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Original Article

Molecular target mechanisms of celecoxib induced liver damage in rats and the potential prophylactic roles of melatonin and/or quercetin

[Mecanismos diana moleculares del daño hepático inducido por celecoxib en ratas y las posibles funciones profilácticas de la melatonina y/o quercetina]

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Abstract

Context: Celecoxib (Cele), a nonsteroidal anti-inflammatory drug (NSAID) is linked with a spectrum of hepatotoxic influences, however the underlying mechanism (s) by which this drug induces liver damage is still unexplored.

Aims: To demonstrate the hepatotoxic mechanism (s) of Cele in rats and the prophylactic roles of melatonin (Mel) and/or quercetin (Qr).

Methods: Rats were divided into eight groups, GI, served as control group; GII, Mel (12 mg/kg/day) treated group; GIII, Qr (10 mg/kg/day) treated group; GIV, Mel and Qr treated group; GV, Cele (50 mg/kg/day) treated group; GVI, Cele treated group concurrently with Mel GVII, Cele treated group concurrently with Qr; GVIII, Cele treated group concurrently with the combination of the two agents. The efficiency of Mel and/or Qr on hepatic histomorphology was also investigated.

Results: Cele significantly reduced hepatic succinate dehydrogenase and adenosine triphosphate and increased adenosine diphosphate versus the control group. Cele also caused rising in hepatic malondialdehyde, nitric oxide, tumor necrosis factor-α, transforming growth factor-β, caspase-3 and hydroxyproline as well as DNA damage along with depletion in catalase and glutathione reductase. Alteration in serum liver function markers and its histologic architecture were also observed in Cele treated group. Co-treatment of Cele treated rats with Mel and/or Qr, effectively ameliorated the deteriorations in the studied parameters as well as the histomorphologic liver pictures.

Conclusions: Mel and/or Qr could protect the liver from Cele, toxicity, which was more pronounced in rats treated with the combination of the two agents.

Keywords: celecoxib; inflammation; liver; melatonin; oxidative stress; quercetin.

Resumen

Contexto: Celecoxib (Cele), un fármaco antiinflamatorio no esteroideo (AINE) está vinculado con un espectro de influencias hepatotóxicas; sin embargo, el mecanismo o mecanismos subyacentes por los que este fármaco induce daño hepático aún no se ha explorado.

Objetivos: Demostrar los mecanismos hepatotóxicos de Cele en ratas y las funciones profilácticas de melatonina (Mel) y/o quercetina (Qr).

Métodos: Las ratas se dividieron en ocho grupos, GI, sirvió como grupo control; Grupo tratado con GII, Mel (12 mg/kg/día); Grupo tratado con GIII, Qr (10 mg/kg/día); Grupo tratado con GIV, Mel y Qr; Grupo tratado con GV, Cele (50 mg/kg/día); GVI, grupo tratado con Cele al mismo tiempo que Mel GVII, grupo tratado con Cele al mismo tiempo que Qr; GVIII, grupo tratado con Cele al mismo tiempo que la combinación de los dos agentes. También se investigó la eficacia de Mel y/o Qr en la histomorfología hepática.

Resultados: Cele redujo significativamente la succinato deshidrogenasa hepática y el trifosfato de adenosina y aumentó el difosfato de adenosina en comparación con el grupo de control. Cele también provocó un aumento de malondialdehído hepático, óxido nítrico, factor de necrosis tumoral α, factor de crecimiento transformante β, caspasa 3 e hidroxiprolina, así como daño en el ADN junto con el agotamiento de la catalasa y la glutatión reductasa. También se observaron alteraciones en los marcadores de función hepática en suero y su arquitectura histológica en el grupo tratado con Cele. El tratamiento conjunto de ratas tratadas con Cele con Mel y/o Qr mejoró eficazmente el deterioro de los parámetros estudiados, así como las imágenes histomorfológicas del hígado.

Conclusiones: Mel y/o Qr podrían proteger al hígado de la toxicidad de Cele, que fue más pronunciada en ratas tratadas con la combinación de los dos agentes.

Palabras Clave: celecoxib; estrés oxidativo; hígado; inflamación; melatonina; quercetina.

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INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are broadly used to alleviate multiple symptoms in clinical indications, including, pain, inflammation, rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis (Burke et al., 2006). NSAIDs suppress cyclooxygenase (COX), an enzyme with two isoenzymes: COX-1 and COX-2. Non-selective NSAIDs prohibit both isoforms while the selective agents mainly repress COX-2, thus decreasing side effects caused by COX-1, such as stomach ulcer (Vane and Botting, 1998).

Evidence from clinical trials have shown that treatment with NSAIDs is associated with severe adverse side impacts on body vital body organs, including liver (Gómez-Lechón et al., 2003; Sriuttha et al., 2018). The hepatic injury linked to NSAIDs was found to be greatly variable, ranging from mild cholestasis to severe hepatocellular injury along with biochemical changes (Sriuttha et al., 2018). Studies showed that these drugs could induce hepatocyte injury via uncoupling oxidative phosphorylation, mitochondrial permeability transition (MPT), mitochondrial swelling, a decrease in hepatic adenosine triphosphate (ATP), generation of reactive oxygen species and apoptosis of hepatocytes (Gómez-Lechón et al., 2003).

