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ORIGINAL ARTICLE

Quercetin and tin protoporphyrin attenuate hepatic ischemia reperfusion injury: role of HO-1

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Abstract Ischemia reperfusion (IR) injury occurs in many clinical situations such as organ transplantation and hepatectomies resulting in oxidative stress and immune activation. Heme oxygenase-1(HO-1) is the rate-limiting step in the heme-degradation pathway and has a critical cytoprotective role. Induction of HO-1 improves liver I/R injury. Quercetin, a plant pigment (flavonoid), is an antioxidant and HO-1 inducer. Tin protoporphyrin (SnPP) is a HO-1 inhibitor. This study was designed to investigate the protective effect of quercetin in hepatic I/R injury and the role of HO-1. Wister rats were randomly divided into four groups (sham, I/R, quercetin, and SnPP). Liver ischemia was induced for 45 min then reperfusion was allowed for 1 h. Quercetin and surprisingly SnPP ameliorate the deleterious effect of I/R by reducing the oxidative stress and hepatocyte degeneration. Both agents decreased the elevated inflammatory cytokines and improved the inhibition of the antiapoptotic marker, Bcl2. They induced HO-1 content and expression. Quercetin has better cytoprotective effect than SnPP. These findings suggest that quercetin has a hepatoprotective effect against I/R injury via HO-1 induction and unexpectedly, SnPP showed the similar effect. Quercetin has more prominent protective effect than SnPP because of its superior ability to induce HO-1.

Keywords Ischemia reperfusion · Ho-1 · Quercetin · Tin protoporphyrin

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Introduction

Ischemia reperfusion (I/R) injury is a cellular damage in a hypoxic organ following the restoration of oxygen delivery (Teoh and Farrell 2003). I/R injury may occur during surgeries such as organ transplantation, trauma, and hepatectomies (Jeyabalan et al. 2008). Reperfusion is complicated by microcirculatory failure, followed by necrosis and cell death (Terajima et al. 2000, Kupiec-Weglinski and Busuttil 2005). Hepatic I/R injury is accompanied by oxidative and inflammatory processes (Sahin et al. 2013). The HO system is one of the most critical cytoprotective mechanisms activated during cellular stress, exerting antioxidant and anti-inflammatory functions (Bedirli et al. 2004).

Quercetin (3,3′,4′5,7-penthydroxyflavone) is a plant pigment (flavonoid) found in many plants such as onions, blueberry, and apples (Tang et al. 2013). Red onions were traditionally used in the Mediterranean cuisine and as a remedy in folk medicine for their strong antioxidant properties which can be attributed to its high quercetin content (Corzo-Martínez et al. 2007). Quercetin shows a powerful anti-inflammatory, antioxidant, and anti-fibrotic properties (Uzun and Kalender 2013). One mechanism of the antioxidant action of quercetin involved in scavenging free radicals, such as superoxide

radicals generated by xanthine and xanthine oxidase (Peluso 2006). It was also reported to inhibit TNF-α production and gene expression (Hirpara et al. 2009). Quercetin is able to stimulate HO-1 expression at both mRNA and protein levels, and also induces HO-1 activity (Liu et al. 2012).

Tin protoporphyrin IX dichloride (SnPP) ($C_{34}H_{32}N_4O_4Sn$. 2 Cl) is a potent inhibitor of HO-1; it regulates HO by a dual mechanism. It increases the synthesis of new protein but effectively hinders the enzyme by acting as a competitive substrate for heme at the catalytic site (Marinissen et al. 2006).

The aim of the present study was to investigate the role of quercetin and SnPP against ischemia/reperfusion in rat liver and the implication of HO-1 induction in their effects.

Methods

Chemicals

Quercetin was purchased from Sigma Aldrich (Taufkirchen, Germany). SnPP was purchased from Frontier Scientific (Utah, USA).

Animals

Thirty-two adult male Wister rats (200–250 g) purchased from an animal house, Zagazig University, were used for this study. They were individually housed under a 12/12-h light/dark cycle, with free access to food and tap water. Room temperature and humidity were maintained at 23 ± 1 °C and 55 ± 5%, respectively. The experimental procedures were approved by the Institutional Animal Ethics Committee of the Faculty of Pharmacy, Zagazig University (approval number P2-3, 2016), and animals were handled following the International Animal Ethics Guidelines, ensuring minimum animal suffering.

Ischemia reperfusion model

Ischemia was induced by occluding the hepatic portal vein, artery, and bile duct using the weight hanging method (Zimmerman et al. 2012). All rats were anesthetized with ketamine/xylazine (100 and 10 mg/kg). Incision was made to the linea alba up to the upper end to easily expose the liver. The portal triad was exposed and a needle was passed with suture underneath it carefully and pulled from the other side gently. The suture was then crossed without knotting. An Eppendorf tube filled with water was attached to each of the suture ends. Then, the suture ends were placed over the sides of the box. By applying such weights, the triad was immediately occluded. During the period of ischemia, normal saline 37 °C was injected in the abdominal cavity every 10 min. It was applied for 45 min then removed the weights and uncrossed the suture, and the suture was pulled out gently to allow blood reperfusion. Rats were then wrapped in plastic wrapping to maintain visceral hydration and were kept on a warm plate adjusted to 37 °C to maintain body temperature for 60 min before euthanasia. All drugs were administered 1 h prior to ischemia.

