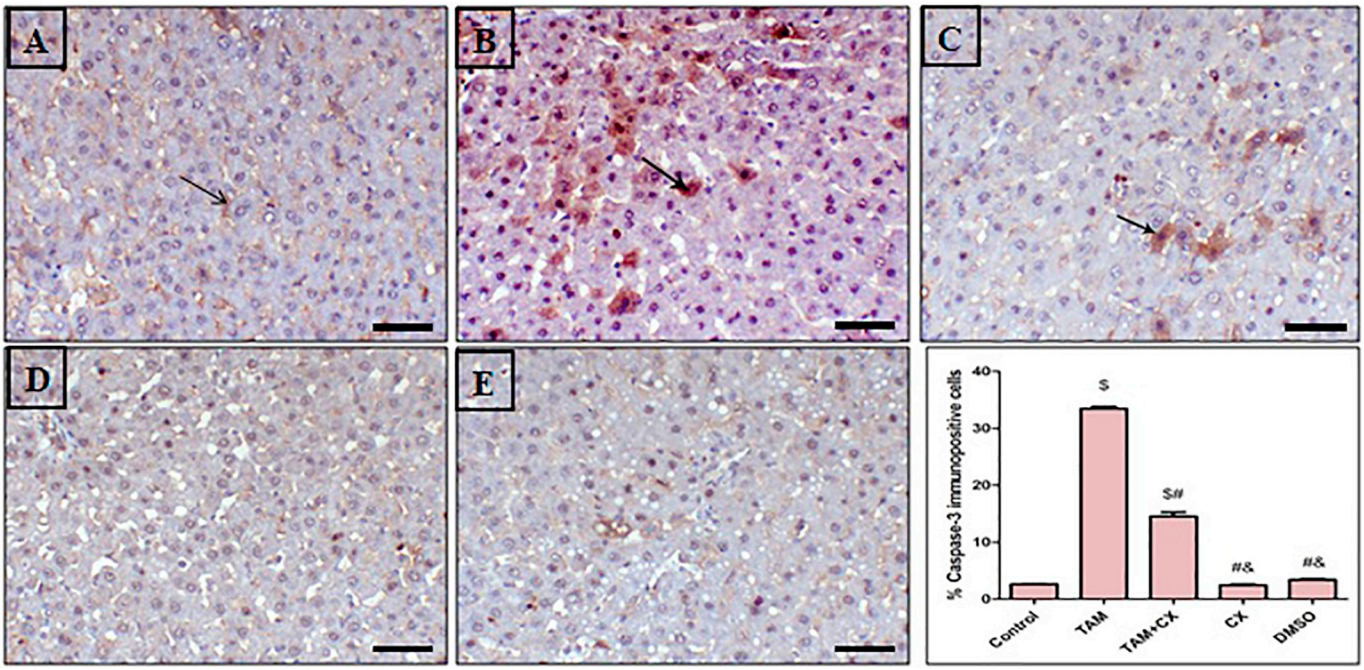


**Fig. 5.** Effect of celecoxib (CX) on tamoxifen (TAM)-induced alteration in caspase-3 in liver (IHCX200).
A) Liver of control animal revealed few positive hepatocytes (arrow); B) Liver of TAM group exhibited marked increase of positive hepatocytes (arrow); C) Liver of TAM + CX group showed a decrease in the positive-immunostained hepatocytes (arrow); D) & E) Livers of rats from groups CX and DMSO, respectively, showed minimal number of caspase immunopositive cells. Semi-quantitative analysis of caspase-3 immuno-positive cells. Bar = 100 μm.
Control group, rats received 0.5% carboxymethyl cellulose (CMC) orally for 14 days; TAM group, rats were injected with TAM (45 mg/kg/day in 0.1% dimethylsulfoxide (DMSO), i.p., for 7 successive days, TAM + CX: rats received celecoxib (15 mg/kg/day in 0.5% CMC orally) for 14 successive days and TAM was injected daily from day 8 to day 14; CX, rats were orally administered celecoxib only for 14 successive days; DMSO, rats received only 0.1% DMSO, i.p., for 14 days. Data expressed as means ± SEM (n = 6). Analyses performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test. $ $p < 0.05$ vs. control; # $p < 0.05$ vs. TAM; & $p < 0.0.05$ vs. TAM + CX.

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Declaration of Competing Interest

Authors declare no conflict of interests.

## Acknowledgment

Authors acknowledge Dr., Walid Abdo, Assistant prof of Pathology, Faculty of Veterinary medicine, Kafr-AlSheikh University for his help in histopathological analysis.