Celecoxib (Cele, celebrex) is a selective COX-2 inhibitor. Clinical and experimental studies have shown that treatment with this drug is associated with hepatotoxicity (Zinsser et al., 2004; Maddrey et al., 2007), ranging from increases in the serum liver function enzymes to severe hepatitis and focal necrosis (Somanath and Sri Sowmya, 2014). Although the hepatotoxic impact of Cele is documented, the underlying mechanism(s) by which this drug induces liver damage is still unexplored. Also, mitigation of the liver damage caused by Cele is a vital clinical issue to be solved. Using of therapeutic agents, especially from natural origin to prevent or reduce the undesirable hepatotoxic side effects of this drug, is considered as an urgent strategy to combat Cele-induced liver damage.

Quercetin (Qr, 3,5,7,30,40-pentahydroxyflavone) is a medicinal polyphenolic flavonoid, found in a large amount in many foods, including fruit, vegetables, and many other dietary sources (Nijveldt et al., 2001). This compound has reported to have many therapeutic activities, including antioxidants, anti-inflammatory, anti-angiogenesis, antitumor anti-apoptosis, and hepatoprotective activities (Choi et al., 2009; D'Andrea, 2015; Ansar et al., 2016). Qr has protective effects against cadmium-induced cytotoxicity via ameliorating lipid peroxidation, enhancement of antioxidants and mitigating apoptosis by inhibiting caspase-3 activity (Jia et al., 2011).

Melatonin (Mel, N-acetyl-5-methoxytryptamine), a secretary neuro-hormone, product of the pineal gland, is a potential endogenous antioxidant. It has several beneficial pharmacological impacts in treatment of many diseases, including liver ailment (Zhang et al., 2017). Mel has reported to have many therapeutic properties, such as anti-inflammatory, antioxidant, antifibrotic, anti-apoptosis, anticancer and hepatoprotective (Tahan et al., 2010; Zhang et al., 2017). Mel has the ability to neutralize free radicals and inhibit the production of inflammatory cytokines (Tahan et al., 2010). It stimulates the antioxidant defense systems, such as glutathione peroxidase, superoxide dismutase and glutathione (Tahan et al., 2010), and minimizes the levels of proinflammatory cytokines (IL-1β, IL-6 and TNF-α) in liver damage experimental animal model. Further, it has cytostatic effects on neutrophils and hepatic stellate cells, which may lead to the suppression of reactive radicals and fibrogenesis (Tahan et al., 2004).

The current study was designed to explore the molecular mechanism(s) of Cele induced liver damage and the potential hepatoprotective roles of Qr and/or Mel in rats.

MATERIAL AND METHODS

Chemicals

Mel, Qr and other chemicals utilized in this study were bought from Sigma Chemical Co. (St.

Louis, MO, USA). Cele (National Agency for Food and Drug Administration and Control registration No. 12/102/13) is a product of Pfizer company (Inc., NY, USA).

Animals and treatment

Eighty adult male Wistar albino rats (150-170 g) were utilized for this research. The rats were get from Laboratory Animal Production, King Fahd Research Centre, King Abdulaziz University. Animals were housed under controlled conditions (23-25^{o}C, humidity 50-65%, 12 h dark/light cycles) and provided by standard rat pellet food and water *ad libitum*. Experimental design was performed according to the roles provided by the Experimental Animal and accepted by the Animal Care and Use Committee of King Abdulaziz University, Faculty of Science (Approval number 28-18). The animals were left for seven days for adaptation and then classified into eight groups (10 rats/group) as follows:

Group I: Normal rats.

Group II: Rats treated orally with Mel (12 mg/kg, Barlas et al., 2017) daily for 30 days.

Group III: Rats treated orally with Qr 10 mg/kg (Chan et al., 2014) daily for 30 days.

Group IV: Rats treated orally with the combination of Mel (12 mg/kg) and Qr (10 mg/kg) daily for 30 days.

Group V: Rats treated orally with Cele 50 mg/kg (Koçkaya et al., 2010) for 30 successive days.

Group VI: Cele treated rats concurrently with Mel 12 mg/kg (Barlas et al., 2017) daily for 30 days.

Group VII: Cele treated rats concurrently with Qr 10 mg/kg (Chan et al., 2014) daily for 30 days.

Group VIII: Cele treated rats concurrently with both Mel (12 mg/kg) and Qr (10 mg/kg).

Cele, Qr and Mel were administered orally. Cele and Qr were prepared as suspensions in sodium carboxy methyl cellulose (0.5% w/v), while Mel was dissolved in PBS before administration. After the experimental period, rats were fasted overnight (12-14 hours). Blood specimens were gathered for clotting and serum separation. The animals were then decapitated, and the livers were collected, washed with cold saline and used for biochemical studies and histopathological examination.

Biochemical analysis

Serum analysis

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and albumin were measured as biomarkers of liver injury, utilizing an automated analyzer.