Study protocol

Animals were divided into four experimental groups (each containing eight animals) as follows: Sham: received vehicle then anesthetized and an incision was made to the linea alba up to the upper end and the portal triad was exposed; I/R: received vehicle then anesthetized then subjected to I/R; quercetin: received quercetin (50 mg/kg) 1 h prior to anesthesia then subjected to I/R; and SnPP: received SnPP (50 μmol/kg) 1 h prior to anesthesia then subjected to I/R.

Blood and tissue sampling

Blood was collected from the retro-orbital plexus and centrifuged (3000×*g*, 4 °C, 20 min) for separation of serum. The obtained serum was used to measure the liver enzyme activities and total bilirubin level. Thereafter, animals were euthanized, livers were separately dissected, and blood was washed off with cold saline; then, livers were divided into two parts: one part was immediately flash frozen in liquid nitrogen and kept at −80 °C for measurement of tissue parameters and the other part was kept in 10% formalin for histopathological examination.

Biochemical analysis

Liver enzymes

The serum alanine amino transferase (ALT) and aspartate amino-transferase (AST) level were measured using commercially available analytical kit (Diamond diagnostic, Egypt). Alkaline phosphatase level was measured using commercially available analytical kit (Vitro Science, Egypt).

Determination of oxidative stress

The generation of reactive oxygen species in response to hepatic ischemia/reperfusion injury was determined in liver tissues by the measurement of the lipid peroxidation product content using commercially available kits and by quantitative ELISA analysis. Malondialdehyde (MDA) (OxiSelect™ TBARS Assay Kit, Cell Biolabs Inc.) (San Diego, USA), glutathione (GSH) (OxiSelect™ Total Glutathione (GSSG/GSH) Assay Kit, Cell Biolabs Inc.) (San Diego, USA), and superoxide dismutase (SOD) (Superoxide Dismutase Assay Kit, Trevigen Inc.) (Gaithersburg, USA)

were measured by commercially available kits. Catalase was detected using rat catalase, CAT ELISA Kit, Eiaab® (Wuhan, China).

Inflammatory cytokines and BcL2

1. Determination of TNF-α

The TNF-α level was detected in liver by quantitative ELISA using RayBio® Rat TNF-alpha ELISA Kit, Georgia, USA.

2. Determination of NF-κB

NF-κB (nuclear factor-kappa B) was detected by quantitative ELISA using rat nuclear factor-kappa B, NF-κB ELISA Kit, Eiaab®, Wuhan, China.

Determination of Bcl2

The Bcl-2 was detected by quantitative ELISA using Rat B-cell leukemia/lymphoma 2 (Bcl2) ELISA kit, Eiaab®, Wuhan, China.

Determination of iNOS and eNOS

The iNOS was detected by quantitative ELISA using rat-inducible nitric oxide synthase, iNOS ELISA Kit, Eiaab®, Wuhan, China. The eNOS was detected by quantitative ELISA using rat endothelial nitric oxide synthase, eNOS ELISA Kit, Eiaab®, Wuhan, China.

Determination of HO-1

1. Determination of enzyme content

(a) Enzyme linked immunosorbent assay

Enzyme-linked immunosorbent assay kit was used to measure heme oxygenase-1 content, (Decycling (HO1), Organism: *Rattus norvegicus* (Rat). USCN, Life Science Inc).

(b) Western blot

For Western blotting, RIPA lysis buffer PL005 was provided by Bio BASIC INC. (Marhham Ontario L3R 8 T4 Canada). The stored lysed samples were brought to complete protein extraction, and the lysate was kept on ice for 30 min on shaker. Then, cell debris was removed by centrifugation. Supernatant was then transferred to a new tube. Bradford Protein Assay Kit (SK3041) for quantitative protein analysis was provided by BIO BASIC INC. Markham Ontario, L3R 8T4, Canada. A Bradford assay was performed according to manufacture instructions. In our study, we used TGX Stain-Free™ FastCast™ Acrylamide Kit (SDS-PAGE) which was provided by Bio-Rad Laboratories, TNC, USA, catalog. no. 161-0181. Samples are then loaded on the running buffer, and a molecular weight marker (BLUelf pre-stained protein ladder, Gene DireX, Taiwan, cat no. PM008-0500) was used to enable the determination of the protein size and also to monitor the progress of an electrophoresis run. The membrane was blocked in Tris-buffered saline with Tween 20 (TEST) buffer and 3% bovine serum albumin (BSA) at room temperature for 1 h. The primary antibodies for MAPK were diluted in TEST. Incubation was done in the HRP-conjugated secondary antibody (Goat antirabbit IgG- HRP-lmg Goat mab-Novus Biologicals) solution against the blotted target protein for 1 h at room temperature. The blot separation was visualized immediately and imaged using stain-free blot technology and ChemiDoc™ imager.

