The Hepatoprotective Effect of Nitric Oxide Modulators and Antioxidants in Hepatic Ischemia/Reperfusion Injury in Albino Rats

*Salah El Deen Aziz Elsayed and Amr A. Fouad**
Physiology & Pharmacology Depts* Faculty of Medicine,
El-Minia University

ABSTRACT

Warm ischemia/reperfusion injury (IRI) of the liver is a major determinant of the outcome of a variety of clinical conditions. During the ischemic phase, tissue injury occurs due to oxygen deficiency. However, much injury arises with the restitution of the circulation (reperfusion injury), in which the generation of reactive oxygen species (ROS) plays a fundamental role. Several studies have emphasized the positive effect of antioxidants in attenuating IRI in many tissues. The role of nitric oxide and its action as an oxidant/antioxidant is considered. The aim of the present work was to investigate the potential of NO in modulating warm IRI of the liver and its possible interaction with different antioxidants in a trial to minimize that injury.

Seventy two albino rats were used in the present study. Rats were classified into the following groups (8 rats each): The 1st group of rats was subjected to 30min of ischemia followed by 60min of reperfusion. The 2nd group was Sham-operated rats underwent the same surgical procedure without interruption of the blood flow to the liver and served as controls. 3rd group L-nitro-arginine methyl ester (L-NAME) treated group, 50mg/kg (Sigma), the 4th, 5th, and 6th groups were the NO-donor, L-Arginine (Sigma) treated groups, in 3 different doses (100, 300, 500mg/kg). The drugs were administered intravenously 5min before ischemia-induction. The 7th group allopurinol (Sigma) treated group (50mg/kg), the 8th group desferrioxamine treated group (desferal) (Sigma) 100mg/kg and the 9th group vitamin C treated group (Sigma) 100mg/kg. Allopurinol was administered intraperitoneally 10min before the induction of ischemia, whereas desferal and vitamin C were administered intravenously 5min before the induction of ischemia. The left lobe of the liver of albino rats was subjected to 30 min warm ischemia followed by 60 min of reperfusion. Serum liver enzymes (AST, ALT, and LDH) were determined as a measure of tissue injury. Plasma nitrite level was assessed as a measure of nitric oxide production. Hepatic levels of malondialdehyde were quantified to evaluate the oxidative stress, while those of superoxide dismutase, catalase, and glutathione served as a determinant of the antioxidant status. Thirty minutes of warm ischemia followed by reperfusion for 60 min inflicted signs of tissue injury accompanied by oxidative stress and exhaustion of tissue antioxidants and plasma nitrite level. This was exacerbated with the nitric oxide blocker L-NAME. On the other hand, the nitric oxide donor (L-arginine) its lowest and intermediate doses (100, 300mg/kg) could attenuate that injury; while in its highest dose (500mg/kg) accentuated the injury.

Treatment with the xanthine oxidase inhibitor (allopurinol) was accompanied with a mild protective effect. Treatment with the iron-chelating agent (desferrioxamine) was accompanied with a substantial protective effect, while the treatment with vitamin C caused a moderate protection. The results clearly demonstrated that nitric oxide supply during IRI is up to a certain limit beneficial in ameliorating hepatic IRI, while its paucity was accompanied with accentuation of the injury. It seems that too high or too low level of NO is detrimental and aggravates IRI and its fine-tuning is a prerequisite to protect at least hepatic tissue. Furthermore, the results revealed that the antioxidant allopurinol did not practically improve the deteriorated livers subjected to IRI. Also, the results proved that iron-chelator (desferrioxamine) exhibited a very strong protective effect. The role of vitamin C warrants further elucidation, as its sole administration could confer protection to the liver against IRI.

INTRODUCTION

Ischemia-reperfusion injury (IRI) is a major determinant in a variety of clinical conditions such as during liver resections and under conditions of hypoperfusion as in cases of hemorrhagic and other types of shock, as well as in liver transplantation. Generation of ROS and exhausion of oxidative defense mechanisms are the main factors implicated in the pathgenesis of ischemia/reperfusion-induced liver damage[1&2]. Also, several studies have demonstrated the beneficial effect of antioxidant in protecting the liver against IRI[3,4&5]. The present study aimed to elucidate the pathomechanisms of pre-ischemic treatment with nitric oxide modulators (donor and inhibitor) and antioxidant (Allupurinol, iron-chelator (desferrioxamine) and vitamin C.

MATERIAL & METHODS

Animals

Seventy two Albino rats of both sexes, weighing about 220-320g, were used. Rats were housed one per cage at 22-24°C with a 12-h dark and light cycle, and were kept on water and standard laboratory chow *ad libitum*.

The rats of the present study were subjected to warm *in vivo* ischemia-reperfusion injury

Surgical procedure

A well established surgical technique was followed as described by **KOOIJ *et al*.**[6]. Following pentobarbital-anesthesia (50mg/kg i.p.), a mid-line abdominal incision was performed and the liver hilum was gently exposed. A fine a traumatic vascular clip was applied at the pedicle supplying the left lobe of the liver. This allowed for selective interruption of the blood supplying the left lobe of the liver, whilst preserving that to the right lobe, thus avoiding the gastrointestinal congestion and the hemodynamic instability accompanying complete occlusion of the hepatic pedicle. Warm saline was injected in the abdomen, which is temporarily closed. The blood flow to the left hepatic lobe was occluded for 30min (ischemic phase), and the clip was removed -after reopening the abdomen- and this signaled the beginning of the reperfusion phase. The reperfusion lasted for 60min, after

which the animal was sacrificed and sampling was done.

Methods and Techniques

1-Sampling and assays

Sampling was done through right atrium puncture and blood was aspirated with a heparin coated syringe. Following blood sampling, the left lobe of the liver was promptly resected and stored at -80°C for subsequent evaluation of the oxidant-antioxidant status. The blood was centrifuged at 3000 rpm for 15min and the plasma was stored at -20°C till biochemical assay.

2-Biochemical assessment of liver integrity

To assess hepatic tissue integrity, plasma samples were analyzed for the activity of the intracellularly located hepatic enzymes aspartate aminotransferase (AST), alanine aminotransferse (ALT), and lactate dehydrogenase (LDH), denoting their release and thereby cellular disintegration. Their activity was spectrophotometrically measured using standard commercial kits according to the manufacturer's instructions.

3-Assessment of nitric oxide production

It is measured using biodiagnostic nitrite assay kit[(7)]. The concentration was expressed in µmol/L

4-Assessment of the oxidant-antioxidant status

Rat livers were homogenized in 0.05mol/L phosphate buffer. The homogenates were centrifuged at 3500 rpm for 30min at 4°C and the supernatant was taken for the assays of malondialdehyde (MDA) and antioxidant enzymes activities[(8)].

To assess the oxidative stress, MDA in liver tissue was measured with **OHKAWA'S**[9] method using thiobarbituric acid (Sigma) reaction. The unit of measurement was nmol/g liver tissue.

Catalase activity in liver tissue was measured using the method described by **BEERS and SIZER**[10]. The enzyme activity expressed as U/mg protein.

Tissue Cu-Zn SOD activities were determined by the method of **SUN *et al.*[11],** the result expressed as U/g protein.

