EVALUATION OF ANTI-ISCHEMIC ACTIVITY OF QUINAPRIL AND LYCOPENE IN HEPATIC ISCHEMIA REPERFUSION INJURY IN RATS.

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Summary

The objective of this study is to investigate the protective effect of Quinapril (QP) and Lycopene (LYC) on hepatic ischemia reperfusion (HIR) injury using rat liver model. Albino Wistar rats were the experimental animals. They were administered QP 1.5, 3, 5, LYC 10 and combination of QP 5 & LYC 10 mg/kg (i.g.) respectively. Ischemia of left and median lobes was induced by clamping the common hepatic artery and left branch of portal vein for 45 minutes followed by reperfusion for 3 hrs.

Liver biochemical analysis, anti oxidant activity and histopathological analysis was performed. Different doses of QP and LYC and combination of both drugs showed significant reduction in liver enzyme activity. Elevated levels of SOD, CAT and GSH ($p<0.001$, for all) were found in QP 5 & LYC 10 mg/kg treated groups. The maximum elevation of CAT and GSH level achieved in combination group (closer to normal control), whereas, SOD and MDA level were found near to values of sham control group. Ischemia-induced liver cell injury after reperfusion was ameliorated by LYC, higher dose of QP and the combination of QP and LYC. In conclusion, considering the dosage used, LYC appears to be significantly more potent than QP in reversing oxidative damage induced by HIR. Our finding show that LYC & QP have beneficial effects against the HIR injury and due to their synergistic effect, when administered in combination, may have more pronounced beneficial effects on liver .

Key Words: Ischemia reperfusion, Renin angiotensin system, Oxidative Stress, Quinapril, Lycopene.

Introduction

Liver is frequently exposed to IR injury under different clinical conditions like circulating shock, intravascular coagulation, liver transplantation and surgery involving this organ. Hepatic ischemia reperfusion (HIR) injuries cause high morbidity and consume substantial health care capacities in patients with primary hepatic injury and systemic injury[1, 2].

Recently the role of the renin-angiotensin system (RAS) shown to be involved in some models of acute inflammation in liver [3]. In liver, stellate cells have been shown to respond to AT-II to stimulate proinflammatory and profibrotic cytokines and chemokines[4], adhesion molecules (*e.g.*, ICAM-1) [5], proinflammatory transcription factors (*e.g.*, activator protein-1 and NFκB) [6] and reactive oxygen species (ROS). Also RAS, known for its regulation of blood pressure and fluid homeostasis, in both IR injury and liver regeneration after partial hepatectomy. However, generation of ROS in the reperfusion phase plays the fundamental role or we can say it is central event for cellular damage in hepatic ischemia[7-10]. Among the currently tested

therapeutic strategies, ischemic preconditioning (IPC) by inhibition of RAS and free radical ablation for the treatment of reperfusion injury has found its first pharmacological interventions that prevent the formation of ROS and/or promote their detoxification have the greatest potential to eliminate the post ischemic oxidative stress [11, 7].

Some studies suggesting that ACE inhibitor and some antioxidants possess good anti ischemic activity[12, 13, 14]. Inspired from them we have decided to investigate the usefulness of Angiotensin converting enzyme inhibitor (ACEI) Quinapril & an antioxidant Lycopene on acute ischemia and reperfusion injury in rat liver.

Materials and Methods

Drugs and Chemicals:

Quinapril and lycopene were kindly provided as a gift sample by Zydus Health Care Ltd. Himachal Pradesh and Comed chemical Ltd. Baroda respectively.

Animal Husbandry and Treatment:

Wistar rats of either sex (200-250 g) used as experimental animal. They were housed in cages and provided food and water *ad libitum*. The experimental protocol was approved by CPSCEA. Animals were randomly divided in to following groups,

Group1: Normal control
Group2: Sham control
Group3: Disease control (HIR)
Group4: QP-1.5 mg/kg (QP-1.5 + HIR)
Group5: QP-3 mg/kg (QP-3 + HIR)
Group6: QP-5 mg/kg (QP-5 + HIR)
Group7: LYC 10 mg/kg treated (LYC-10 + HIR)
Group8: Combination of QP-5 & LYC-10 mg/kg (QP-5 & LYC-10 + HIR)

Animals fasted for 12 h before starting of surgical procedure and they received different dose of Quinapril before 2 days, Lycopene before 5 days or vehicle (saline) by oral route. Systolic blood pressure was measured in the conscious state before surgery by noninvasive tail cuff blood pressure recorder (MLT125/R Rat tail cuff/Pulse transducer; AD Instruments Ltd, Australia) attached to the Power Lab (a multiple data acquisition system; AD Instruments Ltd., Australia).

