

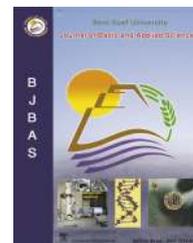
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Full Length Article

Quercetin modulates iNOS, eNOS and NOSTRIN expressions and attenuates oxidative stress in warm hepatic ischemia-reperfusion injury in rats



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ABSTRACT

The pathogenesis of hepatic ischemia/reperfusion (I/R) is mediated through the generation of oxidative and nitrosative stress-induced cell injury. Hence, the present study was designed to evaluate the hepatoprotective effect of quercetin (QR) compared to N-acetylcysteine (NAC) against hepatic I/R injury in rats and to assess iNOS, eNOS and NOSTRIN protein expressions, as a possible mechanism of its hepatoprotective effect. Hepatic ischemia was surgically performed by occlusion of hepatic pedicle (hepatic artery, portal vein, bile duct) that supplies the left and medial lobes (approximately 70% of the total liver mass), for 30 min with a vascular clamp followed by releasing the clamp and the liver was reperfused for 30 min. QR-pretreatment increased eNOS protein expression with simultaneous decrease in iNOS and NOSTRIN protein expressions. It also decreased serum aspartate aminotransferase (AST), alanine aminotransferases (ALT) and hepatic myeloperoxidase (MPO) activities. In addition, it restored the depleted content of reduced glutathione (GSH) and decreased malondialdehyde (MDA) and nitric oxide (NO) levels. A notable finding is that QR alleviated I/R-induced histopathological changes. Therefore, the present study illustrates the hepatoprotective effect of quercetin compared to N-acetylcysteine against ischemia/reperfusion-induced liver injury by inhibiting oxidative stress and by modulating iNOS, eNOS and NOSTRIN protein expressions.

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1. Introduction

Ischemia/reperfusion (I/R) is a phenomenon whereby delivery of oxygen upon reperfusion to an organ after a period of hypoxia leads to accentuated cellular injury. In 1975, this form of injury in the liver was recognized, during experimental liver transplantation studies, as a clinically important pathological disorder (Teoh and Farrell, 2003). Also, I/R is a common problem encountered in a variety of clinical conditions such as hepatic trauma, sepsis (Yildiz et al., 2008), hepatic tumors resection and hepatic pedicle clamping (Pringle maneuver) during a liver surgery (van Gulik et al., 2007). I/R injury could be severe enough leading to significant morbidity and mortality (Siriussawakul et al., 2010). During reperfusion, excessive reactive oxygen species (ROS) generation (Jaeschke, 2003), Kupffer cells activation, interstitial edema, neutrophils infiltration and release of inflammatory cytokines were reported (Montalvo-Jave et al., 2008). In addition, various mediators such as nitric oxide (NO) are involved in the ischemic injury. NO plays a paradoxical role in liver physiology where a cytoprotective effect can result from small amounts of NO induced by endothelial nitric oxide synthase (eNOS) isoform while overproduction of NO induced by inducible nitric oxide synthase (iNOS) isoform may be lethal to hepatic tissues (Lin et al., 2004). Its byproduct, peroxynitrite (ONOO⁻) also causes further hepatic injury due to its potent oxidative effect (Jaeschke, 2003). Zimmermann et al. (2002) stated that eNOS is translocated by eNOS traffic inducer (NOSTRIN) from the plasma membrane to vesicle-like subcellular structures leading to strongly attenuated eNOS-dependent NO production. There is an imbalance between pro-oxidants which significantly increase and the endogenous antioxidant levels which significantly decrease during reperfusion (Montalvo-Jave et al., 2008). Therefore, the administration of exogenous antioxidants can significantly decrease the severity of I/R injury. Quercetin (QR) is one of the most abundant natural flavonol type flavonoids (Kuo et al., 1998; Pawlikowska-Pawłęga et al., 2003) in food and beverage sources that are part of the human diet such as apples, onions, broccoli, lettuce, tea, coffee, and tomatoes (Yousef et al., 2010). It is the most potent antioxidant of the flavonoid family and possesses strong anti-inflammatory properties (Manach et al., 2005; Yousef et al., 2010). It was also reported that QR has protective effects against I/R-induced renal injury (Kahraman et al., 2003), ultraviolet light-induced oxidative stress (Inal and Kahraman, 2000), spontaneous hypertension (Duarte et al., 2001), secondary biliary cirrhosis (Peres et al., 2000), lipopolysaccharide (LPS) (Wadsworth and Koop, 2001) and carcinogenesis (Yang et al., 2000). N-acetylcysteine (NAC), used as a standard treatment in the present study to compare and evaluate the hepatoprotective effect of quercetin, is a sulfhydryl-containing compound and capable of rapidly restoring depleted intracellular reduced glutathione (GSH) content (Glantzounis et al., 2004). It has antioxidant property by acting as a powerful scavenger of free radicals (Aruoma et al., 1989) and anti-inflammatory properties (Zafarullah et al., 2003). Also, it is extensively used as a specific antidote for paracetamol overdose (Dodd et al., 2008). Moreover, it protects against oxidative stress and ischemic injury, that's why it is used for the treatment of several ill-

nesses such as schizophrenia, bipolar disorder (Dodd et al., 2008), heart disease, cancer (Yousef et al., 2010) and as a mucolytic agent for the treatment of bronchopulmonary disease (Dodd et al., 2008). In addition, it is used as a hepatoprotective agent in liver transplantation (Thies et al., 1998). Therefore, the present study aimed to assess the potential protective effects of quercetin compared to N-acetylcysteine in an experimental model of I/R-induced liver injury in rats and to evaluate iNOS, eNOS and NOSTRIN expressions as a possible mechanism of its hepatoprotective effect.

2. Materials and methods

2.1. Chemicals

Quercetin (QR), N-acetylcysteine (NAC), Thiobarbituric acid, N-(1-Naphthyl) ethylenediaminedihydrochloride (NEDD), Sulfanilamide and Ellman's reagent (5-5'-dithiobis-(2-nitrobenzoic acid)) (DTNB), 4,6-diamidino-2-phenylindole (DAPI), O-dianisidine were purchased from Sigma-Aldrich, USA. Hexadecyltrimethylammonium bromide (HTAB) was purchased from MP Biomedicals, Inc., USA. Vanadium trichloride (VCl₃) was purchased from Acros, Belgium. Aspartate aminotransferase (AST) reagent kit and Alanine aminotransferase (ALT) reagent kit were purchased from Spinreact, Spain. Mouse polyclonal anti-inducible nitric oxide synthase (anti-iNOS) and Mouse polyclonal anti-endothelial nitric oxide synthase (eNOS) were purchased from BD-Bioscience, USA. Rabbit polyclonal anti-eNOS trafficking inducer (anti-NOSTRIN) was purchased from Proteintech, USA. Rabbit anti-mouse Alexafluor 488 secondary antibody was purchased from Invitrogen, USA. Goat anti-rabbit Cy3 secondary antibody was purchased from Jackson ImmunoResearch, USA. Other chemicals were selected to be with the highest analytical grades.