Liver tissue analysis

Adenosine triphosphate (ATP) was estimated enzymatically using hexokinase and glucose -6-phosphate dehydrogenase by a spectrophotometric method (Lamprecht and Trautschold, 1974). Adenosine diphosphate (ADP) was determined enzymatically, using pyruvate kinase and lactate dehydrogenase following the method of Jaworek et al. (1974). Nitrite level (as a signal of NO generation) (Green et al., 1982) and malondialdehyde (MDA, marker of oxidation of lipids) (Buege and Aust, 1978) were assayed as markers of oxidative stress. Catalase (CAT) (Aebi, 1984) and glutathione reductase (GR) (Erden and Bor, 1984) were estimated as antioxidant markers. Rat Elisa kits were used for measuring tumor necrosis factor–α (TNF–α) and transforming growth factor-β (TGF-β) (ABCAM, UK), according to the instructions supplied by the manufacturer. 4-Hydroxyproline (HydroxyP) was estimated utilizing Ehrlich's reagent, according to the assay method described by Jamall et al. (1981). DNA damage was estimated by a comet assay (Singh et al. 1988). Succinate dehydrogenase (SDH) activity was assayed by the method of Munujos et al. (1993). Caspase 3-like protease was measured by the method of Vaculova and Zhivotovsky (2008).

Histopathological studies

Liver specimens were fixed in 10% formaldehyde for 24 hours, then embedded into paraffin,

sectioned (3–4-µm in thickness) and stained with hematoxylin and eosin (H&E 400×) for histopathological investigation, utilizing a light microscope.

Statistical analysis

Data were statistically analyzed via IBM SPSS software, version 21. Results were analyzed by comparing the mean values for different Cele groups with the mean values of controls utilizing one-way analysis of variance (ANOVA) and Bonferroni's test as post-ANOVA. Data are calculated as mean ± SD. Values were regarded statistically significant at $p \leq 0.05$.

RESULTS

Hepatic mitochondrial damage markers

The impacts of Mel and/or Qr on hepatic mitochondrial damage markers (SDH, ATP and ADP) in Cele intoxicated rats are shown in (Fig. 1). The data revealed that intake of Cele to rats daily for 30 days, significantly reduced the hepatic SDH activity and ATP concentration. The decrease in these markers was accompanied with an increase in ADP concentration *versus* the control rats ($p \leq 0.001$). Co-ingestion of Mel and/or Qr to Cele intoxicated rats, effectively ameliorated the alterations in these parameters with respect to Cele treated group ($p \leq 0.001$). Treatment with the combination of Mel and Qr was the efficient in modulating the mitochondrial damage indices compared to the treatment with each one lonely. Non-significant changes in SDH, ATP and ADP were observed in rat groups treated with Mel and/or Qr.

Hepatic oxidative stress and antioxidant markers

Hepatic oxidative stress parameters (MDA and NO) and antioxidant indices (CAT and GR) in control and Cele intoxicated rat groups are depicted in (Fig. 2). The data demonstrated that Cele pronouncedly boosted the hepatic MDA (an index of lipid peroxidation) and NO (a marker of nitrosative stress) concentration accompanied with decreases in the antioxidant enzymes *versus* the control rats ($p \leq 0.001$). Coadministration of Mel and/or Qr to Cele administered rats, effectively modulated the alterations in hepatic oxidative stress and antioxidant indices with respect to Cele intoxicated group ($p \leq 0.001$). Treatment with the combination of Mel and Qr was the effective one in ameliorating the levels of these parameters. Non-significant changes were observed in oxidative stress indices in rat groups treated with Mel and/or Qr.

Hepatic inflammatory and fibrosis markers

The influences of Mel and/or Qr on hepatic inflammatory and fibrosis markers (TNF-α, TGF-β and HyroxyP) in Cele treated rats are demonstrated in Fig. 3. Cele significantly caused elevation in the levels of TNF-α, TGF-β and HyroxyP *versus* control rats ($p \leq 0.001$). Oral co-ingestion of Mel and/or Qr to Cele intoxicated rats, pronouncedly reduced the increases in these markers in comparison to Cele treated group ($p \leq 0.001$). The combination of Mel and Qr was the beneficial one in downmodulating the levels of these indices. Non-significant changes were seen in TNF-α, TGF-β and HyroxyP in rat groups treated with Mel and/or Qr.

Hepatic DNA fragmentation and apoptosis indices

Fig. 4 demonstrated that significant increases in the DNA fragmentation indices in terms of DNA tail length and tail moment as well as apoptosis enzyme, namely caspase-3 in hepatic of Cele–treated rats with relation to control ones ($p \leq 0.001$). Oral intake of Mel and/or Qr simultaneously with Cele, effectively reduced the increases in these indices when compared with Cele treated group ($p \leq 0.001$). Treatment with the combination of Mel and Qr simultaneously with Cele was the efficient one in reducing the levels of DNA damage and apoptosis biomarkers *versus* treatment with each one lonely. Non-significant changes were noticed in DNA fragmentation indices and caspase-3 in rat groups treated with Mel and/or Qr.