Surgical Procedure and Sampling:
For induction of hepatic ischemia animals were anesthesthetised with Ketamin (60 mg/kg) and Diazepam (5 mg/kg) intrperiotenially and body temperature was maintained at 37± 0.5°C with a lamp. The left branches of portal vein and common hepatic artery were clamped by vascular clamp to induce complete ischemia of the median and left hepatic lobes. Sham surgery was identical to above in the absence of clamping of the vessels. After 45 min of ischemia, reperfusion was allowed for 3 hrs by removing the clamp. Now animals anesthetized by ether. Blood samples were collected just before scarify and liver was rapidly removed for further analysis.

Liver Biochemical Analysis:
Serum separated from blood by cooling centrifugation at 2500 rpm for 10 minutes for measurement of SGPT and SGOT which was done by using standard kit by semi-auto analyzer (photometer 5010, Nicholas India Pvt. Ltd).

Anti Oxidant Activity:
Liver tissue samples homogenized in ice-cold 150 mM KCl and centrifuged at 2-8°C (10500 rpm for 20 minutes). Supernant was used for the estimation of Super oxide dismutase (SOD) [15], Catalase (CAT) [16], reduced glutathione (GSH) [17] and Lipid peroxidation (lipid Malonaldehyde) [18] activities with the help of UV spectrophotometer.

Histopathological Analysis:
After scarifying the animals, liver tissue placed in 10% (V/V) formaline solution and processed routinely by embedding in paraffin. Tissue sections were stained with Hematoxylin and Eosin and examined under a light microscope (Olympus-BH-2). An experienced histologist who was unaware of the treatment conditions made histological assessments.

Statistical Analysis:
Results were expressed as Mean ± S.E.M. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple column comparisons test using computer based fitting program (Graph pad prism, version 5.0). Differences were considered to be statistically significant when $p < 0.05$.

Results

Effect of QP & LYC on marker enzymes of liver Function:

SGPT Level:

The level of SGPT was found significantly higher ($p<0.001$) in group exposed to HIR injury as compared with normal and sham control group . Pre treatment with different doses of Quinapril caused significant reduction ($p<0.001$) in SGPT in QP-1.5 + HIR, QP-3 + HIR & in QP-5 + HIR treated group .Also reduced level of SGPT was evaluated in LYC-10 + HIR group as compared to HIR group. When both drugs given in combination SGPT level reduced up to 78.17 ± 6.60 U/L which was signifiant with QP-5 + HIR ($p<0.001$) and LYC-10 + HIR ($p<0.01$) group (fig 1).