2. Determination of enzyme expression by quantitative real-time PCR

Total RNA was isolated using Qiagen tissue extraction kit (Qiagen, USA) according to instructions of the manufacturer. The purity (*A*260/*A*280 ratio) and the concentration of RNA were obtained using spectrophotometry (dual wavelength Beckman, spectrophotometer, USA). HO-1 expression was assessed and related to a reference gene, β-actin. The primer sequence for HO-1 was forward primer: 5′-TTC-TAC-CTG-TTC-GAG-CAT-GTG-G-3′, reverse primer:5′-TGT-TAG-CAT-GGA-GCC-AGC-CT-3′ and for β-actin forward primer: 5′-TATCCTGGCCTCACTGTCCA-3, reverse primer: 5′-AACGCAGCTCAGTAACAGTC-3.

3. Determination of enzyme activity by measuring total bilirubin

Serum total bilirubin (tBil) was measured to assess the HO-1 activity using commercially available analytical kit (BioMed Diagnostics, Egypt).

Histopathological examination

Liver tissue samples were fixed in 10% formalin saline for 24 h. Then, tissues were washed with tap water and serial dilutions of alcohol (methyl, ethyl, and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 °C in hot air oven for 24 h. Paraffin beeswax tissue blocks were prepared for sectioning at 4-μm-thickness by sludge microtome. The obtained tissue sections were collected on glass slides and deparaffinized and stained by hematoxylin and eosin (H&E) (Bancroft and Stevens 1990). Slides were mounted using Entellan and covered with cover slips prior to viewing and photographed by a Nikon

Eclipse E-200 light microscope. Histopathological alterations were classified also using scores from 0 to 3, where 0 = no alteration, 1 = slight alteration, 2 = moderate, and 3 = severe alteration, according to Hose et al. (1996)) modified from Poleksic and Mitrovic-Tutundzic (1994)), and characterized as follows: (1) slight alteration—alterations that do not damage organ tissues and restricted to small areas, (2) moderate alteration—alterations that are more severe with pathological effects on tissues, and (3) severe alteration—marked irreversible pathological changes.

Statistical analysis

All data are presented as mean ± S.E.M. Statistical analysis was performed using ANOVA followed by Tukey's post hoc test. Values were analyzed using a computer-based curve fitting program (Prism 5, GraphPad, CA, USA). P value of <0.05 was considered statistically significant.

Results

Effect on liver enzymes

Quercetin significantly suppressed elevation in liver enzyme activities ($P < 0.05$) when compared to the I/R group. SnPP significantly suppressed the elevation in ALT and ALP enzyme activities (Table 1). SnPP showed less prominent suppression to elevated liver enzymes (AST and ALP) when compared with quercetin ($P < 0.05$).

Effect on oxidative stress biomarkers

Ischemia followed by reperfusion resulted in a significant increase in MDA and reduction of GSH, SOD, and catalase compared to the sham group. Quercetin significantly reduced MDA by 62% and significantly increased GSH, SOD, and catalase by 251, 216, and 250%, respectively, compared to the I/R group while it showed non-significant difference in MDA levels comparing to the sham group. Treatment with SnPP before I/R reduced MDA by 45% but significantly increased GSH, SOD, and catalase by 126, 123, and 120%, respectively, compared to the I/R group (Fig. 1).

Pretreatment with quercetin has showed more prominent reduction of elevated MDA and more significant elevation of suppressed GSH, SOD, and catalase than of SnPP.

Effect on inflammatory cytokines and Bcl2

Ischemia followed by reperfusion resulted in significant increase in TNF-α and in NF-κB and deteriorates the levels of Bcl2 compared to the sham group ($P < 0.05$). Both quercetin and SnPP showed significant decrease in the elevated TNF-α and NF-κB and increase in the depressed Bcl2 compared to the I/R group ($P < 0.05$). Quercetin showed more prominent reduction of inflammatory cytokines and increased Bcl2 SnPP (Table 2).

Effect on iNOS and eNOS

Ischemia followed by reperfusion resulted in a significant increase in iNOS expression by 479%. Quercetin significantly decreased iNOS expression by 69% compared to the I/R group. SnPP administration decreased iNOS by 45% compared to I/R.

I/R group showed significant decrease in eNOS expression by 82% compared to the sham group. Quercetin significantly increased eNOS expression by 197% while it showed non-significant difference in iNOS levels comparing to the sham group. However, SnPP administration before I/R caused significant increase of eNOS expression by 67% compared to the I/R group (Fig. 2).

Quercetin showed more potent reduction of elevated iNOS and more elevation of suppressed eNOS than that of SnPP.

Effect on heme oxygenase 1

Compared to the I/R group, both quercetin and SnPP caused elevation of suppressed HO-1 protein content, expression, and activity (Fig. 3 and Table 3). Quercetin had more potent effect on HO-1 content and expression than that of SnPP.