Serum hepatic function indicators

Hepatic serum function tests (Table 1) showed that significant increment in the serum ALT and

AST coupled with a decrement in albumin level in Cele treated rats *versus* the control ones (p≤0.001). Treatment with Mel and/or Qr markedly ameliorated the alterations in hepatic serum function parameters *versus* Cele intoxicated rats (p≤0.001). The two agents in a combination were the most beneficial one in ameliorating the levels of the aforementioned indices. Non-significant changes were noticed in these indices in rat groups treated with Mel and/or Qr.

Histopathological observation

Light microscopic examination of H&E stained sections of rat livers in the control and Mel and/or Qr treated groups showed normal histological structure of hepatic lobule with normal hepatocytes (Figs. 5A-D). Liver sections of rats treated with Cele showed disfiguration of liver architecture as evident by vacuolation of many hepatocyte and others with pyknotic nucleoli, congestion of hepatic sinusoids and central vein and infiltration of inflammatory cells (Fig. 5E-F). Liver sections of rats treated with Cele in concurrent with either Mel (Figure 5G), Qr (Fig. 5H) or their combination (Fig. 5I) showed apparently normal liver histologic pictures.

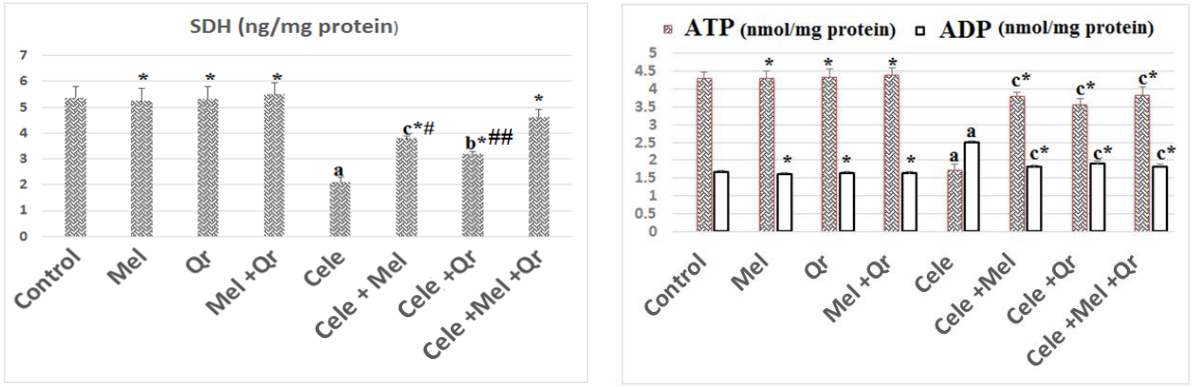

Figure 1. Influence of melatonin (Mel) and/or quercetin (Qr) on hepatic mitochondrial damage indices (SDH, ATP and ADP) in celecoxib (Cele) treated rats.

Values are calculated as mean ± SD of 10 rats. [a]P≤0.001, [b]p≤0.01, [c]p≤0.05 *vs.* control rats; *p≤0.001 *vs.* Cele treated animals; [#]p≤0.05, [##]p≤0.01 *vs.* the combination group (Cele + Mel + Qr).

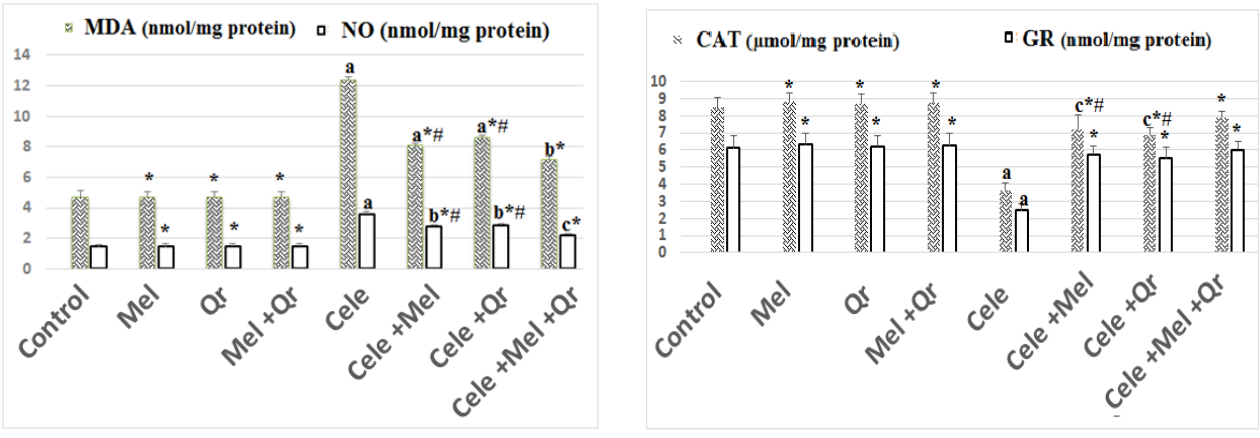

Figure 2. Influence of Mel and/or Qr on hepatic oxidative stress markers (MDA and NO) and antioxidant indices (CAT and GR) in Cele treated rats.