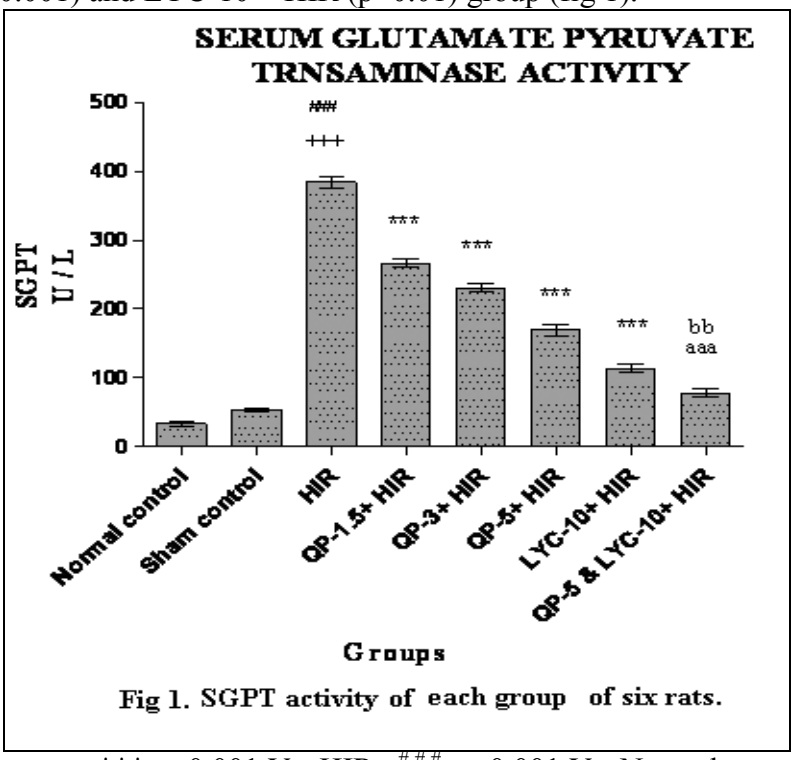

Fig 1. SGPT activity of each group of six rats.

***p< 0.001 Vs. HIR , $^{###}$ p< 0.001 Vs. Normal control, $^{+++}$ p<0.001 Vs. Sham control, ^{aaa} p<0.001 Vs. QP-5 + HIR, ^{bb} p< 0.01 Vs. LYC-10 + HIR.

SGOT Level:

There was significant increase ($p<0.001$) in SGOT level found in HIR group as compared to sham control and normal control group. While in QP-1.5 + HIR, QP-3 + HIR and QP-5 + HIR group, enzyme level was significantly decresed ($p<0.001$) respectively compared to HIR group. It was also reduced up to 131.5 ± 7.25 in the group treated with Lycopene. Combined treatment of both (QP-5 + LYC-10), enzyme level was closure to the values of sham control group & was extremely signifiant with QP-5 ($p<0.001$) and LYC-10 ($p<0.01$) alone (fig 2).

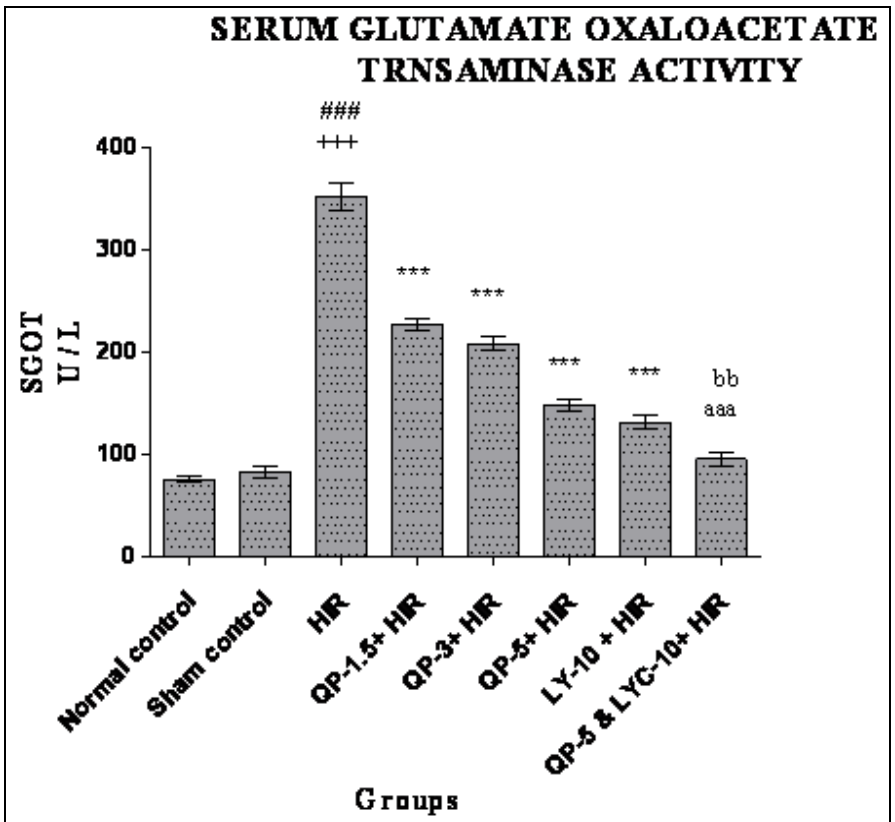

Fig 2. SGOT activity of each group of six rats.