Total glutathione was determined in liver homogenates according to methods described by **ANDERSON**[12]. The unit of measurement was µmol/g liver tissue.

Experimental Protocol

1-Effect of warm ischemia-reperfusion injury

Rats were classified into 2 main groups (n = 8) according to the surgical procedure applied. The 1st group of rats was subjected to 30min of ischemia followed by 60min of reperfusion. Sham-operated rats underwent the same surgical procedure without interruption of the blood flow to the liver and served as controls.

2-Effect of NO-modulators

Rats were classified into 2 main groups (n = 8) according to the type of the NO-modulator used: the NO-inhibitor, L-nitro-arginine methyl ester (L-NAME), 50mg/kg (Sigma), and the NO-donor, L-Arginine (Sigma), in 3 different doses (100, 300, 500mg/kg). The drugs were administered intravenously 5min before ischemia-induction.

3-Effect of antioxidants

Rats were classified into 3 groups (n = 8) according to the type of the antioxidant used: allopurinol (Sigma) 50mg/kg, desferrioxamine (desferal) (Sigma) 100mg/kg, and vitamin C (Sigma) 100mg/kg. allopurinol was administered intraperitoneally 10min before the induction of ischemia, whereas, desferal and vitamin C were administered intravenously 5min before the induction of ischemia.

Statistical Analysis

All data were expressed as mean ± SEM. Differences between groups were assessed using the Student`s *t*-test. Overall statistical significance was set at $p<0.05$.

RESULTS

1-Effect of warm IRI on measured parameters (figure 1, 2)

Plasma activities of liver enzymes: Following 30min warm ischemia and after the 1-h period of reperfusion, the liver enzyme activities increased from a base-line activity to reach high levels at the end of the reperfusion period. Analysis of the enzymatic activity in plasma revealed significantly higher levels after the 1-h reperfusion following the 30-min of ischemia in comparison to sham-operated animals.

Plasma nitrite level: Sham-operated animals had a plasma NO_2-level of 310±12µmol/l. On the other hand, rats subjected to ischemia-reperfusion had significantly lower level of plasma nitrite of 203±8µmol/L.

Hepatic lipid peroxidation: Thirty minutes of hepatic ischemia followed by 60min of reperfusion significantly induced lipid peroxidation in the liver, as manifested by the nearly 2-fold increase in the hepatic MDA-level from 152±7nmol/g in sham-operated livers to 320±3nmol/g.

Hepatic antioxidants: Ischemia-reperfusion caused exhaustion of the hepatic antioxidant defense, that is mirrored by the fall in the activity of the antioxidant enzymes catalase and superoxide dismutase on one hand and the decline in the content of the antioxidant substance glutathione on the other hand.

2-Effect of NO-modulation on warm ischemia/reperfusion injury (figure 3, 4)

A- Inhibition of NO-production

Plasma activities of liver enzymes: Treatment of rats with L-NAME while subjecting them to 30min warm ischemia followed by 1h of reperfusion caused a significant increase in the release of liver enzymes.

Plasma nitrite level: Estimation of the plasma NO_2-level revealed that the treatment with the NO-inhibitor, L-NAME, at the time of ischemia-reperfusion significantly suppressed the production of NO_3 (161±8µmol/l) in comparison to the I/R alone (203±8µmol/l).

Hepatic lipid peroxidation: A significant augmentation of hepatic lipid peroxidation was observed as a result of suppression of the NO-generation using L-NAME (400±11nmol/g), when compared to 320±3nmol/g in I/R-group.

Hepatic antioxidants: Suppression of the NO-production was accompanied with a manifest depletion and exhaustion of the

hepatic antioxidant defense as a result of ischemia-reperfusion. This attained significant level only in case of SOD, although the same trend was, also, observed in glutathione level and to a lesser degree in the catalase activity.

B- Stimulation of NO-production

Plasma activities of liver enzymes: Thirty minutes warm ischemia followed by 1h reperfusion induced the release of liver enzymes. Treatment of the animals with the NO-precursor (L-arginine) resulted in attenuation of the liver enzyme activities in a dose dependent manner. This protective effect was abolished applying the highest dose (500mg/kg), which even inflicted cellular injury.

Plasma nitrite level: Rats treated with L-arginine had a higher plasma nitrite level than those subjected to ischemia-reperfusion. It should be noted that the highest dose used was accompanied with a lower level of plasma nitrite compared to the intermediate (300mg/kg) and even the lowest (100mg/kg) dose.

Hepatic lipid peroxidation: both the lowest and intermediate doses of the nitric oxide generator (L-argininge) significantly counteracted the ischemia-reperfusion injury and suppressed the lipid peroxidation. On the other hand, using that agent at the highest dose level failed to protect the liver.

Hepatic antioxidants: L-arginine did not practically influence catalase activity, while in its lowest and intermediate doses induced significant elevation of SOD. The same was observed in case of glutathione, although it did not reach statistical significance.

3-Effect of antioxidants on warm ischemia/reperfusion injury (figure 5, 6)

Plasma activities of liver enzymes: Subjecting rats to antioxidant treatment while exposed to 30min warm ischemia followed by 1h of reperfusion diminished significantly the release of liver enzymes. That reduction was mostly noticeable with desferrioxamine treatment, and least with allopurinol..

Plasma nitrite level: Estimation of the plasma NO_3-level revealed that the treatment with the antioxidant, allopurinol, at the time of ischemia-reperfusion could not significantly modulate the production of NO_2 (205±6µmol/l) in comparison to I/R alone (203±8µmol/l). Conversely, both desferrioxamine and vitamin C proved to be effective in boosting the plasma nitrite level (278±3; 260±5µmol/l respectively). That increase was statistically significant.

Hepatic lipid peroxidation: A significant repression of the hepatic lipid peroxidation was observed as a result of the treatment with the antioxidants desferrioxamine and vitamin C (170±11; 183±13nmol/g), when compared to 320±3nmol/g in I/R-group. Suppression of the xanthine oxidase pathway using allopurinol did not influence the lipid peroxidation in the liver.

Hepatic antioxidants: Suppression of the XO-pathway failed to influence the antioxidant defense of the liver. On the contrary, desferrioxamine and vitamin C were accompanied with a manifest repletion of the exhausted hepatic antioxidant defense as a result of ischemia-reperfusion. This reached significant

level only in case of SOD, although the same trend was also observed in glutathione level and to a much lesser degree in the catalase activity. It should be noted that desferrioxamine is efficacious in counteracting the manifestation of I/R.