2.2. Animals and experimental protocol

Forty male adult Wister albino rats weighing 300-350 gm were used. This study has been approved by the Research Ethics Committee of the Faculty of Pharmacy, Beni-Suef University, Egypt in accordance with the guidelines of the National Institutes of Health (NIH). Rats were caged at a controlled temperature of 25 °C under 12 h/12 h light/dark cycle, allowed free access to food and tap water ad libitum and left for two weeks in animal house conditions for acclimation. After that, animals were randomly assigned to four groups each consisting of 10 rats. The first group served as sham-operated control (received 0.9% saline and 1% tween80 orally), and underwent operation similar to the other groups without actual clamping of the vessels. Group 2 was subjected to ischemia/reperfusion injury (I/R) (30 minutes ischemia followed by 30 minutes reperfusion) (Su et al., 2003). Groups 3 and 4 were pretreated with N-acetylcysteine (NAC) (300 mg/kg freshly dissolved in 0.9% saline) (Hemalatha et al., 2013) and quercetin (QR) (20 mg/kg suspended in 0.9% saline and 1% tween80) (Yousef et al., 2010), respectively. Rats were daily administered their respective doses per oral (p.o) for 10 days before subjection to I/R.

2.3. Induction of ischemia/reperfusion in liver

The abdomen was opened through a midline incision under intraperitoneal ketamine (50 mg/kg) and xylazine HCl (10 mg/kg) anesthesia (Ara et al., 2005). Dissection of all attached ligaments connecting the liver, diaphragm and neighboring organs and liver hilum was exposed to reveal the common hepatic artery and portal vein. Hepatic ischemia was induced by occlusion of hepatic pedicle (hepatic artery, portal vein, bile duct) which supplies the left and medial lobes (70% of the total liver mass) for 30 minutes with a vascular clamp. Occlusion was evidenced by immediate liver color change into a paler shade. The vascular clamp was released and the liver was reperfused for 30 minutes. 0.5 ml 0.9% saline was given intraperitoneally during the period of ischemia (Karabulut et al., 2006) and the open abdomen was covered by plastic wrap to avoid dehydration. The body temperature was recorded by rectal thermometer and maintained at 37 ± 0.5 °C by placing the animals under heating lamp. At the end of reperfusion, blood samples were collected from the retro-orbital plexus for the determination of serum aminotransferase, then the animals were sacrificed and liver was excised and cut into smaller pieces. Then, it was fixed in Davidson-Solution for immunofluorescence assay and in 10% formalin saline for histopathological evaluation or stored at -80 °C until used for preparation of 25% liver homogenate for various biochemical determinations.

2.4. Determination of serum aspartate aminotransferase (AST)

Serum AST was assayed according to the method described by manufacturer's instructions using test reagent kits (Spinreact, Spain). The absorbance was spectrophotometrically measured at 340 nm and its activity was expressed in U/L.

2.5. Determination of alanine aminotransferase (ALT)

Serum ALT was measured by the method described by manufacturer's instructions using test reagent kits (Spinreact, Spain). The absorbance was spectrophotometrically measured at 340 nm and its activity was expressed in U/L.

2.6. Determination of malondialdehyde (MDA) level

Malondialdehyde, as lipid peroxides end product, was measured according to the method described by Uchiyama and Mihara (1978). The standard curve was constructed by using 1,1,3,3-tetramethoxypropane. The difference in absorbance, spectrophotometrically measured at 535 and 520 nm, was used to calculate the MDA level in each sample and its level was expressed in nmol/g tissue.

2.7. Determination of reduced glutathione (GSH) content

Reduced glutathione content was assayed by the method of Beutler et al. (1963). The resulting yellow color absorbance was spectrophotometrically measured within 5 min at 412 nm and its content was expressed in mg/g tissue.

2.8. Determination of myeloperoxidase (MPO) activity

Myeloperoxidase activity was assayed using the method of Krawisz et al. (1984). The absorbance was spectrophotometrically measured at 460 nm using UV-Double Beam spectrophotometer. MPO activity was calculated according to the following equation: MPO activity (U/g tissue) = $\Delta A/\text{min} \times D.F./\text{extinction coefficient}$; where dilution factor = reciprocal of the relative sample volume in the reaction mixture \times dilution factor of the homogenate \times 1000. Extinction coefficient = $1.13 \times 10^4 \text{ Cm}^{-1} \text{ M}^{-1}$.

2.9. Determination of nitric oxide (NO) content

The content of NO was measured according to the method of Miranda et al. (2001). In the presence of molecular oxygen, NO was relatively unstable and rapidly oxidized to nitrite and nitrate totally designated as (NO_x). The absorbance was spectrophotometrically measured at 540 nm. NO content was calculated according to the standard curve using NaNO₃ and its production was expressed in $\mu\text{M/g}$ tissue.

2.10. Examination of histopathological sections

Liver samples were sliced and immediately fixed in 10% formalin saline for 72 h. Then, they were processed according to Bancroft and Gamble (2008) and stained with conventional hematoxylin and eosin (H&E) stain. Finally, microscopical examination and photomicrography of various groups was performed.

2.11. Immunofluorescence assay of iNOS, eNOS and NOSTRIN expressions

Paraffin tissue sections were deparaffinized by heating at 56 °C for 20 min, then cleaned using xylene 100% two times, 15 min each and rehydrated using gradient concentrations of ethanol 100% for 5 min two times, 90%, 70%, 50% and 30%, 5 min each. After rehydration, tissue was washed with distilled water for 5 min and incubated in Dako solution (citrate buffer, pH 6) in microwave at 500 watt for 20 min for antigen retrieval. After cooling of the solution, slides were washed three times by phosphate buffered saline (PBS), pH 7.4 containing 0.5% tween 20. Fixation was done using absolute methanol for 30 min. After washing, sections were blocked with PBS containing 10% horse serum and 1% bovine serum albumin at room temp. for 1 h to block the non-specific binding of antibodies. The primary antibodies (Mouse polyclonal iNOS or eNOS or Rabbit polyclonal NOSTRIN antibodies) were incubated with tissue sections overnight in 4 °C in the concentration of 1:50. Bound antibodies were detected by rabbit anti-mouse Alexafluor 488 (Green) or goat anti-rabbit Cy3 (Red) (Molecular Probes) secondary antibodies for 30 min, respectively. Nuclei were stained with DAPI and washed by PBST for 30 min. Slides were mounted with Fluoromount, covered by covering slips and allowed to be evaluated by fluorescence microscopy (Leica DM5000 B).