***$p< 0.001$ Vs. HIR, $^{+++}$ $p<0.001$ Vs. Normal control, $^{\#\#\#}$ $p< 0.001$ Vs. Sham control, ^{aaa} $p<0.001$ Vs. QP-5 + HIR, ^{bb} $p< 0.01$ Vs. LYC-10+ HIR.

Effect of QP & LYC on Oxidative stress markers:

Liver Superoxide Dismutase Activity:

SOD level significantly decreased in HIR group compared to sham and normal control ($p<0.001$) group. The enzyme activity in QP-1.5 + HIR was not significant compared to HIR group. While slight improvement in SOD activity was seen in QP-3 + HIR ($p<0.05$) and QP-5+ HIR ($p<0.001$) respectively. Pretreatment with Lycopene alone, highly significant enzyme activity was found ($p<0.001$) compare with HIR group. In QP-5 & LYC-10 + HIR group, SOD level were close to sham control but not significant compared with group QP- 5 + HIR & LYC-10 + HIR alone (fig 3).

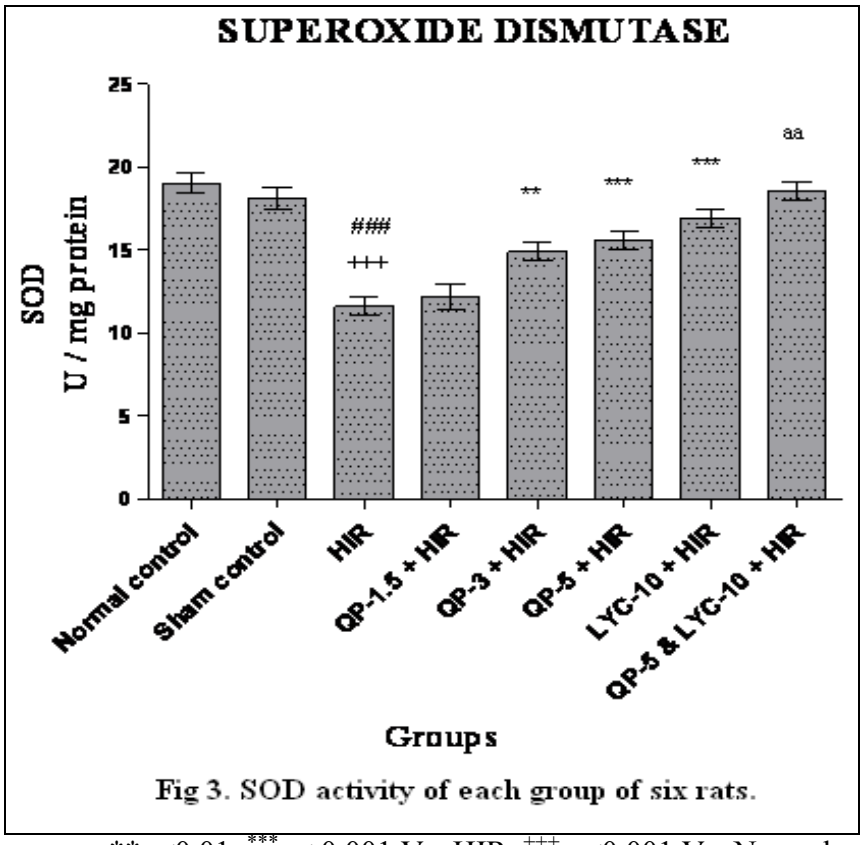

Fig 3. SOD activity of each group of six rats.

**p<0.01, *** p< 0.001 Vs. HIR, +++ p<0.001 Vs. Normal control, ### p< 0.001 Vs. Sham control, aa p<0.05 Vs. QP-5 + HIR

Liver Catalase Activity:

In HIR group, significant fall in Catalase activity was found compare to sham and normal control group ($p < 0.001$). In group QP-1.5 + HIR & QP-3 + HIR, CAT enzyme levels were were significantly higher compare to HIR group ($p<0.05$ & $p < 0.01$) respectivly. Whereas in group QP-5+ HIR and LYC-10 + HIR, value were extremly significant compared to HIR group ($p < 0.001$) respectively. While combined treatment of both elevates enzyme activity 2.486 ± 0.11 which was highly significant ($p<0.001$) compared to QP-5+ HIR group & significant ($p<0.05$) compared to LYC-10 + HIR group (fig. 4).