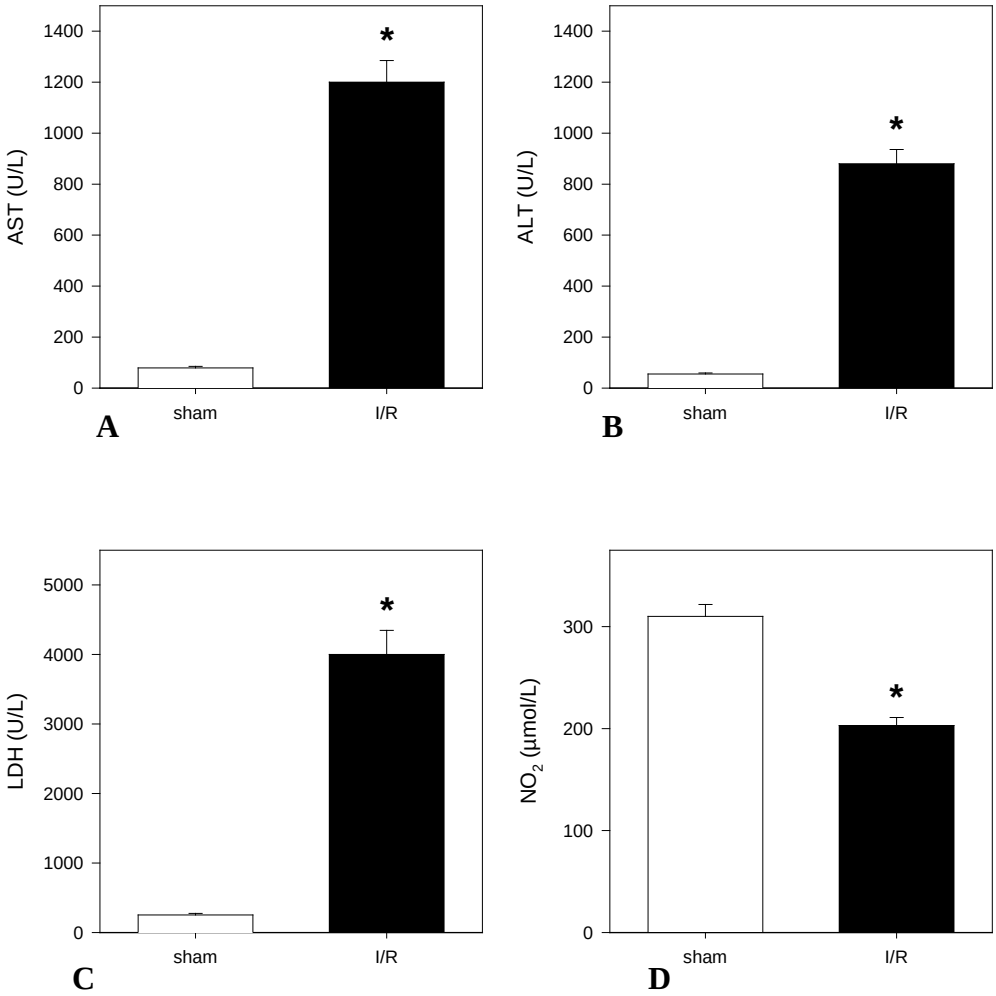

Figure 1: Effect of ischemia-reperfusion on plasma levels of AST, ALT, LDH, and NO_2 in Rats. Livers were subjected to 30min of warm ischemia followed by 60min of reperfusion. Sham-operated animals served as controls. Data are expressed as mean ± SEM, * p<0.05 vs. sham-operated.

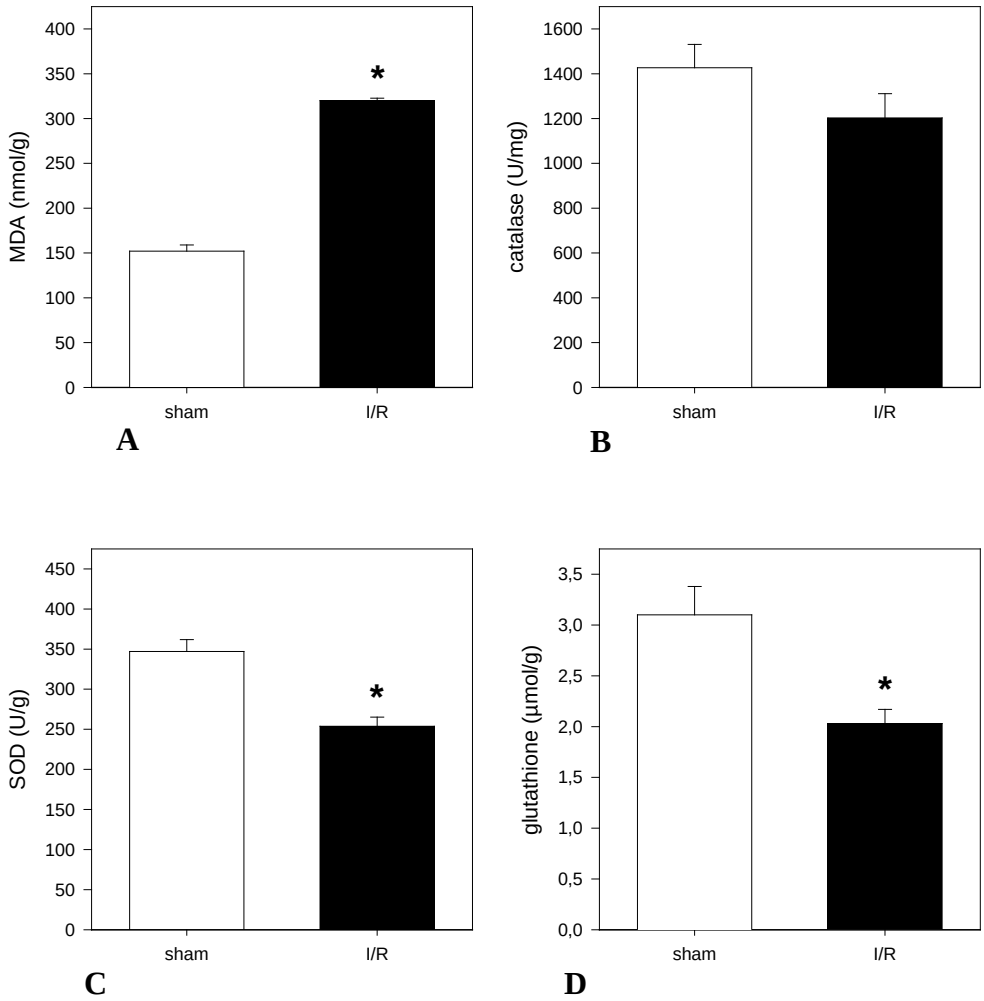

Figure 2: Effect of ischemia-reperfusion on hepatic levels of MDA, catalase, SOD, and glutathione in Rats. Livers were subjected to 30min of warm ischemia followed by 60min of reperfusion. Sham-operated animals served as controls. Data are expressed as mean ± SEM, * p<0.05 vs. sham-operated.