Table 1 Effect of ischemia/reperfusion-induced liver injury and i.p. administration of quercetin (50 mg/kg) and tin protoporphyrin (SnPP, 50 μmol/kg) on serum liver enzymes (serum ALT, AST, and ALP activities) in rats

Parameters	Sham	I/R	Quercetin	Snpp
ALT (U/L)	79 ± 6.48	142.66^{a} ± 2.29	$100.9^{a,b}$ ± 3.56	$138^{a,c}$ ± 6.37
AST(U/L)	23.5 ± 6.06	82.5^{a} ± 2.09	$47.50^{a,b}$ ± 2.98	$51.16^{a,b}$ ± 2.52
ALP(U/L)	74.33 ± 2.8	154.33^{a} ± 2.7	$101^{a,b}$ ± 3.72	$186.8^{a,b,c}$ ± 5.21

Results are presented as mean ± SEM (n = 6–8)

[a] $P < 0.05$ (significantly different from the sham group) by one-way ANOVA and Tukey's post hoc test

[b] $P < 0.05$ (significantly different from the I/R group) by one-way ANOVA and Tukey's post hoc test

[c] $P < 0.05$ (significantly different from the quercetin) by one-way ANOVA and Tukey's post hoc test

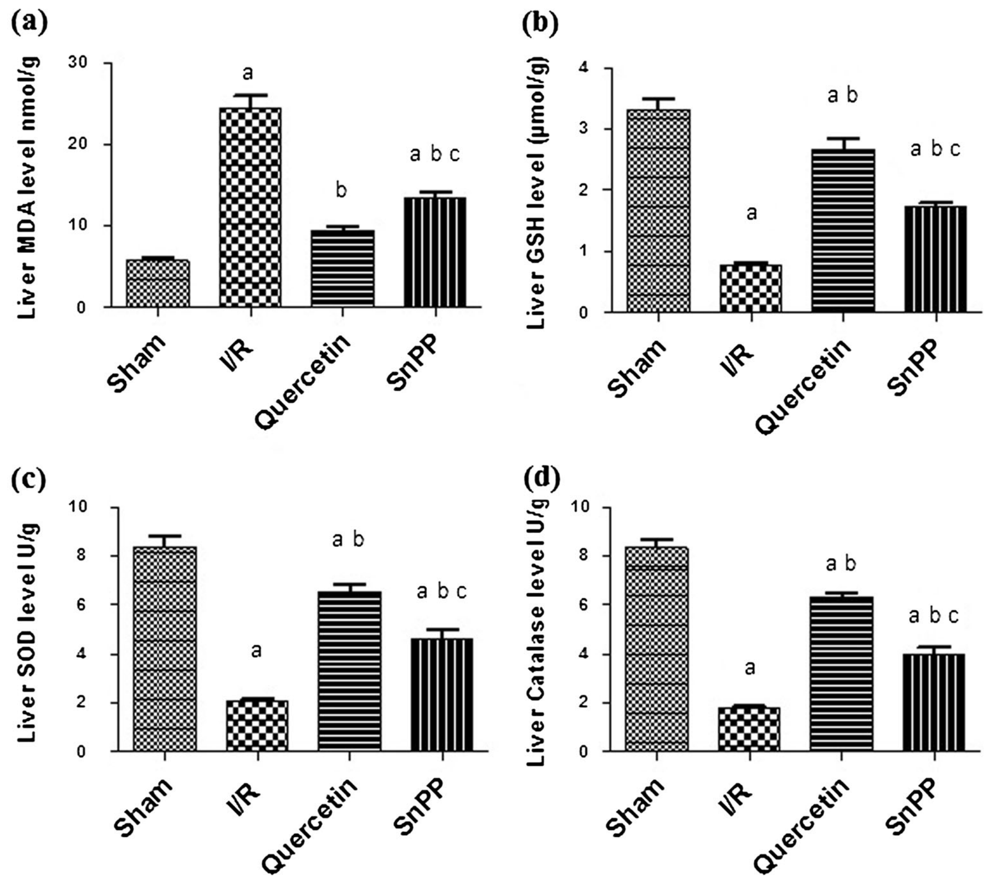

Fig. 1 Effect of ischemia/reperfusion-induced liver injury and i.p. administration of quercetin (50 mg/kg) and SnPP (50 μmol/kg) on **a** MDA, **b** GSH, **c** SOD, and **d** catalase in liver tissue. Results are presented as mean ± SEM (n = 6–8); $^{a}P < 0.05$ (significantly different from sham group), $^{b}P < 0.05$ (significantly different from I/R group), and $^{c}P < 0.05$ (significantly different from Quercetin); by one-way ANOVA and Tukey's post-hoc test

Histopathological results

Light microscopic examination of liver sections of control rats revealed that the hepatic parenchymal cells are arranged as sheets intimately associated with a venous portal system in an uncomplicated and consistent way throughout the organ. The hepatocytes were arranged in cords radiating from the central veins. The hepatocytes form columns of cells adherent to each other by one or more surfaces with well-preserved cytoplasm and prominent round nucleus with fine arrangement of Kupffer cells. The hepatic sinusoids were seen as narrow spaces in between the hepatic cords (Fig. 4 (1) and Table 4).