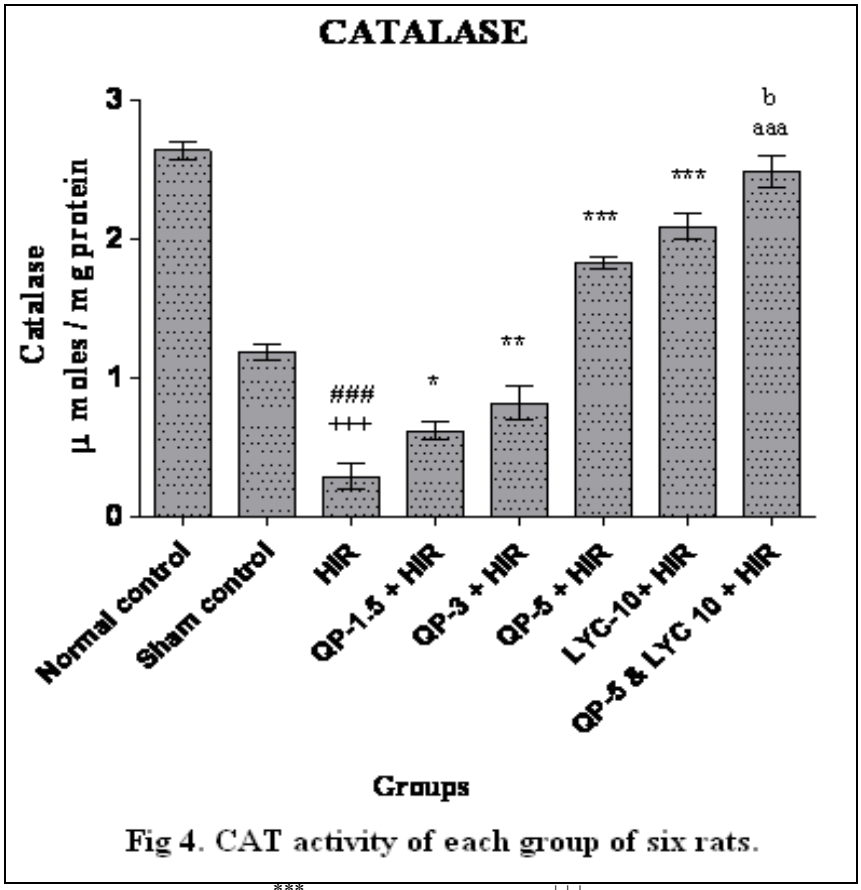

Fig 4. CAT activity of each group of six rats.

*p<0.05, **p< 0.01, ***p< 0.001 Vs. HIR, $^{+++}$ p<0.001 Vs. Normal control, $^{\#\#\#}$ p< 0.001 Vs. Sham control, ^{aaa} p<0.001 Vs. QP-5 + HIR, ^{b} p< 0.05 Vs. LYC-10 + HIR

Liver Reduced Glutathione Activity:

The levels of Reduced glutathione (GSH) in the liver tissue showed a tendency to decrease after HIR, which were significantly higher in sham & normal control ($p<0.001$) groups. Although pretreatment in QP-1.5 mg/kg did not show any beneficial effect on GSH concentrations yet it was found that QP-3 and QP-5 mg/kg significantly elevated the GSH levels ($p<0.01$ & $p<0.001$) respectively, however, it achieve near to sham control only in combination treated which is significant compared to QP-5 + HIR ($p<0.001$) and LYC-10 + HIR ($p<0.01$) groups (fig 5).