Values are calculated as mean ± SD of 10 rats. [a]P≤0.001, [b]p≤0.01, [c]p≤0.05 *vs.* control rats; *p≤0.001 *vs.* Cele treated animals; [#]p≤0.05 *vs.* the combination group (Cele + Mel + Qr).

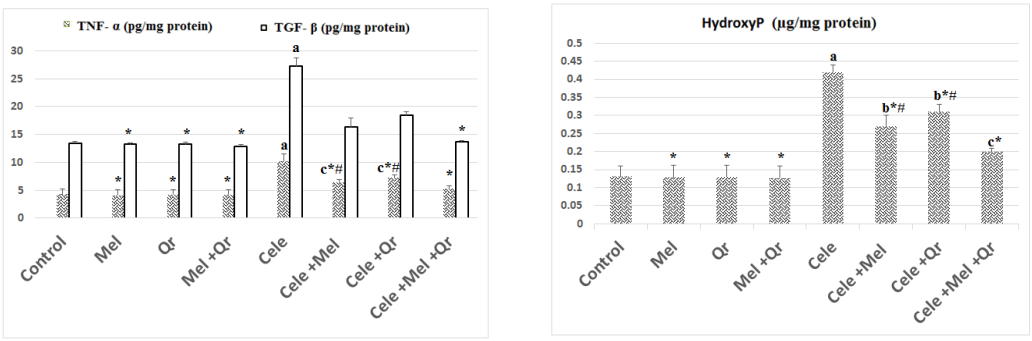

Figure 3. Influence of Mel and/or Qr on hepatic inflammatory, fibrogenic molecules (TNF-α and TGF-β) and index of hepatic fibrosis (HydroxP) in Cele treated rats.

Values are calculated as mean ± SD of 10 rats. [a]P≤0.001, [b]p≤0.01, [c]p≤0.05 *vs.* control rats; *p≤0.001 *vs.* Cele treated animals; #p≤0.05 *vs.* the combination group (Cele + Mel + Qr).

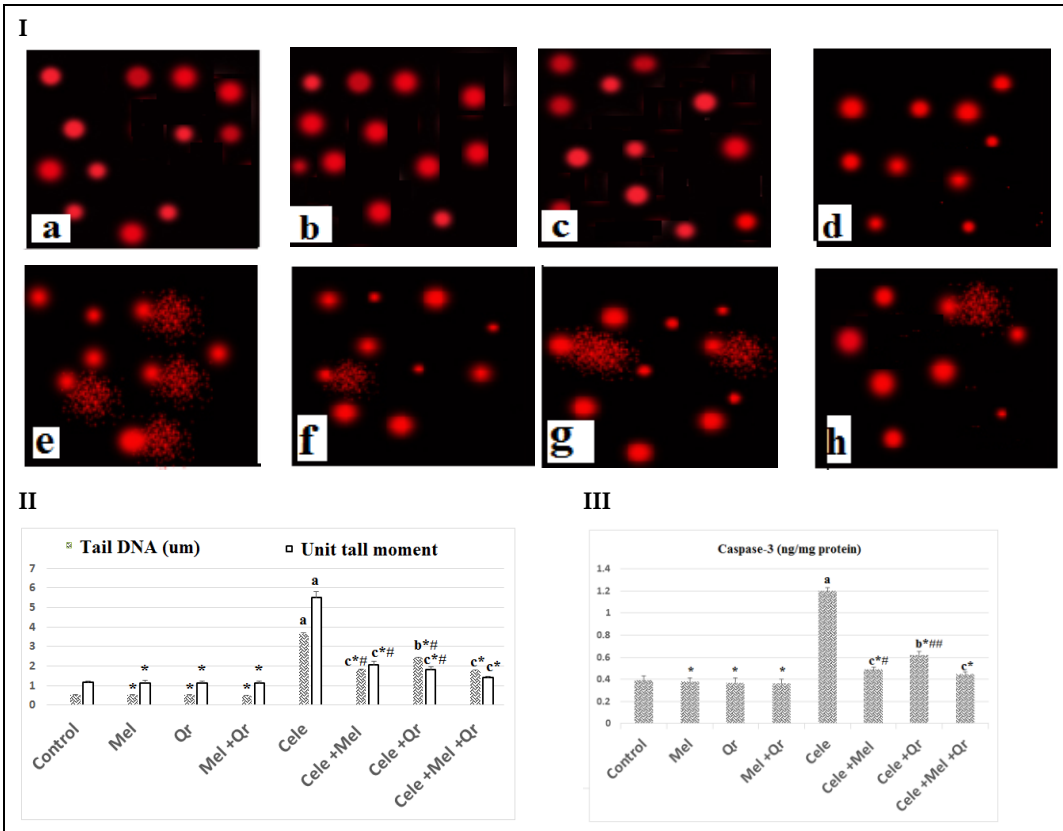

Figure 4. Influence of melatonin (Mel) and/or quercetin (Qr) on hepatic DNA fragmentation markers and apoptosis index (caspase-3).

