Protective Effect of Xanthine Oxidase Inhibition on Ischemia/Reperfusion Injury in Rat Liver

The Department of Physiology, Faculty of Medicine, Cairo University

Abstract

Ischaemia reperfusion (I/R) injury is a major cause of morbidity and mortality in patients undergoing partial liver resection or transplantation. Despite the ongoing research identifying novel approaches of protection against I/R injury such as pharmacologic, genetic & surgical approaches, there is still no accepted therapy used in the clinical transplantation to minimize liver injury. In this study we examined the effects of allopurinol (Allo), ischemic preconditioning (IPC) and postconditioning (IPO) on the extent of I/R injury of rat liver in a trial to evaluate their role as a protective strategy against hepatic I/R injury. The study was conducted on 70 adult male albino rats that were divided into the following 5 main groups: Group I: Control sham-operated group, Group II (I/R): Hepatic ischaemia reperfusion group, Group III: Allopurinol–treated (Allo+I/R) group, Group IV: Conditioning group which included ischemic preconditioning (IPC+IR) subgroup and ischemic postconditioning (IPO+IR) subgroup, Group V: combined allopurinol and conditioning group which included (Allo+IPC+IR) subgroup and (ALLO+IR+IPO) subgroup. Serum alanine aminotransferase (ALT) enzyme, liver tissue malondialdehyde (MDA) and reduced form of glutathione (GSH) were measured. Results revealed significant decrease of serum ALT and liver MDA with significant increase in liver GSH in (Allo+I/R), (IPC+IR), (IPO+IR), (Allo+IPC+IR) and (ALLO+IR+IPO) groups compared to IR group. In conclusion, there are protective and additive effects of allopurinol & different conditioning protocols on I/R injury in rat liver.

Key Words: Liver – Ischemia – Allopurinol – Preconditioning – Postconditioning.

Introduction

OVER the past two decades, liver resection and liver transplantations has increasingly been performed worldwide because of improved postoperative outcomes and evidence that this approach offers the only chance of cure in many patients.

During liver resection surgery, the liver is subjected to ischemia (reduction in blood flow). When the blood flow is restored (reperfusion), the liver is subjected to further injury. The damage caused by ischemia and then reperfusion is called ischemia-reperfusion (I/R) injury [1].

During an ischemic period, several functional changes occur at the cellular level that promotes cell injury. A Decrease in oxidative phosphorylation results in ATP depletion and derangements in calcium homeostasis [2]. The deleterious effects of ATP catabolism modification are further enhanced by the production of several substances, including reactive oxygen species (ROS), cytokines and others [3]. Hepatic cell death occurs due to both necrosis and apoptosis [4].

Excessive reactive oxygen species (ROS) cause tissue damage and cell death by binding and altering cellular macromolecules, including DNA, proteins and lipids, and affect their function. One main chemical source which has been shown to contribute significantly to overall pronounced oxidant stress during hepatic ischemia/reperfusion (I/R) procedure is xanthine oxidase (XO), which generates superoxide anions (O2-) during the conversion of hypoxanthine to xanthine. It is known that much of the sustained injury during liver transplantation is triggered by ROS via activated XO, so allopurinol, an XO inhibitor, may provide some protection against the hepatic I/R-induced injury [8].

Although the mechanisms underlying IR injury have not been defined completely, during the past several years, many investigations have focused on the intervention of IR injury. Ischemic preconditioning (IPC), defined as brief periods of ischemia and reperfusion before sustained ischemia, is a promising approach to minimize hepatic IR injury in animals and humans [6]. Furthermore, recent
researches proved the protective effect of several brief cycles of ischemia and reperfusion at the onset of sustained reperfusion after a prolonged period of ischemia, termed ischemic postconditioning (IPO) [7].

The present study is designed to find out and to compare the possible protective effect of allopurinol, IPC and IPO on liver function and histopathological picture in hepatic I/R rat model.

**Material and Methods**

This study was carried out in the Physiology Department, Faculty of Medicine, Cairo University from 2009-2010. Seventy male albino rats with body weights 180-250 grams were included in this study. The animals were purchased and placed in the animal house of the faculty. They were housed in wire mesh cages at room temperature and had free access to food and water. Rats were fasted for 12 hours before the experiments to avoid diet induced changes in liver enzymes [8].

Animals were randomly divided into the following 5 groups:

**Group I:** Control sham-operated group (n=10): In this group rats were anaesthetized, and then laparotomy & liver exposure for 2.5 hours were performed with no further surgical manipulations [9].