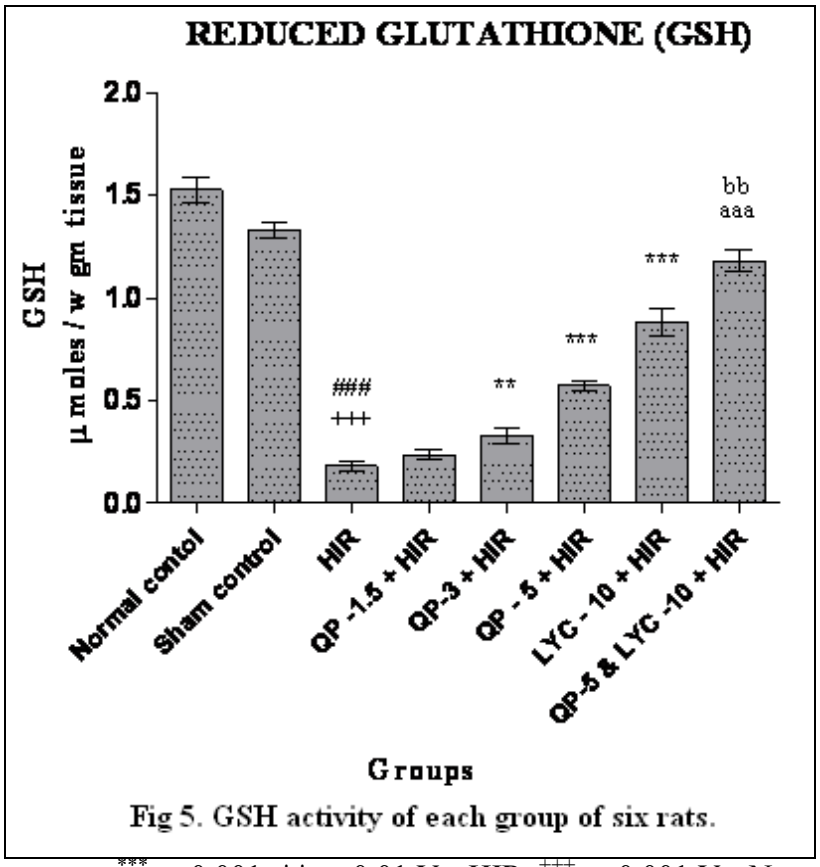

Fig 5. GSH activity of each group of six rats.

$^{***}p< 0.001$, $^{**}p< 0.01$ Vs. HIR, $^{+++}p<0.001$ Vs. Normal control, $^{\#\#\#}p< 0.001$ Vs. Sham control, $^{aaa}p<0.001$ Vs. QP-5 + HIR, $^{bb}p< 0.01$ Vs. LYC-10 + HIR

Liver Malondialdehyde (MDA) level:

MDA level was found significantly higher in the HIR group when compared to sham and normal control groups ($p<0.001$). In group QP-1.5 + HIR, it was not different from HIR whereas in group QP-3 + HIR & QP-5 + HIR significantly decreased ($p<0.01$ & $p<0.001$) levels were found compared to same respectively. With the treatment of combination, significantly decreased MDA level was found compare to QP-5+ HIR group ($p<0.01$) but it was not significant with LYC-10 + HIR group (fig 6).

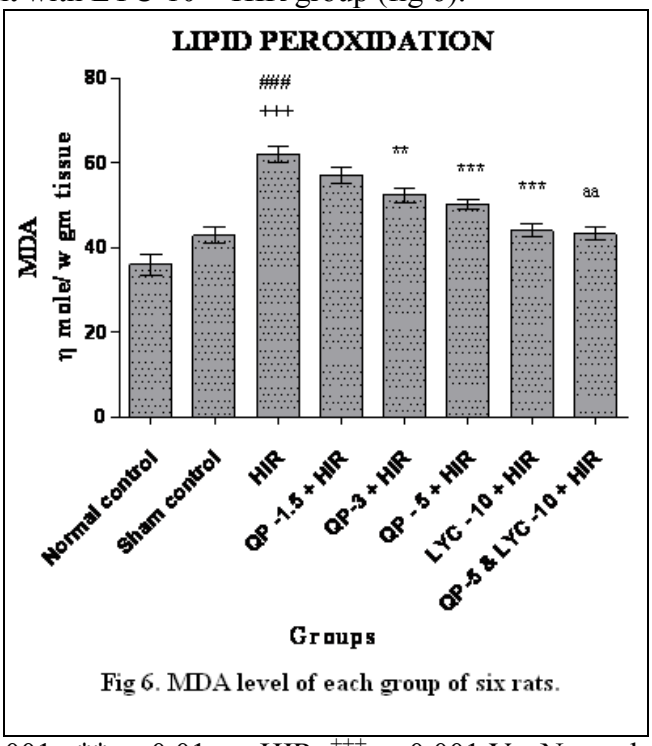

Fig 6. MDA level of each group of six rats.