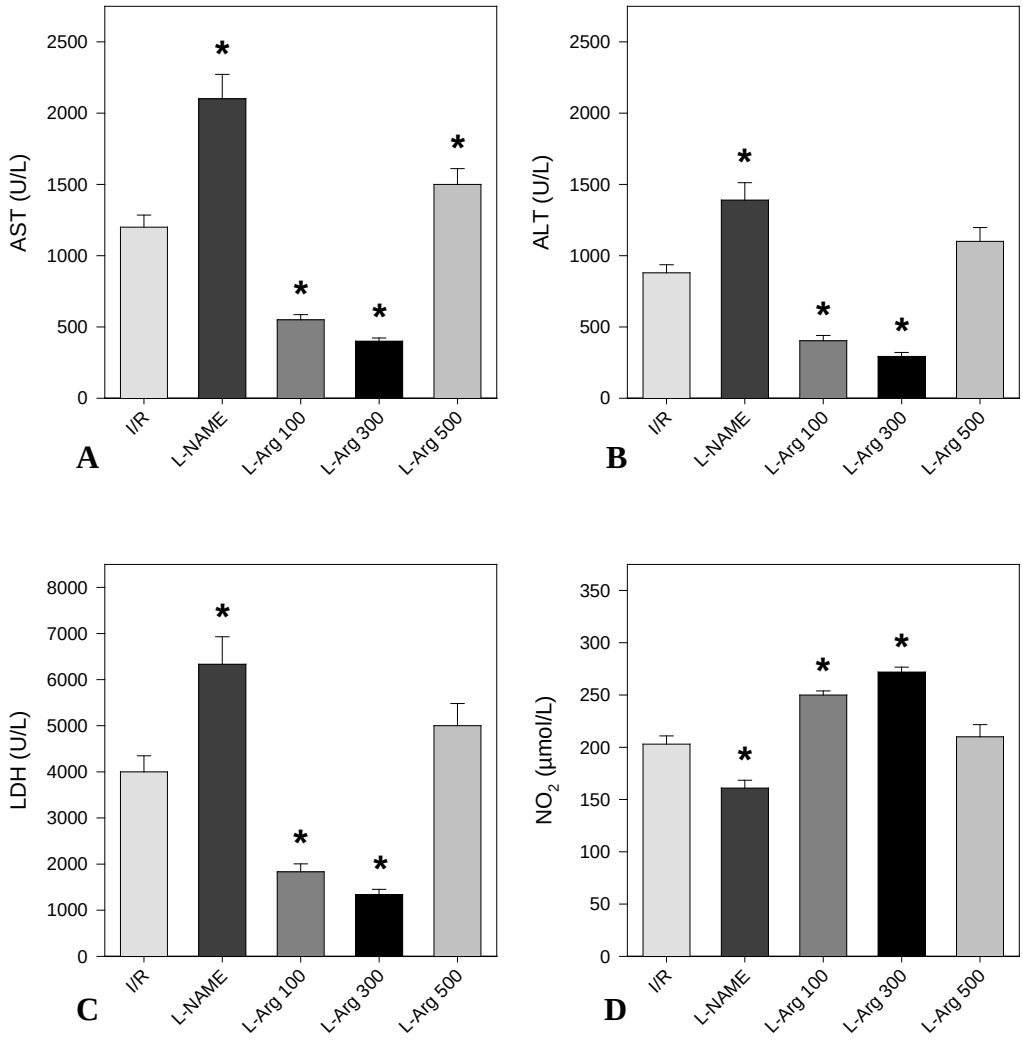

Figure 3: Effect of NO-modulation on plasma levels of AST, ALT, LDH, and NO_2 in Rats. Livers were subjected to 30min of warm ischemia followed by 60min of reperfusion. Data are expressed as mean ± SEM, * p<0.05 vs. I/R.

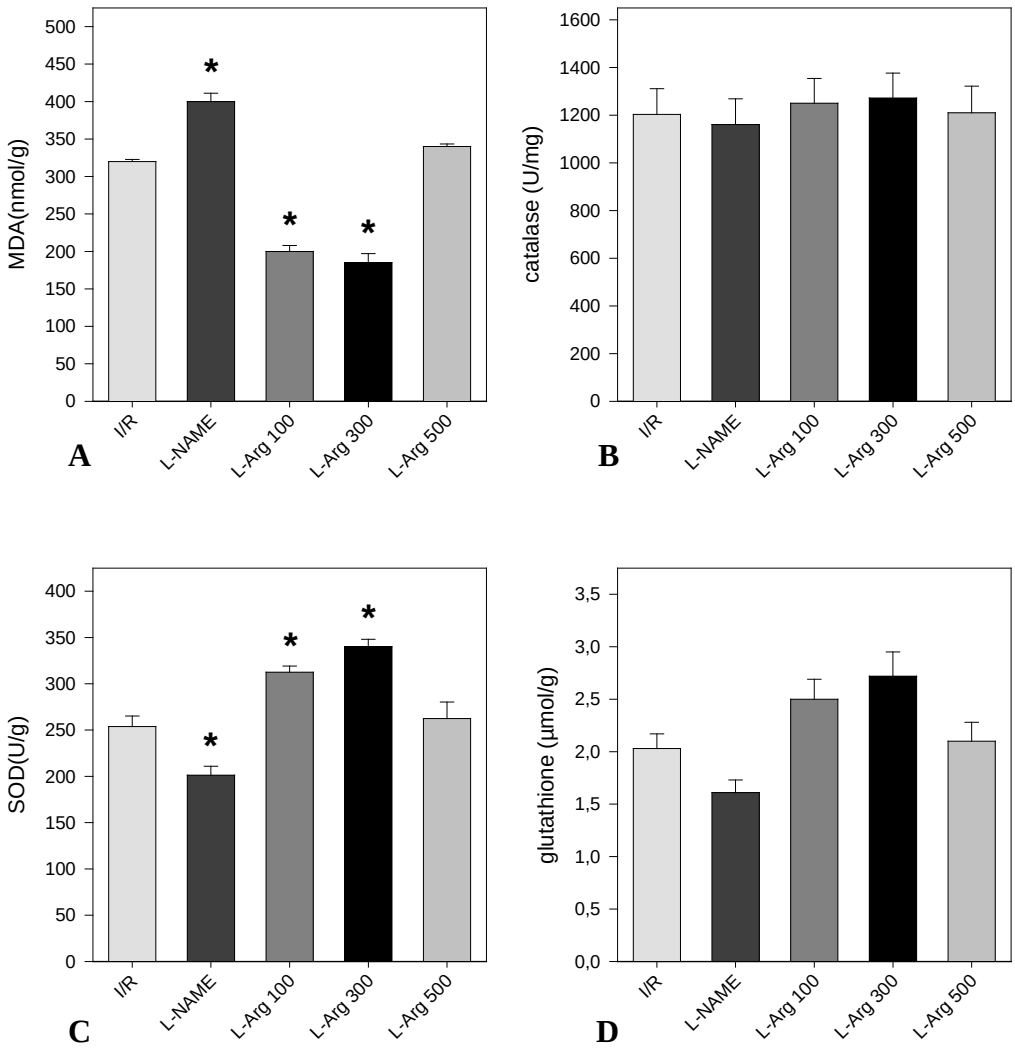

Figure 4: Effect of NO-modulation on hepatic levels of MDA, catalase, SOD, and glutathione in Rats. Livers were subjected to 30min of warm ischemia followed by 60min of reperfusion. Data are expressed as mean ± SEM, * p<0.05 vs. I/R.