(I) Comet assay showed levels of hepatic DNA fragmentation in different celecoxib (Cele) treated group, (a) Control rats; (b) rats treated with Mel; (c) rats treated with Qr; (d) rats treated with the combination of Mel and Qr; (e) Cele-treated rats; (f) Cele- treated rats concurrently with Mel; (g) Cele-treated rats concurrently with Qr; (h) Cele-treated rats concurrently with Mel and Qr.

(II) Levels of DNA damage indices in terms of tail length and unit tail moment in different experimental treated groups.

(III) Levels of hepatic caspase-3 in experimental treated groups. Values are calculated as mean ± SD of 10 rats. [a]P≤0.001, [b]p≤0.01, [c]p≤0.05 *vs.* control rats; *p≤0.001 *vs.* Cele treated animals; #p≤0.05, ##p≤0.01 *vs.* the combination group (Cele + Mel + Qr).

Table 1. Influence of melatonin (Mel) and/or quercetin (Qr) on liver function markers in different treated groups.

Groups	Parameters		
	ALT (U/L)	**AST (U/L)**	**Albumin(mg/dL)**
Control	57.50 ± 2.08	119.00 ± 1.82	3.57 ± 0.09
Mel	53.68 ± 3.50	117.67 ± 2.31	3.6 ± 0.06*
Qr	55.45 ± 2.90	121.73 ± 3.76	3.54 ± 0.05*
Mel +Qr	52.93 ± 4.60	115.67 ± 3.90	3.80 ± 0.030*
Cele	108.25 ± 2.87[a]	269.75 ± 4.42[a]	2.32 ± 0.09[a]
Cele +Mel	67.50 ± 1.73[c*]	131.00 ± 3.26[c*]	3.17 ± 0.09[c*]
Cele + Qr	72.75 ± 3.77[c*]	134.00 ± 3.36[c*]	3.10 ± 0.11[c*]
Cele +Mel +Qr	66.75 ± 1.50[c*]	131.00 ± 1.82[c*]	3.25 ± 0.13[c*]

Values are calculated as mean ± SD of 10 rats. [a]P≤0.001, [c]p≤0.05 *vs.* the control group; *p≤0.001 *vs.* celecoxib (Cele) treated group.

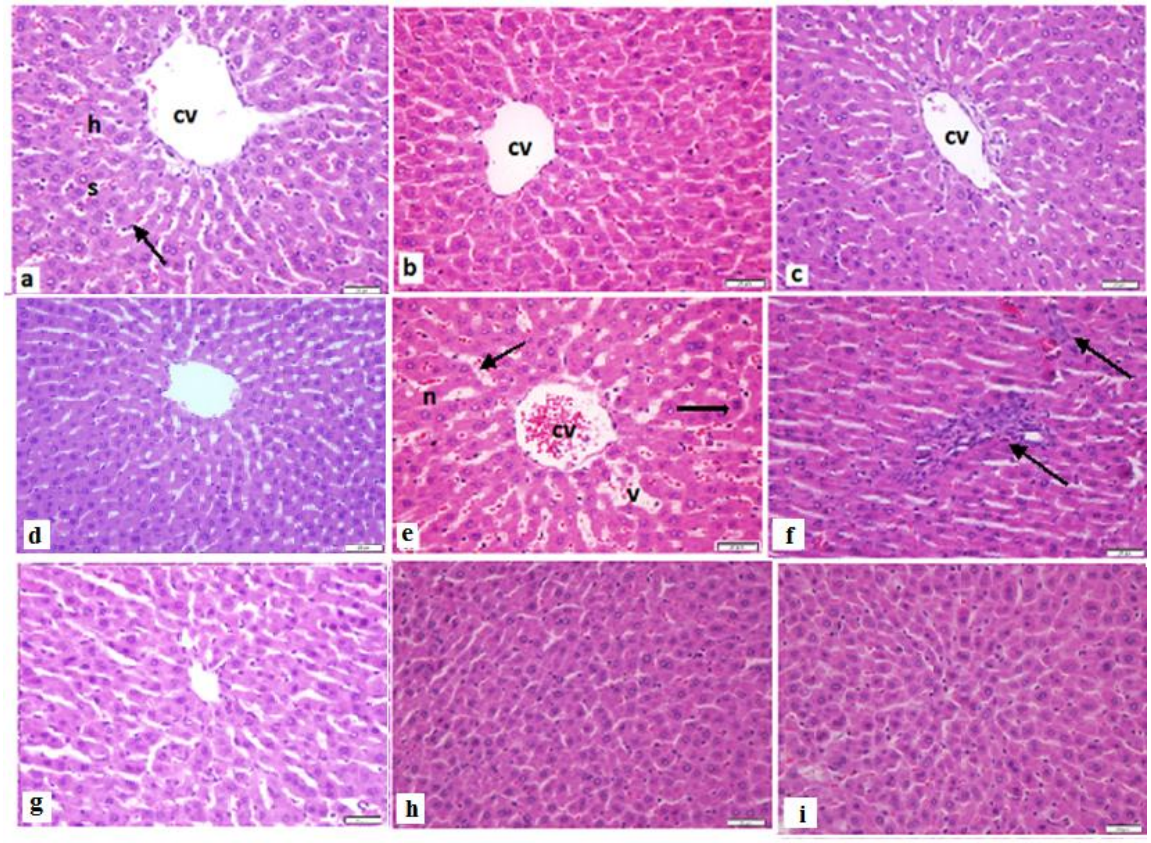

Figure 5. Light micrographs of rat liver sections in different control and Cele treated groups stained with hematoxylin and eosin (H&E, 400×), scale bar = 20 µm.