## References

[1] F. Bray, P. McCarron, D.M. Parkin, The changing global patterns of female breast cancer incidence and mortality, Breast cancer research: BCR 6 (2004) 229–239.
[2] G.M. Suddek, Protective role of thymoquinone against liver damage induced by tamoxifen in female rats, Can. J. Physiol. Pharmacol. 92 (2014) 640–644.
[3] C.Y. Liu, M.H. Hung, D.S. Wang, P.Y. Chu, J.C. Su, T.H. Teng, et al., Tamoxifen induces apoptosis through cancerous inhibitor of protein phosphatase 2A-dependent phospho-Akt inactivation in estrogen receptor-negative human breast cancer cells, Breast cancer research: BCR 16 (2014) 431.
[4] M.P. Ribeiro, A.E. Santos, J.B. Custodio, Mitochondria: the gateway for tamoxifen-induced liver injury, Toxicology 323 (2014) 10–18.
[5] G.M. Suddek, A.E. El-Kenawi, A. Abdel-Aziz, H.A. El-Kashef, Celecoxib, a selective cyclooxygenase-2 inhibitor, attenuates renal injury in a rat model of cisplatin-induced nephrotoxicity, Chemotherapy 57 (2011) 321–326.
[6] E.L. EM, M.S. Zeid, H.S. Kawy, N. Hendawy, W. Baher, Celecoxib attenuates depressive-like behavior associated with immunological liver injury in C57BL/6 mice through TNF-alpha and NF-kappab dependent mechanisms, Life Sci. 163 (2016) 23–37.
[7] S.A. Abdel-Gaber, M.A. Ibrahim, E.F. Amin, S.A. Ibrahim, R.K. Mohammed, A.M. Abdelrahman, Effect of selective versus non-selective cyclooxygenase inhibitors on ischemia-reperfusion-induced hepatic injury in rats, Life Sci. 134 (2015) 42–48.
[8] C. Liu, L. Liu, H.D. Zhu, J.Q. Sheng, X.L. Wu, X.X. He, et al., Celecoxib alleviates nonalcoholic fatty liver disease by restoring autophagic flux, Sci. Rep. 8 (2018) 4108.
[9] I.N. White, F. de Matteis, A. Davies, L.L. Smith, C. Crofton-Sleigh, S. Venitt, et al., Genotoxic potential of tamoxifen and analogues in female Fischer F344/n rats, DBA/2 and C57BL/6 mice and in human MCL-5 cells, Carcinogenesis 13 (1992) 2197–2203.
[10] H. Ohkawa, N. Ohishi, K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem. 95 (1979) 351–358.
[11] G.L. Ellman, Tissue sulfhydryl groups, Arch. Biochem. Biophys. 82 (1959) 70–77.
[12] S.L. Marklund, Superoxide dismutase isoenzymes in tissues and plasma from New Zealand black mice, nude mice and normal BALB/c mice, Mutat. Res. 148 (1985) 129–134.
[13] K.M. Miranda, M.G. Espey, D.A. Wink, A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite, Nitric oxide: biology and chemistry 5 (2001) 62–71.
[14] D.S. El-Agamy, M.A. Elkablawy, H.M. Abo-Haded, Modulation of cyclophosphamide-induced cardiotoxicity by methyl palmitate, Cancer Chemother. Pharmacol. 79 (2017) 399–409.
[15] H. Ye, L.J. Nelson, M. Gomez Del Moral, E. Martinez-Naves, F.J. Cubero, Dissecting the molecular pathophysiology of drug-induced liver injury, World J. Gastroenterol. 24 (2018) 1373–1385.
[16] G.M. Suddek, Allicin enhances chemotherapeutic response and ameliorates tamoxifen-induced liver injury in experimental animals, Pharm. Biol. 52 (2014) 1009–1014.
[17] E.K. Ozer, M.T. Goktas, I. Kilinc, H. Bariskaner, C. Ugurluoglu, A.B. Iskit, Celecoxib administration reduced mortality, mesenteric hypoperfusion, aortic dysfunction and multiple organ injury in septic rats, Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 86 (2017) 583–589.
[18] K. Kotoh, M. Kato, M. Kohjima, M. Tanaka, M. Miyazaki, K. Nakamura, et al., Lactate dehydrogenase production in hepatocytes is increased at an early stage of acute liver failure, Experimental and therapeutic medicine 2 (2011) 195–199.
[19] A. Koul, N. Arora, Celecoxib mitigates cigarette smoke induced oxidative stress in mice, Indian J. Biochem. Biophys. 47 (2010) 285–291.
[20] A. Matsuzawa, H. Ichijo, Redox control of cell fate by MAP kinase: physiological roles of ASK1-MAP kinase pathway in stress signaling, Biochim. Biophys. Acta 1780 (2008) 1325–1336.