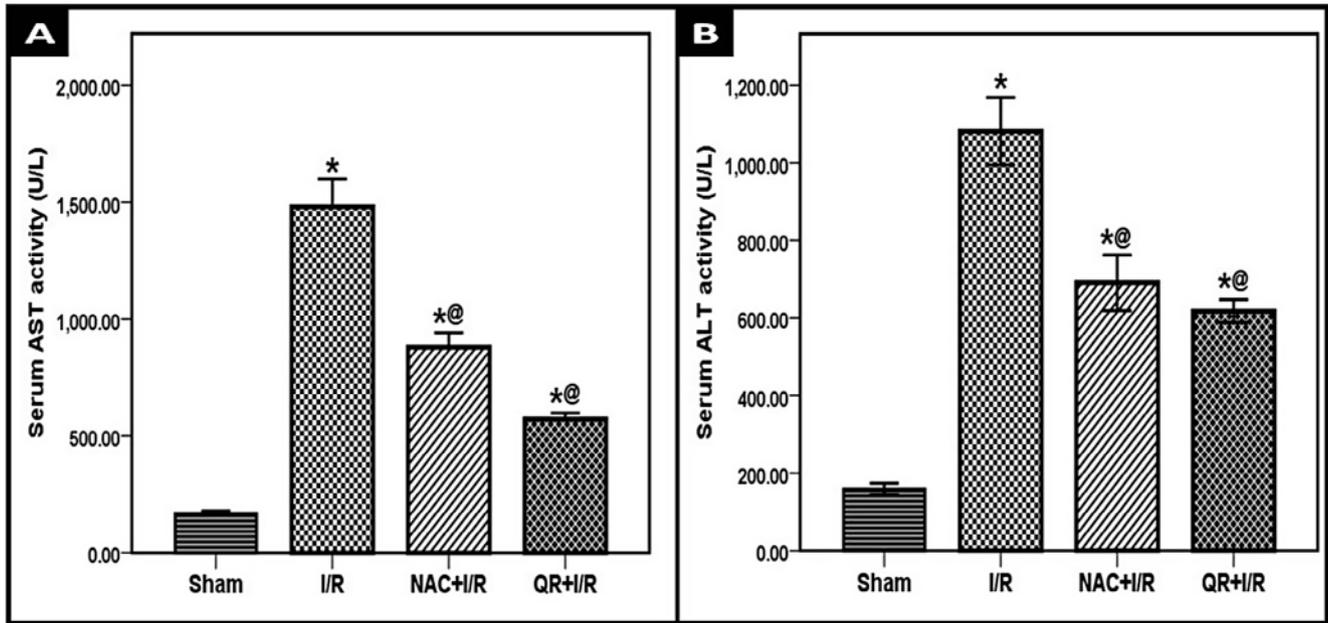


Fig. 1 – Effect of QR in I/R subjected rats on serum AST and ALT activities. (A) Serum AST activity of sham-operated control and I/R with or without NAC or QR-pretreatment. (B) Serum ALT activity of sham-operated control, I/R, and of NAC and QR-pretreatment. All data were expressed as means \pm standard error (S.E.). *Significantly different from sham-operated group at $P < 0.05$; @Significantly different from I/R group at $P < 0.05$. Means with the same superscript symbol are not significantly different at $P < 0.05$.

2.12. Statistical analysis

Statistical analysis of the results was performed using the statistical package for social sciences (SPSS) software (version 22.0). Data were expressed as means \pm standard error (S.E.). All parameters were analyzed using one-way analysis of variance (ANOVA) and the Tukey test for post hoc multiple comparison. Results were graphically represented by bar charts. A P value less than 0.05 was considered as statistically significant difference.

3. Results

3.1. Effect of QR in I/R subjected rats on serum AST and ALT activities

Serum liver toxicity markers, AST and ALT, were depicted in Fig. 1A and B, respectively. I/R group significantly increased serum AST and ALT activities to about $914.16 \pm 46.88\%$ and $846.46 \pm 91.92\%$, respectively, compared to the corresponding sham-operated control group. QR-pretreatment exhibited a significant decrease in liver enzymes to about $41.77 \pm 3.09\%$ and $55.91 \pm 2.45\%$, respectively, while NAC-pretreatment showed lowered AST and ALT activities to about $66.04 \pm 2.42\%$ and $67.10 \pm 5.22\%$, respectively, compared to I/R group.

3.2. Effect of QR in I/R subjected rats on hepatic MDA level and GSH content

Hepatic MDA level significantly increased in I/R group to about $240.10 \pm 24.51\%$ while the GSH content significantly depleted

to about $20.24 \pm 0.96\%$ compared to the corresponding sham-operated rats. QR-pretreated rats significantly decreased lipid peroxidation to about $71.72 \pm 3.17\%$ while significantly increased GSH level to reach $411.76 \pm 11.76\%$ compared to I/R group. NAC-pretreatment significantly reduced MDA level to about $79.70 \pm 9.82\%$ while significantly restored the depleted GSH content to reach $476.47 \pm 23.53\%$ compared to I/R group (Fig. 2A and B, respectively).

3.3. Effect of QR in I/R subjected rats on hepatic MPO activity and NO production

The hepatic MPO activity and NO production were represented in Fig. 2C and D, respectively. I/R subjected rats significantly increased MPO activity and NO production to about $617.65 \pm 70.59\%$ and $254.79 \pm 6.99\%$, respectively, compared to sham-operated animals. QR-pretreated I/R rats significantly decreased MPO activity and NO production to about $36.19 \pm 3.81\%$ and $80.19 \pm 4.90\%$, respectively, compared to IR group. Similarly, NAC-pretreated I/R animals significantly decreased MPO activity and NO production to about $43.81 \pm 5.71\%$ and $78.98 \pm 4.03\%$, respectively, compared to I/R group.