$^{***}p< 0.001$, $^{**}p< 0.01$ vs. HIR, $^{+++}p<0.001$ Vs. Normal control, $^{\#\#\#}p< 0.001$ Vs. Sham control, $^{aa}p<0.01$ Vs. QP-5 + HIR

Liver Histopathological Analysis:

Histopathological analysis in normal control group (fig7-a) showing normal hepatic architecture, 1-2 cell thick hepatic plates lined by hepatocytes, sinusoids, central vein and few KCs whereas in sham control group (fig7-b), section shows a markedly dilated central vein, area of hemorrhage, focal necrosis, mixed inflammatory cells,

nuclear pleomorphism and hyperchromasia with Kuffer cells (KCs) proliferation. In HIR group (fig7-c) the section shows a markedly dilated central vein, large area of hemorrhage, dilated sinusoids, hepatocyte swelling, multi focal area of necrosis, mixed inflammatory infiltrate into mainly periportal areas with mild nuclear pleomorphisam and focal area of vacuolization.

In QP-1.5 mg treatment group (fig7 d) hemorrhagic area with hepatocyte swelling, vacuolization, KCs proliferation, periportal necrosis and inflammation was seen. While in group QP-3 + HIR (fig 8-a) sections shows focal area of necrosis, hemorrhage, inflammatory infiltrate into mainly neutrophiles with vacuolization and sinusoidal dilation. Whereas in QP-5 + HIR group (fig 8-b) focal areas of pleomorphisam, central vein and sinusoidal dilation, mild inflammatory infiltrate into mainly periportal areas were observed.

In LYC-10 + HIR group (fig 8-c) the section shows central vein and sinusoidal dilation with nuclear pleomorphisam and hyperchromasia. While in the combination group (fig 8-d) section shows almost normal liver histology with mild central vein dilation.

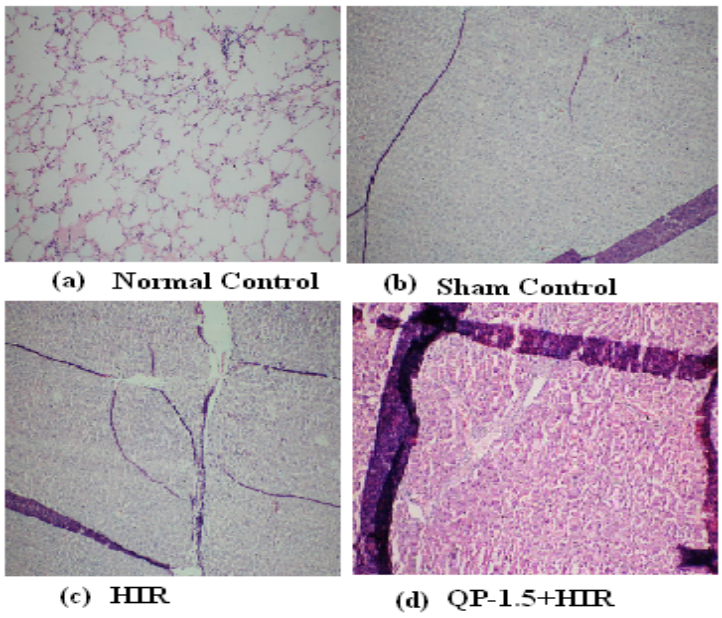

Fig 7. Liver histopahology of Normal control group(a), sham control group (b), HIR group (c), QP-1.5 + HIR group (d)

Discussion

Liver ischemia during arterial occlusion, systemic shock, or organ transplantation is a common cause of frequent clinical complications such as, hepatocyte death, liver failure, liver graft rejection with high morbidity and mortality. Although ischemia can damage cells directly, liver cells have defense mechanisms to protect against such insults if the ischemic time is relatively brief [19]. However, if the liver cells survive even after the ischemic insult, reintroduction of blood flow (reperfusion) often leads to cellular damage due to microcirculation failure that results at the beginning of liver reperfusion from endothelial cell activation, vasoconstriction, platelet aggregation within the sinusoids, and leukocyte recruitment and entrapment [20], this initiates the complex cellular events that eventually results in necrosis and apoptosis of liver cells [21].