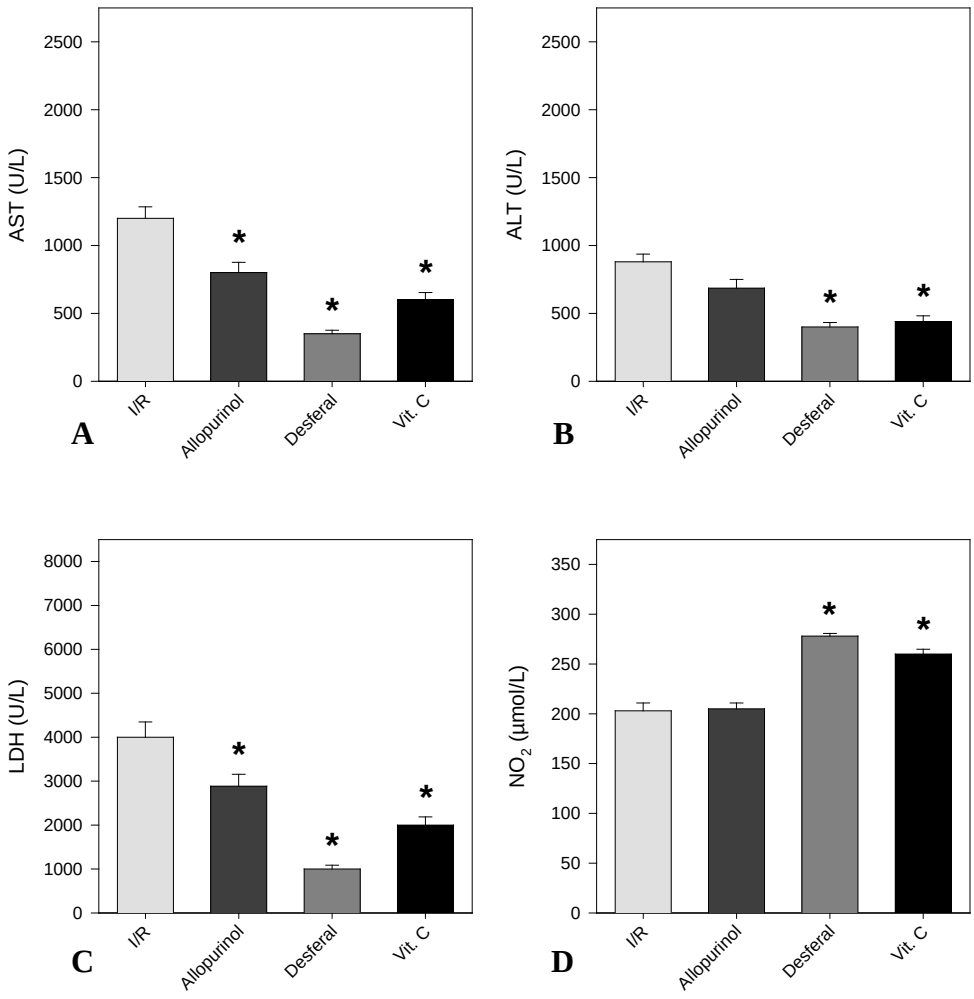

Figure 5: Effect of antioxidant-strategy on plasma levels of AST, ALT, LDH, and NO_2 in Rats. Livers were subjected to 30min of warm ischemia followed by 60min of reperfusion. Data are expressed as mean ± SEM, * p<0.05 vs. I/R.

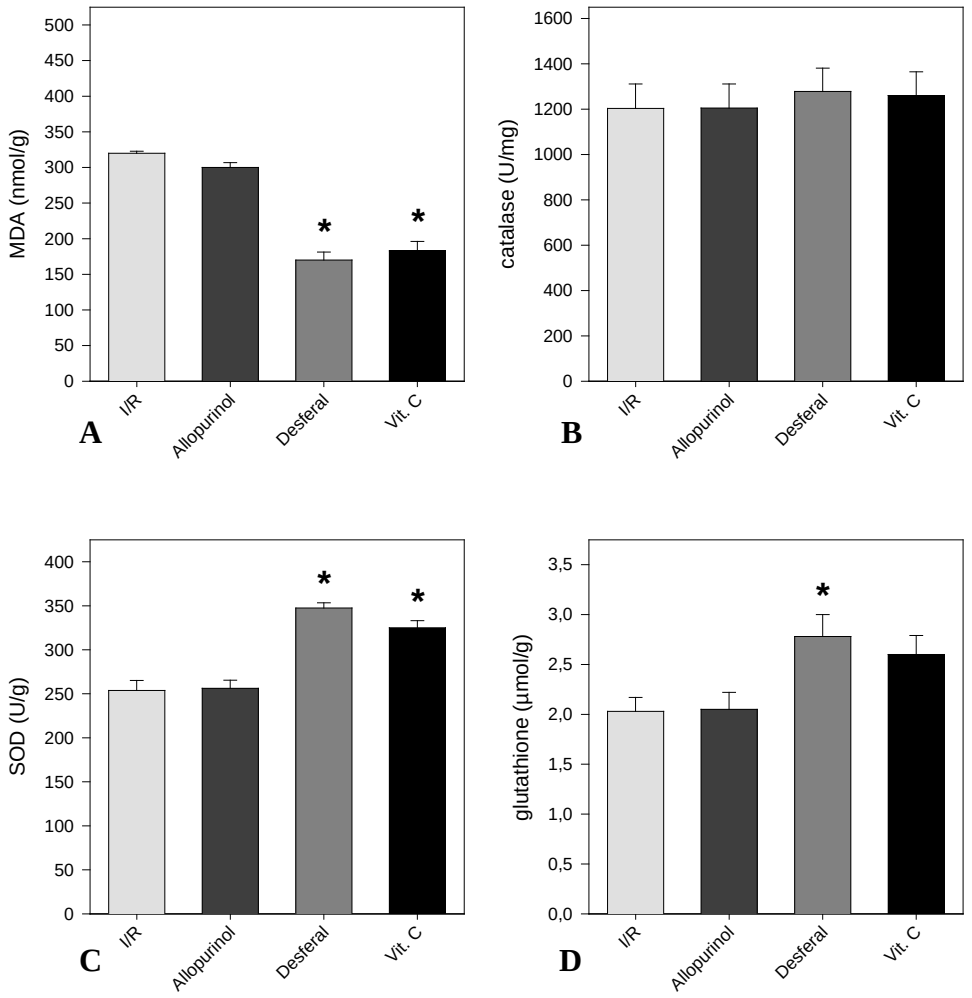

Figure 6: Effect of antioxidant-strategy on hepatic levels of MDA, catalase, SOD, and glutathione in Rats. Livers were subjected to 30min of warm ischemia followed by 60min of reperfusion. Data are expressed as mean ± SEM, * p<0.05 vs. I/R.

DISCUSSION

Reperfusion following a certain period of ischemia imposes cellular injury and tissue damage. It is one of the most important reasons of aggravation of organ injury following a certain period of ischemia. The model of the partial hepatic ischemia/reperfusion is an appropriate model for *in vivo* studying of warm IRI. As the blood flow only to the left

hepatic lobe is interrupted, more blood can flow to the right lobe and thus obviating intestinal congestion, systemic hypotension, and the higher mortality recorded with total clamping of the hepatic pedicle[13]. So, 30min ischemia and 60min reperfusion was made as this has been proven to yield a good and reproducible pattern of injury. Furthermore, ischemia for 30-minutes is considered to be the maximal duration permitted in clinical settings for clamping the hepatic pedicle, beyond which protective measures have to be taken to minimize reperfusion injury[14].