3.4. Histopathological examination of liver sections

Liver sections obtained from sham-operated control group showed normal hepatic architecture with cords of normal hepatocytes radiating from the central vein (CV) and separated by blood sinusoids lined with endothelium and Kupffer cells (KC) (arrow) (Fig. 3A) while I/R subjected rats revealed that many

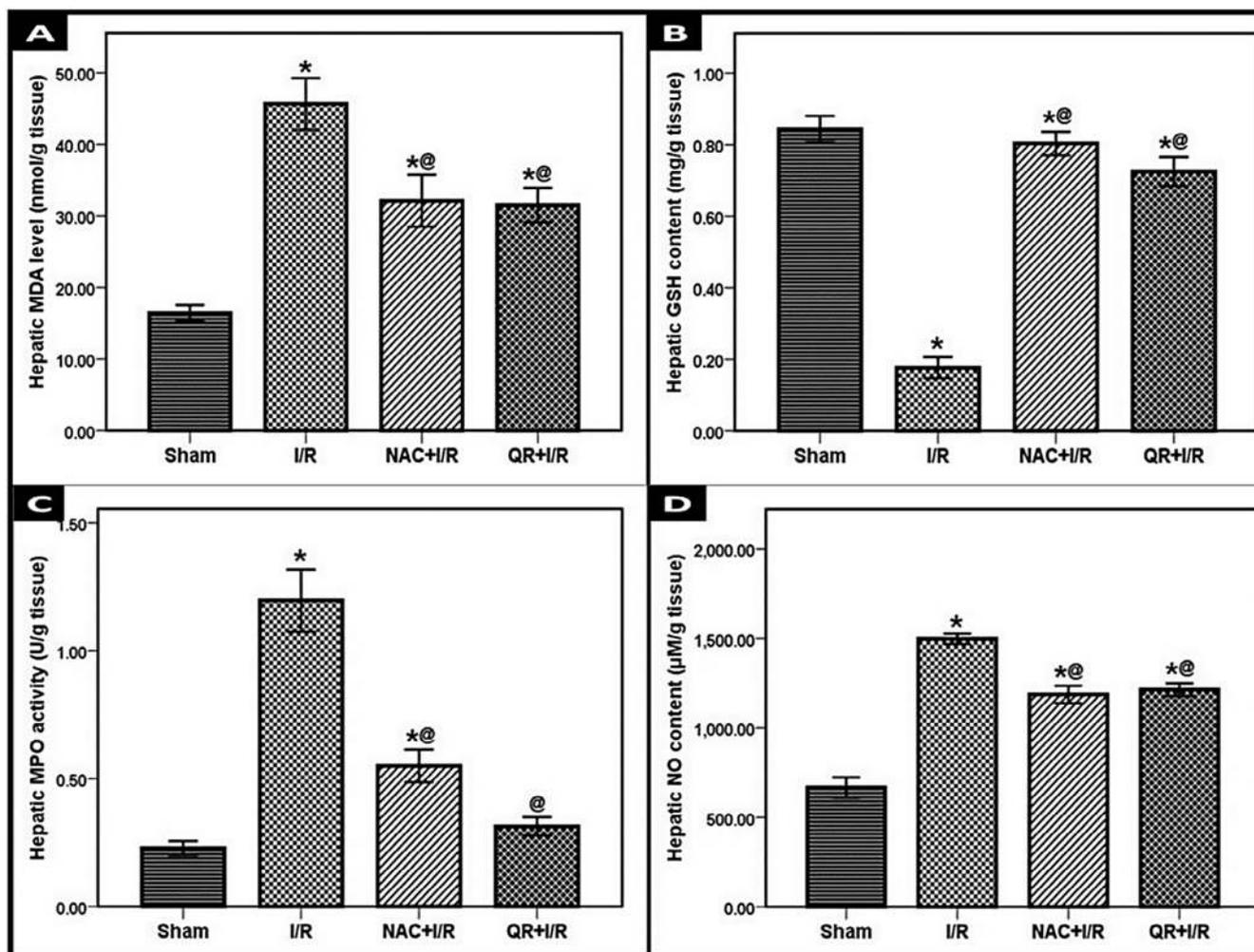


Fig. 2 – Effect of QR in I/R subjected rats on oxidative stress parameters and NO content. (A) Hepatic MDA level of sham-operated control, I/R, I/R with or without NAC or QR-pretreatment. (B) Hepatic GSH content of sham-operated control, I/R, and of NAC and QR-pretreatment. (C) Hepatic MPO activity of sham-operated control, I/R, I/R with or without NAC or QR-pretreatment. (D) Hepatic NO content of sham-operated control, I/R, and of NAC and QR-pretreatment. All data were expressed as means \pm standard error (S.E.). *Significantly different from sham-operated group at $P < 0.05$; @Significantly different from I/R group at $P < 0.05$. Means with the same superscript symbol are not significantly different at $P < 0.05$.

hepatocytes showed cytoplasmic degeneration and inflammatory cells infiltration (head arrow). In addition, massively dilated congested central vein and dilated congested blood sinusoids lined with activated Kupffer cells were noticed (Fig. 3B-1 and 3B-2). QR or NAC-pretreated group maintained normal histopathological picture. Meanwhile, the CV is still dilated and congested (Fig. 3C and 3D).

3.5. Effect of QR in I/R subjected rats on iNOS, eNOS and NOSTRIN protein expressions

Sham-operated rats showed negative iNOS and NOSTRIN protein expressions while I/R subjected animals revealed increase in iNOS and NOSTRIN expressions in the pericentral hepatocytes compared to sham-operated group while QR or NAC-pretreatment showed decrease in iNOS and NOSTRIN

expressions interstitially closed to the central area compared to the I/R group (Figs. 4A and 5A). Semi-quantitative analysis of the immunofluorescence staining expressed as fluorescence intensity stated that I/R group significantly increased iNOS and NOSTRIN expressions in liver tissues to about $153.10 \pm 9.68\%$ and $197.14 \pm 6.50\%$, respectively, compared to the sham-operated group. On the other hand, QR or NAC-pretreatment significantly decreased iNOS and NOSTRIN expressions to about $71.29 \pm 2.42\%$ and $72.56 \pm 4.77\%$ or $72.61 \pm 3.13\%$ and $55.07 \pm 3.18\%$, respectively, compared to I/R processed group (Figs. 4B and 5B).

Sham-operated rats revealed sinusoidal eNOS protein expression. Conversely, liver sections obtained from I/R exposed rats showed decrease in eNOS expression compared to sham-operated group, whereas QR and NAC-pretreatment showed increase in eNOS expression in the sinusoidal areas as well