In contrast, rats subjected to I/R showed extensive pathological changes. Hepatic cell and hepatic microvascular injury were observed. Disordered hepatic lobules, swelled cells, and vacuoles in the liver specimens were visible in this group, which implied that hepatic I/R injury had occurred. The most pronounced pathological abnormalities observed involved degeneration and necrosis of many hepatic cells. Hepatocyte degeneration is mainly associated with cytoplasmic vacuolation with the nuclear contour generally intact, whereas the hepatocyte necrosis is associated with karyorrhexis, in addition to degenerative changes. Most injury was characterized by increased numbers of inflammatory cells. The blood sinusoids were dilated. The striking sign of the liver tissue injury

Table 2 Effect of ischemia/reperfusion-induced liver injury and i.p. administration of quercetin (50 mg/kg) and tin protoporphyrin (SnPP, 50 μmol/kg) on liver inflammatory cytokines (TNF-α and NF-κB) and BCL_2

Parameters	Sham	I/R	Quercetin	Snpp
TNF-α (pg/g)	6.8 ± 0.49	29.46^{a} ± 2.43	10.5^{b} ± 0.43	$16.9^{a,b,c}$ ± 0.98
NF-κb (ng/g)	0.75 ± 0.03	3.17^{a} ± 0.12	$1.12^{a,b}$ ± 0.02	$1.74^{a,b,c}$ ± 0.06
Bcl2 (mg/mL)	27.66 ± 1.39	8.43^{a} ± 0.49	$21.03^{a,b}$ ± 0.86	$14.66^{a,b,c}$ ± 0.58

Results are presented as mean ± SEM (n = 6–8)

^{a} $P < 0.05$ (significantly different from sham group) by one-way ANOVA and Tukey's post hoc test

^{b} $P < 0.05$ (significantly different from I/R group) by one-way ANOVA and Tukey's post hoc test

^{c} $P < 0.05$ (significantly different from quercetin) by one-way ANOVA and Tukey's post hoc test

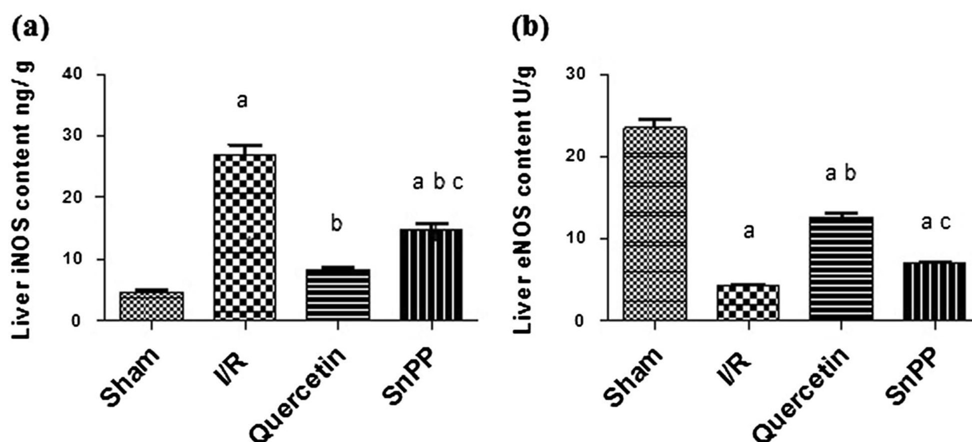

Fig. 2 Effect of ischemia/reperfusion-induced liver injury and i.p. administration of quercetin (50 mg/kg) and SnPP (50 μmol/kg) on **a** iNOs and **b** eNOS in liver tissue. Results are presented as mean ± SEM (n = 6–8); $^{a}P < 0.05$ (significantly different from sham group), $^{b}P < 0.05$ (significantly different from I/R group), and $^{c}P < 0.05$ (significantly different from quercetin); by one-way ANOVA and Tukey's *post-hoc* test

was well discerned at the central vein. Central veins exhibited remarkable dilatation and congestion and its endothelium was eroded (Fig. 4 (2)).

The liver of rat treated by quercetin then subjected to I/R showed mild degeneration of hepatocytes with the presence of some inflammatory cells and mild congestion. Blood sinusoids were less dilated than group II (Fig. 4 (3)).

The liver of rat treated by SnPP then subjected to I/R showed hepatocytes with moderate degeneration with the presence of inflammatory cells. Blood sinusoids and central vein were also moderately dilated with moderate congestion and mild edema (Fig. 4 (4)).

Discussion

In this study, we investigated the effect of quercetin and SnPP on the HO-1 level in hepatic I/R induced in rats. Our findings identify the potential protective effect of quercetin and SnPP on induced hepatic I/R injury. The following findings can explain the ability of both of them to attenuate the hepatic injury when administered prior to ischemia when compared to untreated animals as follows: (a) quercetin and SnPP attenuate the biochemical changes and elevate the suppressed bilirubin level. (b) They both show antioxidant effect through decreasing the elevated MDA and increasing the suppressed antioxidants as GSH, SOD, and catalase. (c) Quercetin and SnPP suppress the elevation in NF-κB expression and TNF-α production and increase the suppressed apoptotic marker BCL2. (d) They both decrease the elevated iNOS and increase the decreased eNOS and HO-1. Such findings prove that quercetin and SnPP protect the liver against I/R injury through their elevation of HO-1 expression and activity and their anti-inflammatory and antioxidant effect.