**Group II (I/R):** Hepatic ischemia reperfusion group (n=10): This group underwent the same procedure as sham operated rats but with the induction of hepatic ischemia/reperfusion (I/R) injury as follows: 30min of ischemia by clamping the hepatic pedicle using a non traumatic microvascular clip, followed by 2 hours of reperfusion [10].

**Group III:** Allopurinol-treated group (Allo+I/R) (n=10): Animals were injected with XO inhibitor, allopurinol (50mg/kg body weight, intraperitoneally 18h and one hour before the hepatic I/R procedure) [11].

**Group IV: Conditioning group (n=20), subdivided into 2 subgroups:**

**Subgroup IVa:** Ischemic preconditioning (IPC+I/R) group (n=10): Animals were subjected to ischemic preconditioning protocol as follows: 10min of ischemia followed by 10 min of reperfusion. It was followed by I/R procedure (30min of ischemia followed by reperfusion for 2 hours) [12].

**Subgroup IVb: Ischemic postconditioning (I/R+IPO) group (n=10):** Animals underwent 30min of ischemia followed by ischemic postconditioning (IPO) as follows: 30 seconds of reperfusion followed by 30 seconds of re-occlusion for 3 cycles. Finally reperfusion was performed for 2 hours [13].

**Group V: Allopurinol and conditioning group (n=20), subdivided into 2 subgroups:**

**Subgroup Va:** Combined allopurinol and ischemic preconditioning (Allo+IPC+I/R) group (n=10): Animals were injected with allopurinol as in group III, then subjected to ischemic preconditioning in combination with hepatic I/R as descried in subgroup IVa.

**Subgroup Vb:** Combined allopurinol and ischemic postconditioning (Allo+I/R+IPO) group (n=10): Animals were injected with allopurinol as in group III, then hepatic I/R was done in combination with ischemic postconditioning as described in subgroup IVb.

At the end of the experimental procedure blood samples were withdrawn from the retro-bulbar plexus using a capillary tube to assess the liver enzyme alanine aminotransferase (ALT) in the serum. Then, animals were sacrificed and livers were rapidly excised for further detection of the lipid peroxidation product malondialdehyde (MDA) & the reduced form of glutathione (GSH). Liver slices were taken for histopathological examination.

Serum ALT was assessed by ALT Enzymatic Assay kit supplied by Lab Biotechnology (USA) catalog (Sup 600). The malondialdehyde (MDA) was measured in liver tissue homogenate by MDA colorimetric Assay Kit from Oxis International, Inc. Foster City, CA 94404 USA, and the reduced form of glutathione (GSH) was measured by Glutathione Assay Kit supplied by Biovision Linda Vista Avenue, CA 94043 USA.

**Statistical analysis:**

Data were analyzed using the statistical package SPSS version 15. Values were expressed as mean ± standard deviation (SD) and % change for the quantitative variables was calculated. Comparisons between groups were done using unpaired student t-test. p-values less than 0.05 were considered as statistically significant.

**Results**

This study examined the effects of allopurinol pretreatment, Ischemic preconditioning (IPC) and ischemic postconditioning (IPO) on liver alanine aminotransferase enzyme (ALT), malondialdehyde (MDA) and reduced form of glutathione (GSH) in addition to histopathological picture in hepatic I/R...
Allopurinol administration prior to I/R (group III) significantly decreased serum ALT (U/L) by 21.26% & MDA level in liver tissue (nmol/mg ptn) by 31.62%, while GSH (nmol/mg ptn) was significantly higher by 47.39% compared to I/R group (group II). However, on comparing the corresponding values of allopurinol and control groups, it is noticed that serum ALT (U/L) & MDA level in liver tissue (nmol/mg ptn) were still significantly elevated in allo+I/R group by 18.54% and 679.79% respectively (Table 1).

Ischemic preconditioning enhanced liver function and offered a degree of protection against I/R injury. This can be observed from Table (2) where serum ALT (U/L) & MDA level in liver tissue (nmol/mg ptn) were significantly decreased in IPC+I/R (subgroup IVa) by 17.22% and 26.03% respectively, compared to I/R group (group II). GSH was significantly increased by 40.62% in subgroup IVa compared to group II. However, there was still significant difference in the corresponding values of measured parameters between subgroup IVa and the control group (Table 2).