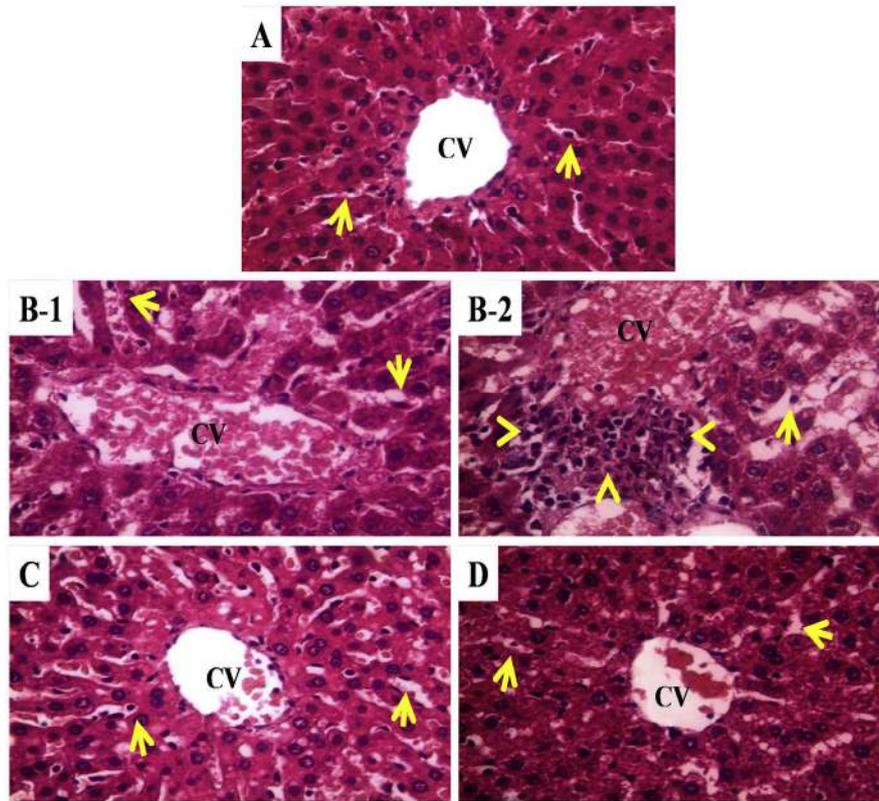


Fig. 3 – Histopathological examination of liver sections. (A) Sham-operated control group: Showed normal hepatic architecture. (B-1) I/R group: Showed massively dilated and congested central vein (CV). Hepatocytes are separated by dilated congested blood sinusoids with aggregated Kupffer cells (arrow). (B-2) IR group: Showed dilated and congested central vein. Many hepatocytes showed extensive inflammatory reaction (head arrow). (C) NAC-pretreated group: Showed slightly dilated and congested central vein. Hepatocytes are separated by slightly dilated congested blood sinusoids with Kupffer cells (arrow). (D) QR-pretreated group: Showed dilated and congested central vein. Normal hepatocytes are separated by slightly dilated congested blood sinusoids with Kupffer cells (arrow). (H&E 400 \times).

as in periportal and pericentral hepatocytes compared to I/R group (Fig. 6A). Fluorescence intensity of eNOS stated that I/R operated group significantly decreased eNOS protein expression in liver tissues to about $51.75 \pm 2.29\%$ compared to sham-operated control. On the other hand, pretreatment with QR or NAC significantly increased eNOS expression to about $166.54 \pm 7.82\%$ and $153.27 \pm 7.48\%$, respectively, compared to I/R group (Fig. 6B).

4. Discussion

In a variety of clinical conditions such as liver surgery, particularly in transplantation and hepatic trauma of diverse origins and hepatic failure after shock, ischemia/reperfusion is one of the major obstacles faced. Oxidative and nitrosative stress-induced cell damage and inflammatory reactions play an essential role in the pathogenesis of I/R injury. In the present study, hepatic I/R showed significant rises in the serum AST and ALT activities. In addition, it significantly increased hepatic MPO activity, MDA level and NO production. On the other hand,

it significantly depleted GSH content along with marked histopathological changes in liver tissues. Moreover, it strongly increased hepatic iNOS and NOSTRIN expressions in addition to decreased eNOS expression. Analysis of our data revealed the fact that pretreatment with QR prior to ischemia seems to be required in order to obtain the hepatoprotection against I/R, as showed by a significant decrease in serum levels of AST and ALT compared to IR group. These results are in a good agreement with a recent study which demonstrated that QR treatment significantly decreased ALT and AST activities in rats subjected to liver injury induced by ethanol (Chen, 2010). Concerning the lipid peroxidation (assessed by the hepatic MDA level) and the reduced GSH content, QR-pretreatment significantly reduced the MDA level while markedly restored GSH depletion compared to animals subjected to I/R, supporting its antioxidant effects as reported by other studies (Tokyol et al., 2006; Yousef et al., 2010). Interestingly, pretreatment with QR significantly decreased hepatic MPO activity, suggesting that the hepatoprotective effect of this compound is related as well to its anti-inflammatory properties. These findings were confirmed by marked improvement in the liver cell structure and decreased inflammatory cells infiltration as shown by