Our findings showed that the induction of ischemia for 45 min followed by 1 h reperfusion results in elevation of liver enzymes, ALP, and histopathological lesions in liver sections obtained from I/R rats. These findings come in agreement with the results found in previous studies of hepatic I/R injury in rats (Tanrikulu et al. 2013, Savvanis et al. 2014). On the other hand, significant decreases of ALT, AST, and ALP in group pretreated by quercetin and which in turn are confirmed by results of histopathological examination. For the SnPP group, there was a significant decrease of ALT compared to I/R rats and non-significant decrease of AST, while there was a significant elevation in ALP compared to I/R group.

MDA is an end product of lipid peroxidation and reflects the degree of oxidative stress (Shah et al. 2014). GSH is considered the major endogenous antioxidant produced by hepatocytes, participating directly in scavenging free radicals and

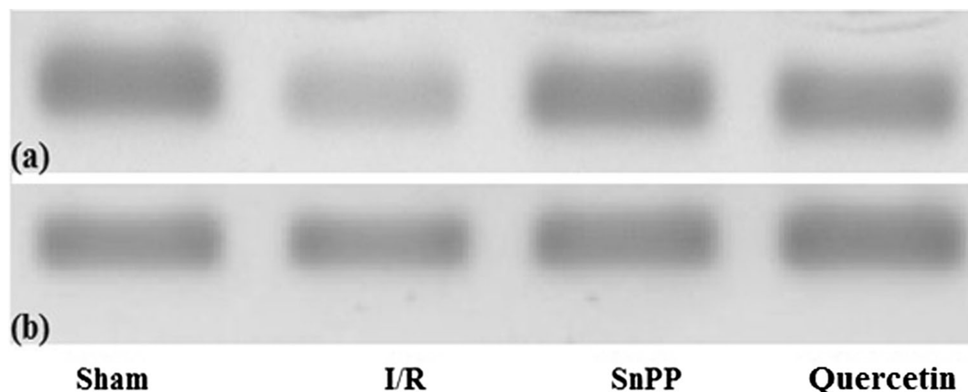

Fig. 3 Western blot analysis show **a** protein expression of HO-1 and **b** beta actin in different studied groups

Table 3 Effect of ischemia/reperfusion-induced liver injury and i.p. administration of quercetin (50 mg/kg) and tin protoporphyrin (SnPP, 50 μmol/kg) on liver heme oxygenase-1 (HO-1) enzyme content detected using quantitative ELISA and Western blot analysis and liver HO-1 mRNA expression detected by RT-PCR and activity using total bilirubin in rats

Parameters	Sham	I/R	Quercetin	Snpp
HO-1 (ng/g)	3.04 ± 0.23	0.77[a] ± 0.06	1.69[a,b] ± 0.06	1.07[a,c] ± 0.02
HO-1 by Western blot	1.12 ± 0.04	0.27[a] ± 0.03	0.72[a,b] ± 0.12	0.65[a,b] ± 0.08
HO-1 by RT-PCR	1.00 ± 0.01	0.21[a] ± 0.01	0.88[b] ± 0.02	0.72[a,b] ± 0.8
Total bilirubin(mg/dL)	0.31 ± 0.01	0.17[a] ± 0.01	0.32[b] ± 0.01	0.52[a,b,c] ± 0.02

Results are presented as mean ± SEM (n = 6–8)

[a] $P < 0.05$ (significantly different from sham group) by one-way ANOVA and Tukey's post hoc test

[b] $P < 0.05$ (significantly different from I/R group) by one-way ANOVA and Tukey's post hoc test

[c] $P < 0.05$ (significantly different from quercetin) by one-way ANOVA and Tukey's post hoc test

ROS and maintains exogenous antioxidants in their active forms (Scholz et al. 1997, Altas et al. 2011). GSH, catalase, and SOD are suppressed in oxidative stress conditions (Tao et al. 2014). This probably is the result of degradation of antioxidants due to an increase in free radical activity (Marubayashi et al. 1987).

In the present study, we observed a higher level of MDA and a lower level of GSH, SOD, and catalase in the liver of I/R-treated rats. However, such oxidative stress was significantly corrected by quercetin pretreatment. Surprisingly, SnPP showed similar effect to quercetin with less prominent correction.

In agreement, quercetin was reported to alleviate oxidative stress in a rat model of hepatic I/R injury (Shizuma 2014). SnPP findings have proven that SnPP has an antioxidant activity based on peroxyl radical scavenging (Williams et al. 1994).

NF-κB plays a critical role in activation of macrophages and the subsequent production of pro-inflammatory cytokines and other mediators (Karin and Ben-Neriah 2000). Incorrect regulation of NF-κB has been linked to inflammatory and autoimmune diseases (Cressman et al. 1994).