Various liver diseases are associated with typical ranges of AST and ALT levels. ALT levels often rise to several thousand U/L in patients with acute viral hepatitis. The highest ALT levels -often more than 10,000U/L- are usually found in patients with acute toxic injury subsequent to, for example, acetaminophen overdose or acute ischemic insult to the liver. AST and ALT levels usually fall rapidly after an acute insult. Lactate dehydrogenase is less specific than AST and ALT as a marker of hepatocyte injury. However, it is worth noting that LDH is disproportionately elevated after an ischemic liver injury[15].

Thirty minutes of ischemia followed by 60 minutes of reperfusion could inflict signs of reperfusion injury, manifested by release of hepatic enzymes into the circulation, depletion of the presumably protective NO, and exhaustion of the antioxidant defense. This proves that 30 minutes of warm hepatic ischemia are sufficient to induce hepatic cellular injury, and that injury manifests itself as early as 60 minutes following the ischemia. This is in accordance with the resul of **Moriisue *et al*.**[16] who reported the same observation.

The present results have demonstrated that NO-depletion using L-NAME was accompanied by deterioration of the liver condition and augmentation of the hepatic injury; while its repletion, up to a certain limit, was associated with amelioration of that injury and restitution of the hepatic antioxidant defense. This could be ascribed to the well known functions of NO as a vasodilator and anti-platelet agent as well as its antioxidant properties, thus affording cellular protection[17].

The enzymatic inhibition of cellular nitric oxide synthase with L-NAME elicited a greater damage, presumably due to other vascular effects of L-NAME on blood flow[18]. Because neutrophils are not involved in early hypoxia-reoxygenation injury, we can rule out an effect of L-NAME on polymorphonuclear leukocytes. Protection of hepatocytes by endogenous NO against hypoxic damage may be related to the conservation of cellular bioenergetics for maintenance of tissue homeostasis during hypoxia *via* NO-mediated inhibition of the use of mitochondrially derived ATP for nonessential metabolic processes[19]. The finding that an exogenous NO donor afforded protection against that injury during reoxygenation indicates that NO is, in fact, cytoprotective in the reoxygenation phase.

It has, also, been reported that NO has anti-apoptotic and anti-necrotic actions *via* many mechanisms, one of which is through

blocking the mitochondrial permeability transition, a very early step in the death pathway[20]. Moreover, NO has a proven inhibitory action on the deleterious effects of Kupffer cells in the setting of hypoxia and reoxygenation[19].

The observation that L-arginine when administered in a high dose induced cellular injury rather than protection, could be explained by the formation of the highly injurious per-oxy-nitrite radical, which is responsible for cellular injury. This assumption is supported by the finding that plasma nitrite level is relatively reduced, denoting its consumption, thus being unavailable to detection. It is worthy to mention that **Ferraz *et al*.,**[21] reported a paradoxical effect of a high dose of L-arginine (500mg/kg) in a model of ethanol-induced gastric mucosal injury. This would substantiate the herein reported findings.

The current results showed that the allopurinol seemed to be ineffective in reducing hepatic injury. This would cast doubt on the mission of xanthin oxidase in liver injury and the cellular defence mechanism in hepatic tissue. In fact, **Metzger *et al*.**[13] reported the same observation and suggested that xanthine oxidase plays a limited role in the generation of reactive oxygen species in the liver.

Another evidence comes from the work of **Fredriks** and **BOSCH**[22], who studied the role of xanthine oxidase in the induction of liver cell damage during reperfusion after ischemia and the potential of the antioxidant system in a model of *in vivo* ischemia of rat liver followed by 1h reperfusion by the use of enzyme histochemistry. Based on decreased lactate dehydrogenase activity in certain areas of liver parenchyma, cell damage could already be detected at 1h reperfusion after ischemia. Incubations performed on serial sections showed that the same areas contained decreased activities of xanthine oxidoreductase, xanthine oxidase, catalase and glucose-6-phosphate dehydrogenase. Some individual cells in the undamaged liver parenchyma expressed a very high glucose-6-phosphate dehydrogenase, which suggests that these cells have a good defence against oxidative stress. It was concluded that oxygen radicals derived from xanthine oxidase do not play a decisive role in the induction of cell damage immediately at reperfusion after ischemia. However, it cannot be excluded that xanthine oxidase present in the blood stream could give rise to the development of additional damage later on. This would support the present finding regarding the ineffectiveness of allopurinol administration in reducing IRI in the present model.

On the other hand, **Rhoden *et al*.**[23] reported a beneficial effect of allopurinol when administered to Wistar rats before subjecting them to 50min of ischemia followed by reperfusion for 1 hour. The severity of ischemia and the difference in strain could account for the observed discrepancy.

A critical point to be considered is the kinetic of conversion of xanthine dehydrogenase to xanthine oxidase. **ENGERSON *et al*.**[24] reported that in response to global ischemia, tissue xanthine dehydrogenase was

converted to xanthine oxidase in all tissues with half-times of conversion at 37°C of ~3.6, 6, 7, and 14h for the liver, kidney, heart, and lung, respectively. They provided evidence that a proteolytic mechanism is responsible for that conversion in ischemic rat liver. Furthermore, **BRASS *et al*.**[25] found that in a functionally intact, isolated perfused rat liver model, the mean % xanthine oxidase activity increased as a function of both the duration (25 to 45% in 3h) and degree of hypoxia. This process was markedly accelerated in ischemic liver by an overnight fast (45 vs. 30% at 2h), and by imposing a short period of *in vivo* ischemia (cardiopulmonary arrest 72%). Moreover, only under these conditions was there a significant rise in the xanthine oxidase activity due to the conformationally altered xanthine dehydrogenase molecule (18%), as well as concomitant morphologic injury. From these two kinetic studies, one can infer that the duration of ischemia and subsequent reperfusion in the present model might be too short to allow for an immense conversion of xanthine dehydrogenase to xanthine oxidase and subsequently for a manifest action of allopurinol.

The results of the current study clearly demonstrated that desferrioxamine is highly effective in attenuating liver injury following warm ischemia and reperfusion. This emphasizes the important role of iron and of FENTON reaction in the amplification of reactive oxygen species generation. By interruption of such an amplification loop, we could substantially reduce hepatic tissue damage following warm ischemia and reperfusion. The present results match those of **OMAR *et al*.**[26] and **COLLETTI *et al*.**[27] who communicated the efficacy of iron-chelation in attenuating hepatic IRI.

Another applied approach was the use of vitamin C in a trial to scavenge the generated ROS. That approach proved successful in ameliorating tissue damage as a result of warm ischemia and reperfusion. In fact, such a beneficial effect of vitamin C was previously described by **Seo and Lee**[28]. Vitamin C exerts an antioxidant action both by itself and by interacting with reduced glutathione or vitamin E**(29&30)**. It repairs vitamin E, thereby permitting vitamin E to recycle(31). Ascorbic acid scavenges reactive oxygen species such as superoxide radical ($O2^-$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), singlet oxygen, and hypochlorous acid (HOCl), which is produced from H_2O_2 and Cl^- *via* myeloperoxidase present in neutrophils, *in vitro***(32&33)**.