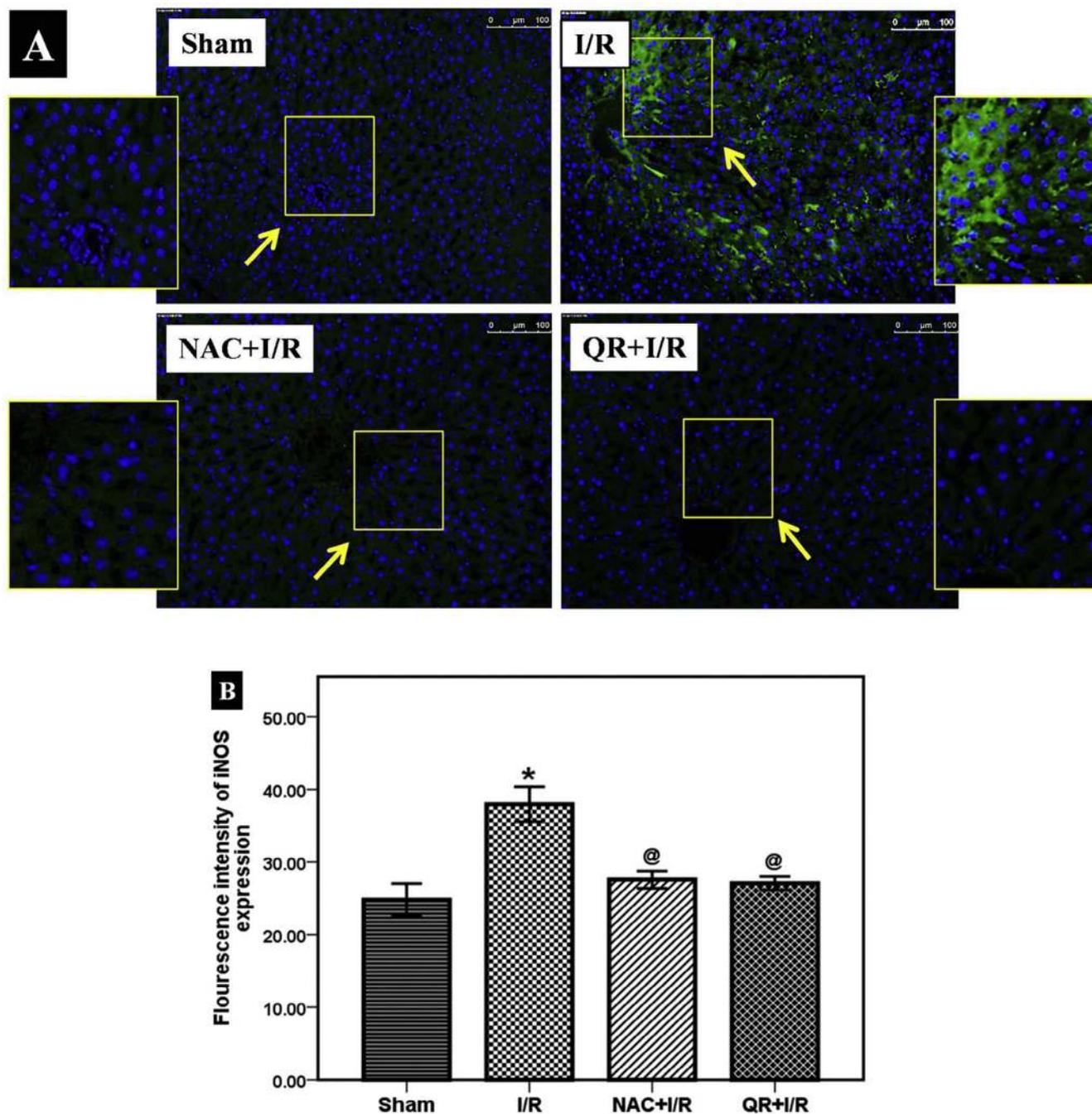


Fig. 4 – Effect of QR in I/R subjected rats on iNOS protein expression. (A) iNOS protein expression in the liver sections. It was detected by binding with mouse polyclonal iNOS antibody, then this bound antibody was detected by rabbit anti-mouse Alexafluor 488 (green) as a secondary antibody and the nuclei were counterstained using DAPI (blue). Figures were captured using immunofluorescence microscope (Leica DM5000B) and analyzed using Image J software (NIH). Scale bar = 100 μ M. (B) Semi-quantitative analysis of the immunofluorescence staining expressed as fluorescence intensity of iNOS protein expression of sham-operated control and I/R with or without NAC or QR-pretreatment. All data were expressed as means \pm standard error (S.E.). *Significantly different from sham-operated group at $P < 0.05$; @Significantly different from IR group at $P < 0.05$.

histopathological examination. Similar results were obtained by previous studies of Kahraman et al. (2003) who stated that QR significantly decreased MPO activity in a model of I/R-induced renal injury and Tokyol et al. (2006) who stated that histopathological liver damage scores significantly decreased

in QR-pretreated I/R animals compared to I/R alone. Furthermore, in the current study, QR significantly suppressed iNOS expression and decreased NO production and subsequently its potent oxidative byproduct, peroxynitrite. Therefore, QR inhibited nitrosative stress injury. Similar results have been

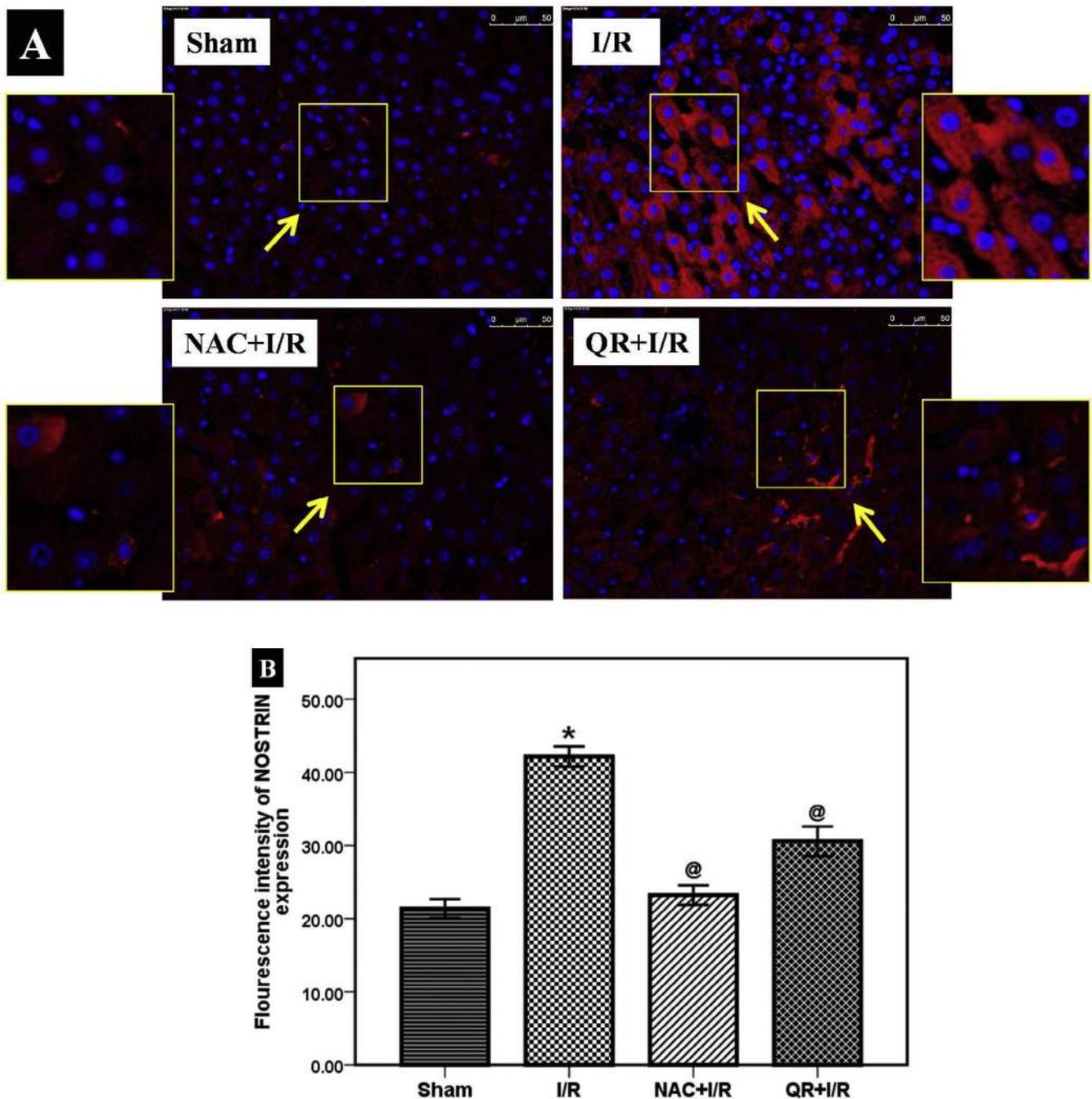


Fig. 5 – Effect of QR in I/R subjected rats on NOSTRIN protein expression. (A) NOSTRIN protein expression in the liver sections. NOSTRIN protein expression was detected in the liver of different groups using rabbit polyclonal iNOS antibody, detected by goat anti-rabbit cy3 (red) and the nuclei were counterstained using DAPI (blue). Scale bar = 50 μM. (B) Fluorescence intensity of NOSTRIN protein expression of sham-operated control and I/R with or without NAC or QR-pretreatment. All data were expressed as means ± standard error (S.E.). *Significantly different from sham-operated group at $P < 0.05$; @Significantly different from IR group at $P < 0.05$.