In this study, the NF-κB induced by I/R was inhibited when rats were pretreated with quercetin and SnPP. Recent studies have attributed the anti-oxidative and anti-inflammatory effects of quercetin to its ability to inhibit the NF-κB pathway (Chen et al. 2005, Kang et al. 2013, Saw et al. 2014). SnPP showed to protect against injury from hypoxic ischemia (Sardana and Kappas 1987) by decreasing the systemic and local inflammatory mediators (Ibanez et al. 2011).

TNF-α was reported to be up-regulated in I/R injury and can initiate additional cellular inflammatory responses ultimately causing organ injury (Marshall et al. 2014). In

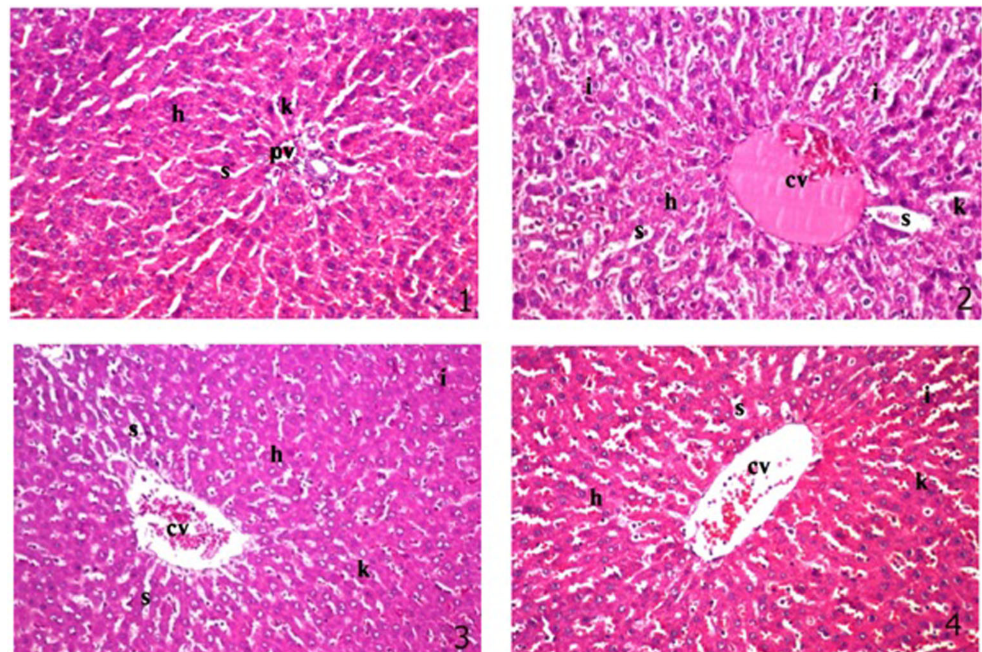

Fig. 4 Photomicrograph of a section in the liver from four rats stained with hematoxylin and eosin. (*1*) Sham group, (*2*) I/R group, (*3*) quercetin group, and (*4*) SnPP group. (h) hepatocytes, (PV) portal vein, (S) blood sinusoids, (k) kupffer cells, (cv) central vein, and (i) inflammatory cells (H&E × 200)

Table 4 The severity of histopathological alterations in hepatic tissue of different experimental groups

Parameters	Sham	I/R	Quercetin	SnPP
Congestion	−	+++	+	++
Degenerative change in hepatocytes	−	++	+	+
Inflammatory reaction in portal area	−	+++	+	++
Oedema in portal area	−	+	−	+
Focal hemorrhage in parenchyma	−	−	−	−

− no alterations, + slight alteration, ++ moderate alteration, +++ severe alteration

accordance with the present results, Camara-Lemarroy et al. (2014)) reported increased level of TNF-α in I/R in rats.

Quercetin and SnPP significantly decreased blood levels of TNF-α; however, SnPP lowered it with less prominent effect. Such findings came with agreement with previous studies that stated that levels of TNF-α in rat serum were inhibited by quercetin treatment, supporting the hepatoprotective effect of quercetin, which is partly attributed to its anti-inflammation action (Ali et al. 2015). SnPP probably produces its protective effects through other inducible pro-inflammatory enzymes such as NOS and COX (Sutherland et al. 2011).

The BCL2 family of proteins regulates the cellular changes during both apoptosis and necrosis (Tsujimoto and Shimizu 2000). Here, the suppression of BCL2 after I/R was marked. Significantly, quercetin and SnPP prior to administration increased BCL2. These observations suggest that the quercetin and SnPP have anti-apoptotic properties which comes in agreement with others who reported that quercetin possesses anti-apoptotic properties (Kanter et al. 2012). High dose of SnPP pretreatment suppressed the caspase-3 activation and decreased the apoptotic cell death (Kaizu et al. 2003).

NOs is released in inflamed tissues (Brenner et al. 2013). Under stressful conditions hepatocytes may express iNOS with consequent production of large amounts of NO (Hon et al. 2002). We detected an increased expression of iNOS and decreased expression of eNOS in hepatic tissue of I/R rats. Quercetin and SnPP significantly suppressed the elevated iNOS expression and elevated the decreased eNOS production. Quercetin treatment significantly increased hepatic eNOS expression in a model of cadmium-induced hepatotoxicity (Vicente-Sanchez et al. 2008). SnPP was associated with a significant up-regulation of total NOS activity and inhibition of iNOS activity (Sutherland et al. 2011). SnPP-exerted protection might be related to the inhibition of iNOS and COX activities, while increasing total NOS activity. SnPP was associated with a significant up-regulation of total NOS activity and inhibition of iNOS activity (Sutherland et al. 2011).