Table (3) showed that serum ALT (U/L) & MDA level in liver tissue (nmol/mg ptn) were also improved following ischemic postconditioning. They were significantly decreased in I/R+IPO (subgroup IVb) by 16.53% and 31.25% respectively, compared to I/R group (group II). Additionally, GSH (nmol/mg ptn) showed significant increase in (subgroup IVb) by 41.61% compared to I/R group (group II). However, there were still significant difference in the corresponding values of measured parameters between subgroup IVb and the control group (group I).

Allopurinol combined with ischemic preconditioning improved liver recovery subsequent to I/R. Table (4) showed that serum ALT (U/L) & MDA level in liver tissue (nmol/mg ptn) were significantly decreased by 35.97% and 48.41% respectively in allo+IPC+I/R (subgroup Va) compared to I/R group (group II). Also, GSH (nmol/mg ptn) was significantly higher in allo+IPC+I/R (subgroup Va) by 76.85% compared to I/R group (group II). Fortunately, serum ALT (U/L) (considered as an index for liver function) showed a non-significant difference in subgroup Va compared to control group (group I), indicating full recovery of the ALT liver enzyme.

Concerning their beneficial effects on liver I/R injury, allopurinol together with ischemic postconditioning had similar results as those obtained with allopurinol together with ischemic preconditioning. Data in Table (5) described that serum ALT (U/L) & MDA level in liver tissue (nmol/mg ptn) were significantly decreased by 35.38% and 51.03% respectively in allo+I/R+IPO (subgroup Vb) compared to I/R group (group II). While GSH (nmol/mg ptn) was significantly increased by 75.11%. Fortunately, serum ALT (U/L) (considered as an index for liver function) showed a non-significant difference in subgroup Vb compared to control group (group I), indicating full recovery of the ALT liver enzyme.

Tables (6, 7) showed that combination of allopurinol with either IPC or IPO had better significant results on ALT, MDA and GSH levels than either Allo, IPC, or IPO alone.

### Table (1): Effect of allopurinol (Allo) on serum alanine aminotransferase (ALT in U/L), liver malondialdehyde (MDA in nmol/mg protein) and reduced form of glutathione (GSH in nmol/mg protein) in hepatic I/R rats.

<table>
<thead>
<tr>
<th></th>
<th>Group I (Control)</th>
<th>Group II (I/R)</th>
<th>Group III (Allo+I/R)</th>
<th>p-value</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ALT (U/L)</td>
<td>53.46±5.55</td>
<td>80.48±6.25</td>
<td>63.37±5.2</td>
<td>0.001 *</td>
<td>18.54% #</td>
</tr>
<tr>
<td>MDA (nmol/mg ptn)</td>
<td>0.94±0.07</td>
<td>10.72±1.4</td>
<td>7.33±1.64</td>
<td>0.000 *</td>
<td>679.79% #</td>
</tr>
<tr>
<td>GSH (nmol/mg ptn)</td>
<td>41.55±6.49</td>
<td>20.09±2.25</td>
<td>29.61±1.86</td>
<td>0.000 *</td>
<td>72.84% @</td>
</tr>
</tbody>
</table>

Values are represented as mean ±SD
* Significant p-value (p<0.05) comparing corresponding values in groups (III) and (I).
• Significant p-value (p<0.05) comparing corresponding values in groups (III) and (II).
# % change between corresponding values in group (III) compared to group (I).
@ % change between corresponding values in group (III) compared to group (II).
Table (2): Effect of ischemic preconditioning (IPC) on serum alanine aminotransferase (ALT in U/L), liver malondialdehyde (MDA in nmol/mg protein) and reduced form of glutathione (GSH in nmol/mg protein) in hepatic I/R rats.

<table>
<thead>
<tr>
<th></th>
<th>Group I (Control)</th>
<th>Group II (I/R)</th>
<th>Subgroup IVa (IPC+I/R)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ALT (U/L)</td>
<td>53.46±5.55</td>
<td>80.48±6.25</td>
<td>66.62±4.92 *•</td>
<td>24.53% #</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>–17.22% @</td>
</tr>
<tr>
<td>MDA (nmol/mg ptn)</td>
<td>0.94±0.07</td>
<td>10.72±1.4</td>
<td>7.93±1.11 *•</td>
<td>743.62% #</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>–26.03% @</td>
</tr>
<tr>
<td>GSH (nmol/mg ptn)</td>
<td>41.55±6.49</td>
<td>20.09±2.25</td>
<td>28.25±2.71 *•</td>
<td>–32.01% #</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40.62% @</td>
</tr>
</tbody>
</table>

Values are represented as mean ±SD
* Significant p-value (p<0.05) comparing corresponding values in groups (IVa) and (I).
• Significant p-value (p<0.05) comparing corresponding values in groups (IVa) and (II).
# % change between corresponding values in subgroup (IVa) compared to group (I).
@ % change between corresponding values in subgroup (IVa) compared to group (II).