reported by Martínez-Flórez et al. (2005) who demonstrated that QR inhibited NO production through the inhibition of iNOS expression in interleukin (IL)-1 β -stimulated hepatocytes. Also, QR significantly increased eNOS along with decreased NOSTRIN expression. Our finding of increased eNOS protein expression is consistent with Vicente-Sánchez et al. (2008) who

reported that QR treatment significantly increased hepatic eNOS expression in a model of cadmium-induced hepatotoxicity. The involvement of NOSTRIN, eNOS and NO in IR with or without QR-pretreatment so far remain least explored. The increased protein expression of NOSTRIN may partly contribute to I/R injury. This finding is in accordance with Mookerjee et al. (2007)

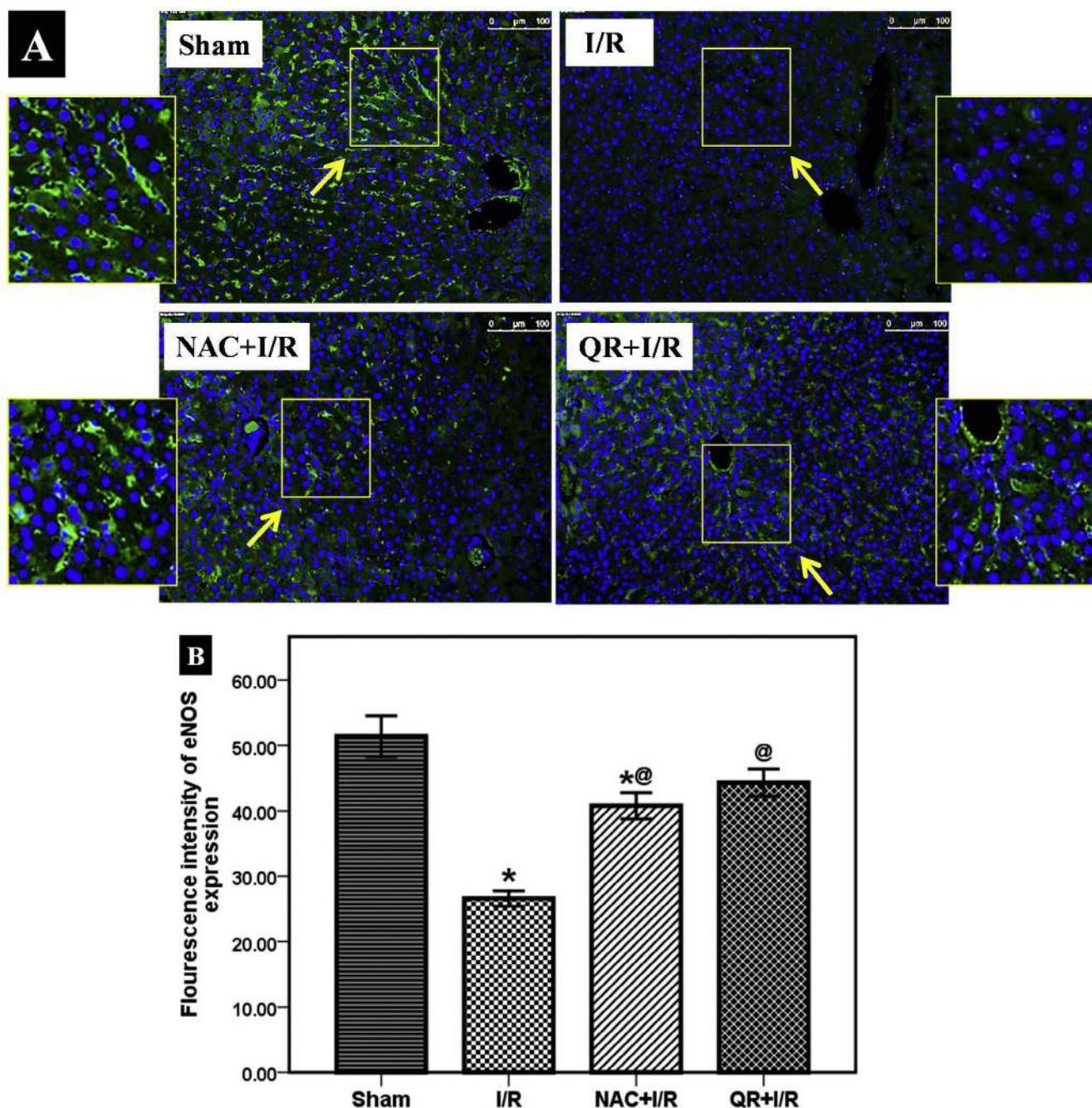


Fig. 6 - Effect of QR in I/R subjected rats on eNOS protein expression. (A) eNOS protein expression in the liver sections. Sham-operated rats' livers showing sinusoidal eNOS expression. Ischemia/reperfusion liver tissues presenting reduced eNOS expression in the sinusoidal area of the liver compared to sham operated rats. NAC and QR-pretreatment showed restoration of eNOS expression interstitially and in hepatocytes compared to IR operated animals. Scale bar = 100 μM. (B) Fluorescence intensity of eNOS protein expression of sham-operated control and I/R with or without NAC or QR-pretreatment. All data were expressed as means ± standard error (S.E.). *Significantly different from sham-operated group at $P < 0.05$; @Significantly different from IR group at $P < 0.05$.

who demonstrated that the increased gene and protein expression of NOSTRIN may partly contribute to the reduced eNOS activity and increased intrahepatic resistance in patients with alcoholic hepatitis. On the other hand, QR might protect against I/R injury by decreasing the increased protein expression of NOSTRIN.

5. Conclusions

Quercetin exhibited a more pronounced hepatoprotection as it successfully restored liver function and architecture. Also, it inhibited oxidative and nitrosative injury induced by I/R

compared with N-acetylcysteine. Still, further investigations should be done to investigate its potential uses in clinical trial.