[21] J.M. Kyriakis, J. Avruch, Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation, Physiol. Rev. 81 (2001) 807–869.
[22] G. Fujino, T. Noguchi, A. Matsuzawa, S. Yamauchi, M. Saitoh, K. Takeda, et al., Thioredoxin and TRAF family proteins regulate reactive oxygen species-dependent activation of ASK1 through reciprocal modulation of the N-terminal homophilic interaction of ASK1, Mol. Cell. Biol. 27 (2007) 8152–8163.
[23] D. Wu, A. Cederbaum, Activation of ASK-1 and downstream MAP kinases in cytochrome P4502E1 potentiated tumor necrosis factor alpha liver injury, Free Radic. Biol. Med. 49 (2010) 348–360.
[24] M.M. Farag, A.A. Khalifa, W.F. Elhadidy, R.M. Rashad, Hepatorenal protection in renal ischemia/reperfusion by celecoxib and pentoxifylline, J. Surg. Res. 204 (2016) 183–191.
[25] R. Liu, Q. Tan, Q. Luo, Decreased expression level and DNA-binding activity of specificity protein 1 via cyclooxygenase-2 inhibition antagonizes radiation resistance, cell migration and invasion in radiation-resistant lung cancer cells, Oncol. Lett. 16 (2018) 3029–3037.
[26] N. Gnesutta, A. Minden, Death receptor-induced activation of initiator caspase 8 is antagonized by serine/threonine kinase PAK4, Mol. Cell. Biol. 23 (2003) 7838–7848.
[27] A.A. Shaaban, D.H. El-Kashef, M.F. Hamed, D.S. El-Agamy, Protective effect of pristimerin against LPS-induced acute lung injury in mice, Int. Immunopharmacol. 59 (2018) 31–39.
[28] D.H. El-Kashef, M.H. Sharawy, Venlafaxine mitigates cisplatin-induced nephrotoxicity via down-regulating apoptotic pathway in rats, Chem. Biol. Interact. 290 (2018) 110–118.
[29] Y. Leng, Y. Feng, L. Cao, Z.P. Gu, Effects of droloxifene on apoptosis and Bax, Bcl-2 protein expression of luteal cells in pseudopregnant rats, Acta Pharmacol. Sin. 22 (2001) 155–162.
[30] G.D. Basu, L.B. Pathangey, T.L. Tinder, M. Lagioia, S.J. Gendler, P. Mukherjee, Cyclooxygenase-2 inhibitor induces apoptosis in breast cancer cells in an in vivo model of spontaneous metastatic breast cancer, Molecular cancer research: MCR 2 (2004) 632–642.
[31] A. Hassanzade, A. Mandegary, E. Sharif, R. Rasooli, R. Mohammadnejad, Y. Masoumi-Ardekani, Cyclooxygenase inhibitors combined with deuterium-enriched water augment cytotoxicity in A549 lung cancer cell line via activation of apoptosis and MAPK pathways, Iranian journal of basic medical sciences 21 (2018) 508–516.
[32] Y.Q. Zhao, H.W. Feng, T. Jia, X.M. Chen, H. Zhang, A.T. Xu, et al., Antiproliferative effects of celecoxib in Hep-2 cells through telomerase inhibition and induction of apoptosis, Asian Pacific journal of cancer prevention: APJCP 15 (2014) 4919–4923.
[33] J.E. Chipuk, T. Moldoveanu, F. Llambi, M.J. Parsons, D.R. Green, The BCL-2 family reunion, Mol. Cell 37 (2010) 299–310.
[34] P. Golstein, G. Kroemer, Cell death by necrosis: towards a molecular definition, Trends Biochem. Sci. 32 (2007) 37–43.
[35] L. Zitvogel, O. Kepp, G. Kroemer, Decoding cell death signals in inflammation and immunity, Cell 140 (2010) 798–804.
[36] R.S. Whelan, K. Konstantinidis, A.C. Wei, Y. Chen, D.E. Reyna, S. Jha, et al., Bax regulates primary necrosis through mitochondrial dynamics, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 6566–6571.
[37] W. Xia, T. Zhao, J. Lv, S. Xu, J. Shi, S. Wang, et al., Celecoxib enhanced the sensitivity of cancer cells to anticancer drugs by inhibition of the expression of P-glycoprotein through a COX-2-independent manner, J. Cell. Biochem. 108 (2009) 181–194.
[38] S. Tacconelli, A. Bruno, R. Grande, P. Ballerini, P. Patrignani, Nonsteroidal anti-inflammatory drugs and cardiovascular safety - translating pharmacological data into clinical readouts, Expert Opin. Drug Saf. 16 (2017) 791–807.