From previous studies, the in vivo expression of HO-1 has been shown to protect livers from ischemia/reperfusion injury caused by either transplantation (Amersi et al. 1999) or hemorrhage/resuscitation (Rensing et al. 1999). The suppressive effect of quercetin against I/R-induced NO production may be related to HO-1 expression induced by quercetin. The results indicated that quercetin-mediated HO-1 induction plays a role in modulating NO production. The inhibitory effect of quercetin against NO production is mediated by the enzymatic by-products of heme catabolism, such as CO and bilirubin (Morse et al. 2003).

Eighty to 85% of bilirubin is derived from heme catabolism in vivo where HO-1 is the rate-limiting enzyme; the amount of formed bilirubin indicates the HO activity (Abraham and Kappas 2008). Bilirubin also represents a physiologically important defense against reactive oxygen species and has cytoprotective effects (Jansen and Daiber 2012). The present study showed decrease of total bilirubin in the I/R group compared to the sham group as indicator that the liver is no longer protected. It also indicates that HO-1 has been suppressed. Total bilirubin was increased in the group pretreated with quercetin and SnPP indicating the activation of HO-1.

SnPP was originally used to inhibit HO-1 expression; surprisingly, it yielded the opposite effect. In our study, SnPP induced HO-1 content, expression, and activity. Bing et al. (1995)) showed that HO activity was reduced during the first 6 h following a single administration of SnPP, but daily SnPP administration for 4 days failed to maintain this HO inhibition. This suggests that repeated administrations or high dose of SnPP might reduce its effect for inhibition of HO. SnPP may induce HO-1 at both the transcriptional and translational levels (Ito et al. 2000). Other researchers suggest that HO-1 induction participates in the protective effects of this protoporphyrin (Kaizu et al. 2003). The present study demonstrated that pretreatment with SnPP improved liver functions, enzymes, and morphological structure compared to I/R group. It could be improved by HO-1 overexpression following preoperative SnPP administration.

Histopathological findings in the present study have shown that hepatocytes were very much negatively affected by I/R. Reperfusion injury mainly derives from toxic reactive oxygen species generated upon reintroduction of oxygen to ischemic tissues. Reactive oxygen species are produced from both intracellular and extracellular sources, with the mitochondria being their major source in liver cells (Caraceni et al. 2005). The protective effect of quercetin was confirmed by histological findings which show intact hepatic architecture and mild sinusoidal congestion and degeneration. Quercetin significantly decreased all histopathological scores. In agreement with the present study, Tokyol et al. (2006)) stated that histopathological liver damage scores significantly decreased in quercetin pretreated I/R rats compared to I/R alone. While pretreatment with SnPP reduced sinusoidal congestion, cytoplasmic vacuolation, inflammatory cells, and hepatocyte necrosis, it was not marked as in the quercetin group. SnPP administration could preserve mitochondrial energetics

and reduce cell death (Sutherland et al. 2011). High dose of SnPP decreases the inflammatory cell number in the exudates in the pleural cavity 24 h after injection of carrageenan (Willis et al. 1996).

SnPP regulates HO by a dual control mechanism, potently inhibiting the HO enzyme activity while enhancing the synthesis of HO enzyme protein (Sardana and Kappas 1987). The relation between SnPP and HO-1 is complicated. First, as a competitive inhibitor of HO-1, SnPP can increase HO-1 mRNA and protein levels either by enzyme or by the induction of a mild pro-oxidant state with counterbalancing HO-1 production (Kaizu et al. 2003). Second, the SnPP may upregulate HO-1 via direct pro-oxidant effects (Barrera-Oviedo et al. 2013). Third, secondary HO-1 induction could potentially be offset by SnPP-induced HO-1 inhibition. We believe that the latest mechanism is the possible explanation for the SnPP results in the present study.

Other HO-1 inhibitors such as zinc protoporphyrin (Hirai et al. 2007) have been previously reported to be an HO-1 inducer through different mechanisms (Yang et al. 2001, Kwok 2013). Other studies have suppressed HO-1 using small interfering RNA (siRNA) and after using HO-1 inducer, they have shown similar anti-oxidant and anti-inflammatory effects to our presented results (Ke et al. 2009, Shen et al. 2011). Also, suppressing HO-1 using heterozygous Hmox-1-knockout mice results in elevated levels of oxidative stress; such results suggest the crucial role of HO-1 as an antioxidant (Yoshida et al. 2001), which suggests the antioxidant effect showed in the groups pretreated with HO-1 inducers in our presented study. Such evidences prove that the attenuation in hepatic I/R injury in the present study was probably due to HO-1 pathway.

Conclusions

These results suggest that quercetin and SnPP have a beneficial effect in protecting the liver against I/R injury during organ transplantation and hepatectomies probably through HO-1 elevation.

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