In addition, vitamin C scavenges peroxynitrite ($ONOO^-$), a highly reactive nitrogen oxide species, which is produced by the reaction of $O2^-$ and nitric oxide *in vitro***(34,35&36)**. It is known that vitamin C scavenges O_2^- generated by activated NADPH oxidase in neutrophils and in the hypoxanthine-xanthine oxidase (XO) system *in vitro***(37&38)**. It is, also, known that vitamin C prevents neutrophil adherence to endothelium by scavenging ROS derived from activated neutrophils *in vitro***(39)**. Furthermore, a stable derivative of vitamin C, ascorbic acid 2-glucoside, was found to possess antiapoptotic properties after cold ischemia-

reperfusion injury in rat liver transplantation[40].

The lowest dose of L-arginine (100mg/kg) was choosen in order to detect minor changes in response. Additional application of allopurinol did not significantly change the response to L-arginine alone. This would raise the question regarding the role of xanthine oxidase in liver injury.

Vitamin C, a known reducing agent, could reduce nitrites (the end product of NO) back to its original form (NO), which in its excess would react with oxygen free radicals to form the highly injurious peroxynitrite radical. That speculation is reinforced by the detected reduction of nitrite level.

It could be concluded that reperfusion following warm ischemia inflicts cellular damage due to the generation of ROS and the exhaustion of the liver defence mechanisms. Activation of the nitric oxide pathway up to a certain limit ameliorates that injury, while its overactivation augments that injury. Xanthine oxidase seems to play a minor role in the evolution of hepatic IRI, whereas as iron-chelation with desferrioxamine markedly inhibits cellular injury. Vitamin C ameliorates IRI. A judicious multimodal approach seems to be an effective tool to abogate warm IRI of the liver.

REFERENCES

1. **Bilzer M. and Gerbes A.L. (2000):** Preservation injury of the liver: mechanisms and novel therapeutic strategies. J. Hepatol., 32: 508-515.
2. **Galaris D.; Barbouti A. and Korantzpoulos P. (2006)**: Oxitaive stress in hepatic ischemia-reperfusion injury: the role of antioxidatants and iron chelating compounds. Curr. Pharm. Dis., 12:2875-2890.
3. **Giakoustidis D.; Papageorgion G.; Iliadis S. and Takoudas D. (2006):** The protective effect of alpha-tocopherol and CdCl, against hepatic ischemia-reperfusion injury. Surg. Today 36:450-456.
4. **Zhang W.H.; Li J.Y. and Zho Y. (2006):** Melatonin abates liver ischemia/reperfusion injury by improving the balance between nitric oxide and endothelin. Hepatobiliary Pancreat. Dis. Int., 5:574-579.
5. **Vali L.; Stefanovits B.E.; Szentmihaly K. and Blazovies A. (2007):** Liver-protecing effects of table beet (Beta vulgaris var rubra) during ischemia-reperfusion. Nutrition 23:172-178.
6. **Kooij A.; Schiller H.; Schijns M.; Noorden C. and Frederiks W. (1994):** Conversion of xanthine dehydrogenase into xanthine oxidase in rat liver and plasma at the onset of reperfusion after ischemia. Hepatology, 19(6):1488-1495.
7. **Montgomery H.A. and Dymock J.F. (1961):** Analyst. 86-414.
8. **Lowry O.H.; Rosebrough N.G.; Farr A.L. and Randall R.J. (1951):** Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193:265-271.
9. **Ohkawa H.; Ohishi N. and Yagi K. (1979):** Assay for lipid

peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.

10. **Beers R.F. and Sizer I.W. (1952):** A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem., 195:133-138.
11. **Sun Y.; Oberley L.W. and Li Y. (1988):** A simple method for clinical assay of superoxide dismutase. Clin. Chem., 34: 497-500.
12. **Anderson M. (1985):** Determination of glutathione and glutathione disulfide in biological samples. In: Methods in Enzymology. ed. Meister, A. New York: Academic Press. Vol 113, pp. 548-555.
13. **Metzger J.; Dore S. P. and Lauterburg B.H. (1988):** Oxidant stress during reperfusion of ischemic liver: no evidence for a role of xanthine oxidase. Hepatology 8:580-584.
14. **Pichlmayr R.; Bretschneider H.J.; Kirchner E.; Lamesch P.; Hauss J.; Niehaus K.J. and Kaukemueller J. (1988):** Ex-situ-Operation an der Leber – eine neue Möglichkeit in der Leberchirurgie. Langenbecks Arch. Chir., 373:106-112.
15. **Gitlin N. and Serio K.M. (1992):** Ischemic hepatitis: widening horizons. Am. J. Gastroenterol., 87:831-836.
16. **Morisue A.; Wakabayashi G.; Shimazu M.; Tanabe M.; Mukai M.; Matsumato K. and Kitajima M. (2003):** The role of nitric oxide after a short period of liver ischemia-reperfusion. J. Surg. Res., 109:101-109.
17. **Moncada S.; Higgs A. and Furchgott R. (1997):** International Union of Pharmacology Nomenclature in Nitric Oxide Research. Pharmacol. Rev., 49:137-142.
18. **Wang Y.; Mathews W.R.; Guido D.M.; Farhood A. and Jaeschke H. (1995):** Inhibition of nitric oxide synthesis aggravates reperfusion injury after hepatic ischemia and endotoxemia. Shock 4:282-288.
19. **Taniai H.; Hines I.N.; Bharwani S.; Maloney R.E. and Grisham M.B. (2004):** Susceptibility of murine peripheral hepatocytes to hypoxia-reoxygenation: role for NO and Kupffer cell-derived oxidants. Hepatology, 39:1544-1552.
20. **KIM J-S.; OHSHIMA S.; PEDIADITAKIS P. AND LEMASTERS J.J. (2004):** Nitric oxide protects rat hepatocytes against reperfusion injury mediated by the mitochondrial permeability transition. Hepatology 39:1533–1543.
21. **Ferraz J.G.; Tigley A. and Wallace J.L. (1994):** Paradoxical effects of L-arginine on gastric mucosal integrity. Eur. J. Pharmacol., 260:107-111.
22. **Frederiks W.M. and Bosch K. S. (1995):** the role of xanthine oxidase in ischemia/reperfusion damage of rat liver. Histol. Histopathol., 10:111-116
23. **Rhoden E.; Pereira L.L.; Lucas M.; Zettler C.; Petteffi L. And Bello K. (2000):** The effects of

allopurinol in hepatic ischemia and reperfusion: experimental study in rats. Eur. Surg. Res., 32:215-222.