REFERENCES

- Ara C, Kirimlioglu H, Karabulut AB, Coban S, Ay S, Harputluoglu M, et al. Protective effect of resveratrol against oxidative stress in cholestasis. *J Surg Res* 2005;127:112-17.
- Aruoma OI, Halliwell B, Hoey BM, Butler J. The antioxidant action of N-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. *Free Radic Biol Med* 1989;6:593-7.
- Bancroft JD, Gamble M. Theory and practice of histological techniques. 6th ed. Philadelphia: PA.: Churchill Livingstone/ Elsevier; 2008.
- Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. *J Lab Clin Med* 1963;61:882-8.
- Chen X. Protective effects of quercetin on liver injury induced by ethanol. *Pharmacogn Mag* 2010;6:135-41.
- Dodd S, Dean O, Copolov DL, Malhi GS, Berk M. N-acetylcysteine for antioxidant therapy: pharmacology and clinical utility. *Expert Opin Biol Ther* 2008;8:1955-62.
- Duarte J, Galisteo M, Ocete MA, Pérez-Vizcaino F, Zarzuelo A, Tamargo J. Effects of chronic quercetin treatment on hepatic oxidative status of spontaneously hypertensive rats. *Mol Cell Biochem* 2001;221:155-60.
- Glantzounis G, Yang W, Koti R, Mikhailidis D, Seifalian A, Davidson B. Continuous infusion of N-acetylcysteine reduces liver warm ischaemia-reperfusion injury. *Br J Surg* 2004;91:1330-9.
- Hemalatha P, Reddy AG, Reddy YR, Shivakumar P. Evaluation of protective effect of N-acetyl cysteine on arsenic-induced hepatotoxicity. *J Nat Sci Biol Med* 2013;4:393-5.
- Inal ME, Kahraman A. The protective effect of flavonol quercetin against ultraviolet a induced oxidative stress in rats. *Toxicology* 2000;154:21-9.
- Jaesckhe H. Molecular mechanisms of hepatic ischemia-reperfusion injury and preconditioning. *Am J Physiol Gastrointest Liver Physiol* 2003;284:15-26.
- Kahraman A, Erkasap N, Serteser M, Koken T. Protective effect of quercetin on renal ischemia/reperfusion injury in rats. *J Nephrol* 2003;16:219-24.
- Karabulut A, Kirimlioglu V, Kirimlioglu H, Yilmaz S, Isik B, Isikgil O. Protective effects of resveratrol on spleen and ileum in rats subjected to ischemia-reperfusion. *Transplant Proc* 2006;38:375-7.
- Krawisz J, Sharon P, Stenson W. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. *Gastroenterology* 1984;87:1344-50.
- Kuo S-M, Leavitt PS, Lin C-P. Dietary flavonoids interact with trace metals and affect metallothionein level in human intestinal cells. *Biol Trace Elem Res* 1998;62:135-53.
- Lin HI, Wang D, Leu F, Chen C, Chen HI. Ischemia and reperfusion of liver induces eNOS and iNOS expression: effects of a NO donor and NOS inhibitor. *Chin J Physiol* 2004;47:121-7.
- Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr* 2005;81:230-42.
- Martínez-Flórez S, Gutiérrez-Fernández B, Sánchez-Campos S, González-Gallego J, Tuñón MJ. Quercetin attenuates nuclear factor- κ B activation and nitric oxide production in interleukin-1 β -activated rat hepatocytes. *J Nutr* 2005;135:1359-65.
- Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide* 2001;5:62-71.
- Montalvo-Jave EE, Escalante-Tattersfield T, Ortega-Salgado JA, Piña E, Geller DA. Factors in the pathophysiology of the liver ischemia-reperfusion injury. *J Surg Res* 2008;147:153-9.
- Mookerjee RP, Wiesenthal A, Icking A, Hodges SJ, Davies NA, Schilling K, et al. Increased gene and protein expression of the novel eNOS regulatory protein NOSTRIN and a variant in alcoholic hepatitis. *Gastroenterology* 2007;132:2533-41.
- Pawlikowska-Pawłęga B, Gruszecki WI, Misiak LE, Gawron A. The study of the quercetin action on human erythrocyte membranes. *Biochem Pharmacol* 2003;66:605-12.
- Peres W, Tuñón M, Mato S, Collado P, González-Gallego J. Hepatoprotective effects of the flavonoid quercetin in rats with biliary obstruction. *J Hepatol* 2000;33:742-50.
- Siriussawakul A, Zaky A, Lang JD. Role of nitric oxide in hepatic ischemia-reperfusion injury. *World J Gastroenterol* 2010;16:6079-86.
- Su J, Guo C-J, Wei J, Yang J, Jiang Y, Li A. Protection against hepatic ischemia-reperfusion injury in rats by oral pretreatment with quercetin. *Biomed Environ Sci* 2003;16:1-8.
- Teoh NC, Farrell GC. Hepatic ischemia reperfusion injury: pathogenic mechanisms and basis for hepatoprotection. *J Gastroenterol Hepatol* 2003;18:891-902.
- Thies J, Teklote J, Clauer U, Tóx U, Klar E, Hofmann W, et al. The efficacy of N-acetylcysteine as a hepatoprotective agent in liver transplantation. *Transpl Int* 1998;11:390-2.
- Tokyo C, Yilmaz S, Kahraman A, Cakar H, Polat C. The effects of desferrioxamine and quercetin on liver injury induced by hepatic ischaemia-reperfusion in rats. *Acta Chir Belg* 2006;106:68-72.
- Uchiyama M, Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal Biochem* 1978;86:271-8.
- van Gulik TM, de Graaf W, Dinant S, Busch O, Gouma DJ. Vascular occlusion techniques during liver resection. *Dig Surg* 2007;24:274-81.
- Vicente-Sánchez C, Egido J, Sánchez-González P, Pérez-Barriocanal F, López-Novoa J, Morales A. Effect of the flavonoid quercetin on cadmium-induced hepatotoxicity. *Food Chem Toxicol* 2008;46:2279-87.
- Wadsworth TL, Koop DR. Effects of Ginkgo biloba extract (EGb 761) and quercetin on lipopolysaccharide-induced release of nitric oxide. *Chem Biol Interact* 2001;137:43-58.
- Yang K, Lamprecht SA, Liu Y, Shinozaki H, Fan K, Leung D, et al. Chemoprevention studies of the flavonoids quercetin and rutin in normal and azoxymethane-treated mouse colon. *Carcinogenesis* 2000;21:1655-60.
- Yıldız F, Coban S, Terzi A, Ates M, Aksoy N, Cakir H, et al. Nigella sativa relieves the deleterious effects of ischemia reperfusion injury on liver. *World J Gastroenterol* 2008;14:5204-9.
- Yousef MI, Omar SA, El-Guendi MI, Abdelmegid LA. Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat. *Food Chem Toxicol* 2010;48:3246-61.
- Zafarullah M, Li W, Sylvester J, Ahmad M. Molecular mechanisms of N-acetylcysteine actions. *Cell Mol Life Sci* 2003;60:6-20.
- Zimmermann K, Opitz N, Dedio J, Renné C, Müller-Esterl W, Oess S. NOSTRIN: a protein modulating nitric oxide release and subcellular distribution of endothelial nitric oxide synthase. *Proc Natl Acad Sci U S A* 2002;99:17167-72.