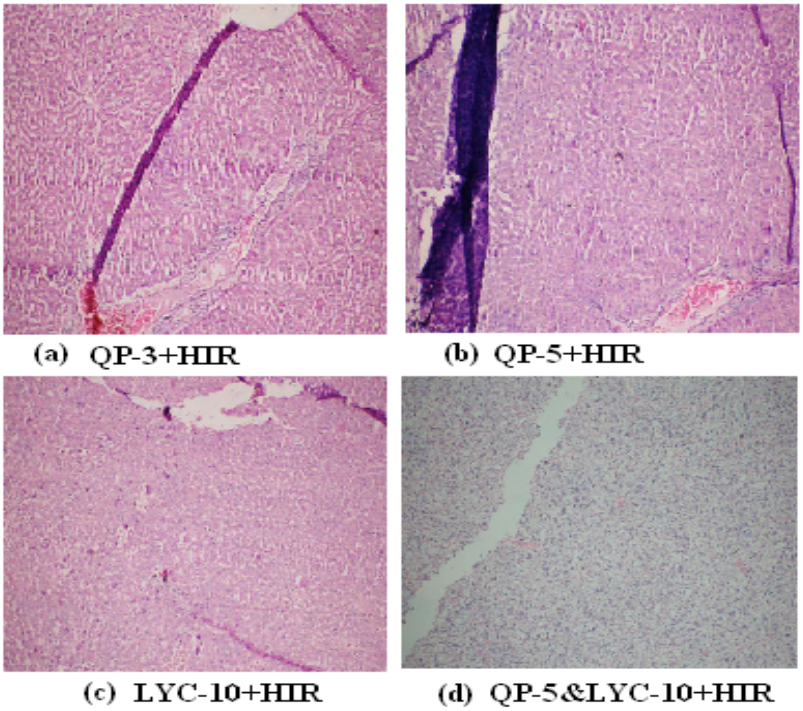

Fig 8. Liver histopathology of QP-3+HIR group (a), QP-5+ HIR group (b), LYC-10+ HIR group (c), QP-5 & LYC-10 + HIR group (d).

LYC, one of the major carotenoids, having potent antioxidant activity may prevent the formation of ROS or detoxification of oxidative stress[22]. Quinapril, have potent ACE inhibitory activity with good free radical scavenging property. The hepatoprotection provided by Quinapril might be due to blocking formation of AT-II, down regulate cellular adhesion molecules (e.g., ICAM-1), inhibition of synthesis of proinflammatory cytokines and chemokines, e.g. TNFα, cytokine-induced neutrophil chemoattractant-1 (CINC-1) and reduce IR injury [10, 13].

It is suggest that suppression of inducible nitric oxide synthase (iNOS) improves HIR injury [23].Quinapril also might be attribute other mechanisms against ROS protection like increasing in NO bioactivity by inhibition of bradykinin degradation[24].It may be increases blood microcirculation at the beginning of liver reperfusion after ischemia, by inhibiting platelet aggregation within the sinusoids, vasoconstriction, leukostasis [20, 25]. Lycopene shows protection seems to be blocking ROS production, as it is well proven antioxidant. When QP & LYC drugs given in combination they offered additional protection by the efficient removal of ROS formed in situ due to IR injury.

Quinapril and Lycopene, offers a safe therapeutic option after hepatic ischemia. However LYC appears to be significantly more potent than all doses of QP in reversing oxidative damage induced by HIR. It is likely that prevention of inflammatory mediators & blocking RAS system, contributed to the observed hepatoprotective activity in HIR injury. However, the complex interactions in this central regulatory system affecting on microvascular perfusion, inflammatory processes and ROS in hepatic ischemia are poorly understood. Further investigations are required in concerning the secondary mediators and the mechanisms of interaction.

Conclusion

QP 5, LYC 10 and combination of both have profound protective effect, produced by significantly decreasing liver biochemical parameters, normalize tissue antioxidant system and providing beneficial effect on hisopathological changes. LYC & QP have

beneficial effects against the HIR injury and due to their synergistic effect, when administered in combination, may have more pronounced beneficial effects on liver.

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