(a) Liver section from control rat, showing normal histological structure hepatocytes (h), central vein (cv), sinusoids (s) and Kupffer's cells lining the sinusoids (arrow). **(b, c & d)** Liver sections from rats treated either with Mel **(b)**, Qr **(c)** or the combination of Mel and Qr **(d)** showing normal liver architecture. **(e & f)** Liver sections from Cele treated rats, **(e)** showing severe congestion in the central vein and sinusoids (thin arrow) as well as hepatocyte vacuolation (v), many hepatocytes showing pyknotic nucleoli (thick arrow) and other showing necrotic changes (n); **(f)** showing infiltrations of inflammatory immune cells (arrows). **(g, h & i)** Liver sections from Cele treated rats in concurrent with either Mel, Qr or their combination, showing normal histological structure of the liver.

DISCUSSION

Cele has reported to have hepatotoxic adverse impacts (Somanath and Sri Sowmya, 2014). The present investigation aimed to study the molecular hepatotoxic mechanism(s) of Cele and the prophylactic impacts of two natural compounds namely quercetin (Qr) and/or melatonin (Mel) in rats.

The current investigation demonstrated that oral administration of Cele to rats, significantly diminished hepatic ATP concentration and SDH activity along with an increase in ADP concentration with relation to control ones. The alterations in these markers may attribute to the damaging impact of Cele on the hepatic mitochondria. No previous studies have shown the adverse effects of Cele on markers of hepatic mitochondrial damage and this study for the first time provides one of the molecular mechanisms of liver damage induced by Cele. The depletion in hepatic ATP and SDH along with an increase in ADP may reflect the high utilization of ATP store with a reduction in its production. This deleterious impact may attribute to the lipophilic ability of Cele with its acidic character to cause uncoupling of oxidative phosphorylation via penetrating the outer mitochondrial membrane easily and act as proton translocator (protonophores), shuttling protons from the intermembranous space across the inner mitochondrial membrane back into the matrix (Moreno-Sanchez et al., 1999). This can cause dissipation of proton gradient, resulting in suppression of ADP phosphorylation by ATP synthetase, causing a drop in ATP synthesis (Ponsoda et al., 1995). The decrease in hepatic SDH activity by Cele may indicate that Cele selectively could inhibit complex II of the electron transport chain (ETC), causing blocking of free energy generation required for ATP production, causing a depletion in ATP biosynthesis (Bonora et al., 2012). The ameliorating influences of Mel and/or Qr *versus* Cele induced alterations in SDH, ATP and ADP may be due to their abilities to prevent the interaction of Cele with mitochondrial complex II (SDH), suggesting that mitochondrial complex II (SDH) of ETC can be seen as likely molecular target for the cytoprotective actions of both Mel and Qr against Cele toxicity. The protective impacts of both agents versus mitochondrial dysfunction have been reported (Kireev et al., 2013; Li et al., 2016).

It has been reports that oxidative stress is one of important adverse mechanisms of Cele promoted liver damage (Sozer et al., 2011). In line with a previous study, significant increment in the levels of hepatic MDA (an index of lipid peroxidation) and NO (an index of nitrosative stress) and decreases in the enzymatic antioxidants, CAT and GR in Cele-treated rats compared with control ones (Sozer et al., 2011). The increase in hepatic lipid peroxidation by Cele administration presented in the current study may be due to over generation of NO and other reactive free radicals in response to Cele toxicity (Stark, 2005; Mahmoud et al., 2015). Somasundaram et al. (2002) found that NSAIDs have the ability to bind to a site near complex I and ubiquinone to generate ROS, which eventually leads to oxidative cell death. The significant depletion in hepatic CAT and GR in Cele treated rats may be due to inactivation caused by excess generation of reactive radicals. So, alterations in the levels of antioxidants by Cele may render the hepatic cells susceptible to oxidative stress and hence cell injury. Modulation of oxidative stress and antioxidant markers upon treatment with Mel and/or Qr concurrently with Cele may propose that both agents could mitigate hepatic oxidative stress via their antioxidant potential action (Ansar et al., 2016; Zhang et al., 2017).

It is well known that reactive species can attack DNA, causing structural modifications to DNA that contributes to mutagenesis and carcinogenesis (Jena, 2012). Comet assay showed marked rising in the tail DNA length and the tail moment in Cele treated rat livers, reflecting DNA fragmentation (Akram, 2016). The damaging impact of Cele on hepatic DNA may relate to lipid peroxidation product (MDA) and reactive radicals, which can interact with DNA causing damage to DNA bases and backbone (Martinez et al., 2003). This result provides the first evidence of hepatic DNA damaging effect of Cele, which may represent another molecular mechanism underlying liver damage in

response to Cele toxicity. The reduction in the DNA tail length and tail moment in rats treated with Mel and/or Qr concurrently with Cele, may reflect the protective effect of both agents versus DNA fragmentation (Ansar et al., 2016, Ferreira et al., 2013).