24. **Engerson T.D.; Mckelvey T.G.; Rhyne D.B. and Jones H.P. (1987):** Conversion of Xanthine Dehydrogenase to Oxidase in Ischemic Rat Tissues. J. Clin. Invest., 79:1564-1570.
25. **Brass C.A.; Narciso J. and Gollan J.L. (1991):** Enhanced Activity of the Free Radical Producing Enzyme Xanthine Oxidase in Hypoxic Rat Liver Regulation and Pathophysiologic Significance. J. Clin. Invest., 87:424-431.
26. **Omar R.; Nomikos I.; Piccorelli G.; Savino J. and Agarwal N. (1989):** Prevention of postischaemic lipid peroxidation and liver cell injury by iron chelation. Gut 30:510-514.
27. **Colletti L.M.; Remick D.G. and Campbell J.R. (1994):** Desferal attenuates TNF release following hepatic ischemia/reperfusion. J. Surg. Res., 57:447-453.
28. **Seo M.Y. and Lee S.M. (2002)**: Protective effect of low dose of ascorbic acid on hepatobiliary function in hepatic ischemia/reperfusion in rats. J. Hepatol., 36:72-77.
29. **Beyer R.E. (1994):** The role of ascorbate in antioxidant protection of biomembranes: Interaction with vitamin E and coenzyme Q. J. Bioenerg. Biomembranes 26:349-358.
30. **Winkler B.S.; Orselli S.M. and Rex T.S. (1994)**: The redox couple between glutathione and ascorbic acid: A chemical and physiological perspective. Free Radic. Biol. Med., 17:333-349.
31. **Buettner G. R. (1993):** The pecking order of free radicals and antioxidants: lipid peroxidation, □-tocopherol, and ascorbate. Arch Biochem. Biophys., 300:535-543.
32. **Rose R.C. (1990):** Ascorbic acid metablosim in protection against free radicals: A radiation model. Biochem. Biophys. Res. Commun., 169:430-436.
33. **Deutsch J.C. (1998):** Ascorbic acid oxidation by hydrogen peroxide. Anal. Biochem., 255:1-7.
34. **Shi X.; Rojanasakul Y.; Gannett P.; Liu K.; Mao Y. and Saffiotti U. (1994):** Generation of thiyl and ascorbic acid radicals in the reaction of peroxiynirite with thiols and ascorbic acid at physiological pH. J. Inorg. Biochem., 56:77-86.
35. **Bartlett D.; Church D.F.; Bounds P.L. and Koppenol W.H. (1995)**: The kinetics of the oxidation of L-ascrobic acid by peroxynitirite. Free Radic. Biol. Med., 18: 85-92.
36. **Whiteman M. and Halliwell B. (1996):** Protection against peroxynitrite-dependent tyrosine nitration and α1-antiproteinase inactivation by ascorbic acid. A comparison with other biological antioxidants. Free Radic. Res., 25:275-283.
37. **Anderson R. and Lukey P.T. (1987)**: A biological role for ascorbate in the selective neutralization of extracellular phagocyte-derived oxidants. Ann. NY Acad. Sci., 498:229-247.

38. **Dwenger A.; Funk M.; Lueken B.; Schweitwer G. and Lehmann U. (1992)**: Effect of ascorbic acid on neutrophil functions and hypoxanthine/xanthine oxidase-generated, oxygen-derived radicals. Eur. J. Clin. Chem. Clin. Biochem., 30:187-191.
39. **Jonas E.; Dwenger A. and Hager A. (1993):** In vitro effect of ascorbic acid on neutrophil-dendotherial cell interaction. J. Biolumin. Chemilumin., 8:15-20.
40. **Liu J.; Yagi T.; Sadamori H.; Matsukawa H.; Sun D-S and Tanaka N. (2003):** Annexin V Assay-proven Anti-apoptotic Effect of Ascorbic Acid 2-glucoside after Cold Ischemia/Reperfusion Injury in Rat Liver Transplantation. Acta Med. Okayama, (57) 5:209-216.

دور معدلات أكسيد النيتريك و مضادات الأكسدة في الوقاية ضد الأصابة بالأفقار واعادة السريان في كبد الفئران البيضاء

*دصلاح الدين عزيز السيد ، د.عمرو أحمد فؤاد**

قسمي الفسيولوجي و الفارماكولوجي*، كلية الطب، جامعة المنيا

تعتبر إصابة الكبد الناشئة عن الإفقار الدافئ ثم إعادة السريان أحد العوامل الكبرى المحددة لمحصلة العديد من الحالات الإكلينيكيةحيث أنه أثناء مرحلة الإفقار تحدث الإصابة نتيجة نقص الأكسجين بينما تحدث مزيد من الإصابة عند إعادة السريان حيث تلعب جزيئات الأكسجين النشطة دوراً أساسياً. وقد أوضحت العديد من الدراسات الدور الإيجابى لمضادات الأكسدة في تقليل الإصابة في العديد من الأنسجة ومع اكتشاف الدور الحيوي لأكسيد النيتريك في معظم الأنسجة وكذلك التفاعلات المحتملة مع مختلف مضادات الأكسدة كان الهذف من هذه الدراسة في محاولة للحد من هذه الإصابة.

تم تعريض الفص الأيسر من الفئران البيضاء (72 فأرا)إلى ثلاثين دقيقة من الإفقار الدافئ وبعد ستين دقيقة تمت إعادة السريان ثم تم قياس نشاط إنزيمات الكبد في المصل كمقياس لإصابة الأنسجة بينما تم تحديد مستوي النيتريت في الدم كمقياس لإنتاج أكسيد النيتريك كما تم قياس مستوىثنائي ألدهايد المالوت في أنسجة الكبد لتقدير معدل الأكسدة بينما ساعد تقدير مستوى السوبر أكسيد الدسميوتيز والكاتاليز والجلوتاثيون في تحديد حالة مضادات الأكسدةوقد أحدثت ثلاثون دقيقة من الإفقار الدافئ ثم ستون دقيقة من إعادة السريان علامات إصابة الأنسجة مصاحبة بجهد الأكسدة واستهلاك مضادات الأكسدة بالأنسجة وكذلك أكسيد النيتريك بالمصل وهذه الإصابة قد تفاقمت عند استخدام مضاد أكسيد النيتريك (L-NAME 50مجم /كجم)وفي الجانب الآخر فقد قلت هذه الإصابة عند استخدام معطى أكسيد النيتريك (L.arginine) في الجرعة القليلة والمتوسطة (100،300 مجم/كجم) وليس عند الجرعة العالية (500مجم/كجم) والتى أحدثت مزيداً من الإصابة. هذا وقد صاحب معالجة الفئران بمثبط الزانثين أوكسيديز (الوبيورينول 50 مجم/كجم)تأثير وقائى ضعيف وقد أحدثت إزالة الحديد (ديسفيروكسامين50مجم/كجم)وقاية ملحوظة بينما أحدث فيتامين ج (50مجم/كجم) وقاية متوسطة. وقد أظهرت النتائج أن إمداد أكسيد النيتريك أثناء الإفقار وإعادة السريان مفيد إلى حد ما فى تقليل إصابة الكبد بينما كانت قلته مصاحبة بازدياد الإصابة.كما يبدو أن مستواه عندما يكون عالياً جداً أو قليلاً جداً ينتج عنه ازدياد الإصابة ومن ثم يتبين لنا أن الضبط الدقيق أساسى لحماية أنسجة الكبد على الأقل. أن نتائج هذا البحث أشارت الي أن مضاد الأكسدة ألوبيورينول لم يحسن عملياً الكبد المتعرض إلى إصابة الإفقار وإعادة السريان وأن قابض الحديد "ديسفيروكسامين"أظهر تأثيراً وقائياًَ فائقاً وكذلك فيتامين ج حيث أن إعطاءه قد وقى الكبد من إصابة الإفقار وإعادة السريان.