The marked rising in hepatic caspase 3 (apoptosis biomarker) in Cele treated rats, suggesting that apoptosis might relate to Cele-induced lipid peroxidation. It has found that lipid peroxidation increases the permeability of mitochondrial membrane, causing loss of mitochondrial integrity those results in the release of cytochrome c into the cytoplasm, causing induction of apoptotic cell death via activation of caspase activity (Crompton 1999). This is supported by previous study has found that diclofenac as one of NSAIDs, can cause apoptotic cell death via releasing mitochondrial cytochrome c and activation of the caspase enzymes (Lim et al., 2006). Ingestion of Mel and/Qr concurrently with Cele, significantly down-modulated the hepatic increase in caspase-3, indicating antiapoptosis of both agents (Choi et al., 2009; Zhang et al., 2017).

In line with clinical studies on NSAIDs, the present study demonstrated that Cele significantly caused rising in hepatic inflammatory cytokines, namely TNF-α and TGF-β in rats (Çağıltay et al., 2015). Some investigations have explained that the reactive metabolite of NSAIDs can bind covalently to important cellular proteins, forming drug-modified protein adducts, which can be recognized by the inflammatory immune cells (neutrophils, lymphocytes and macrophages), leading to production of inflammatory proteins and causes inflammatory tissue injury (Adams et al., 2010). Ingestion of Mel and/or Qr**,** significantly down-regulated the hepatic increases in TNF-α and TGF-β in Cele treated rats, documenting their anti-inflammatory actions (Hu et al., 2009; Li et al., 2018).

Liver fibrosis is a common pathological pathway of chronic liver injury, distinguished by excessive building up of extracellular matrix proteins (ECMPs), particularly collagens as predominant structural components (Kim et al., 2017). HydroxyP is a major component of collagen metabolism. An increase in hepatic hydroxyproline content can consider a valuable indicator of liver fibrosis (Bolarin and Azinge, 2007). The significant increase in hepatic hydroxyP in Cele treated rats, may reflect liver fibrogenesis resulted from excessive collagen deposition in response to liver injury under the effect of Cele toxicity. Similarly, Hui et al. (2006) found that Cele potentiates experimental liver fibrosis induced by CCl_4 in rat. The increment in TNF-α and TGF-β presented in the current study may have the major role in hepatic fibrogenesis in response to Cele toxicity. TNF-α and/or TGF-β1 play a direct key role in liver fibrosis by activating the hepatic stellate cells, which have the role in the production of extracellular matrix proteins, including collagen as well as down-regulating the expression of matrix metalloproteinases (MMPs) and promoting tissue inhibitors of matrix metalloproteinases (TIMPs), leading to excessive deposition of collagenous fibers and hence, participates in the liver fibrogenesis (Cui et al., 2003; 2011; Yang and Seki, 2015). The present result suggests that increased hepatic HydroxyP level along with TNF-α and TGF-β in Cele treated rats may provide another molecular mechanism of fibrogenic liver damage related to Cele toxicity**.** The pronounced depletion in hepatic HydroxyP in rats treated with Mel and/or Qr along with Cele may give a clue to the potential antifibrotic action of both Mel and Qr, which may be attributable to their abilities to suppress oxidative stress and the production of the fibrogenic cytokines (TNF-α and TGF-β**)** (Hu et al., 2009; Li et al., 2018).

The hepatocellular damage presented in the current work is confirmed by the rising in the serum levels of AST and ALT with a concomitant depletion in albumin concentration in Cele treated rats. This result is supported by the liver histopathology, which demonstrated severe degenerative changes. Similarly, clinical studies have shown that chronic ingestion of coxibs can induce liver injury via induction of cholestatic hepatitis and severe hepatocellular necrosis (Papachristou et al., 2004). Prophylactic administration of Mel and/or Qr, significantly ameliorated the alterations in the above-mentioned liver function indices

as well as the histological picture of liver tissue. The potential hepatoprotective influences of both agents against liver tissue injury in different models have been confirmed (Selvakumar et al., 2013; Chojnacki et al., 2017).

CONCLUSIONS

The current investigation demonstrated that treatment with Cele has the potential to cause liver damage. Co-treatment with Mel and/or Qr could protect the liver tissues from the damaging impact of Cele, which was more pronounced in rats treated with the combination of the two agents. The current study may provide a protective strategy against drug related hepatotoxicity that may consider as a promoting and preventive care for the general population. This will reduce the cost for the treatment of liver damage related hepatotoxic medications.

CONFLICT OF INTEREST

The authors declare no conflicts of interests.

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Contribution	Sulimani ES	Yousef JM	MohamedAM
Concepts or ideas			x
Design		x	x
Definition of intellectual content		x	x
Literature search		x	x
Experimental studies	x	x	x
Data acquisition	x		x
Data analysis		x	x
Statistical analysis	x		x
Manuscript preparation	x	x	x
Manuscript editing		x	x
Manuscript review	x	x	x

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