Table (3): Effect of ischemic postconditioning (IPO) on serum alanine aminotransferase (ALT in U/L), liver malondialdehyde (MDA in nmol/mg protein) and reduced form of glutathione (GSH in nmol/mg protein) in hepatic I/R rats.

<table>
<thead>
<tr>
<th></th>
<th>Group I (Control)</th>
<th>Group II (I/R)</th>
<th>Subgroup IVb (I/R+IPO)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ALT (U/L)</td>
<td>53.46±5.55</td>
<td>80.48±6.25</td>
<td>67.18±5.03 *•</td>
<td>25.66% #</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>–16.53% @</td>
</tr>
<tr>
<td>MDA (nmol/mg ptn)</td>
<td>0.94±0.07</td>
<td>10.72±1.4</td>
<td>7.37±0.79 *•</td>
<td>684.04% #</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>–31.25% @</td>
</tr>
<tr>
<td>GSH (nmol/mg ptn)</td>
<td>41.55±6.49</td>
<td>20.09±2.25</td>
<td>28.45±3.17 *•</td>
<td>–31.53% #</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>41.61% @</td>
</tr>
</tbody>
</table>

Values are represented as mean ±SD
* Significant p-value (p<0.05) comparing corresponding values in groups (IVb) and (I).
• Significant p-value (p<0.05) comparing corresponding values in groups (IVb) and (II).
# % change between corresponding values in subgroup (IVb) compared to group (I).
@ % change between corresponding values in subgroup (IVb) compared to group (II).
º Insignificant p-value (p>0.05) comparing corresponding values in groups (IVb) and (I).

Table (4): Combined effect of Allopurinol (Allo) and ischemic preconditioning (IPC) on serum alanine aminotransferase (ALT in U/L), liver malondialdehyde (MDA in nmol/mg protein) and reduced form of glutathione (GSH in nmol/mg protein) in hepatic I/R rats.

<table>
<thead>
<tr>
<th></th>
<th>Group I (Control)</th>
<th>Group II (I/R)</th>
<th>Subgroup Va (Allo+IPC+I/R)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ALT (U/L)</td>
<td>53.46±5.55</td>
<td>80.48±6.25</td>
<td>51.53±6.41 *•</td>
<td>–3.61% #</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>–35.97% @</td>
</tr>
<tr>
<td>MDA (nmol/mg ptn)</td>
<td>0.94±0.07</td>
<td>10.72±1.4</td>
<td>5.53±0.53 *•</td>
<td>488.30% #</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>–48.41% @</td>
</tr>
<tr>
<td>GSH (nmol/mg ptn)</td>
<td>41.55±6.49</td>
<td>20.09±2.25</td>
<td>35.53±1.51 *•</td>
<td>–14.49% #</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>76.85% @</td>
</tr>
</tbody>
</table>

Values are represented as mean ±SD
* Significant p-value (p<0.05) comparing corresponding values in groups (Va) and (I).
• Significant p-value (p<0.05) comparing corresponding values in groups (Va) and (II).
º Insignificant p-value (p>0.05) comparing corresponding values in groups (Va) and (I).
# % change between corresponding values in subgroup (Va) compared to group (I).
@ % change between corresponding values in subgroup (Va) compared to group (II).
Table (5): Combined effect of Allopurinol (Allo) and ischemic postconditioning (IPO) on serum alanine aminotransferase (ALT in U/L), liver malondialdehyde (MDA in nmol/mg protein) and reduced form of glutathione (GSH in nmol/mg protein) in hepatic I/R rats.

<table>
<thead>
<tr>
<th></th>
<th>Group I (Control)</th>
<th>Group II (I/R)</th>
<th>Subgroup Vb (Allo+I/R+IPO)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ALT (U/L)</td>
<td>53.46±5.55</td>
<td>80.48±6.25</td>
<td>52.01±5.87 *</td>
<td>–2.71% #</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>–35.38% @</td>
</tr>
<tr>
<td>MDA (nmol/mg ptn)</td>
<td>0.94±0.07</td>
<td>10.72±1.4</td>
<td>5.25±0.46 **</td>
<td>458.51% #</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>–51.03% @</td>
</tr>
<tr>
<td>GSH (nmol/mg ptn)</td>
<td>41.55±6.49</td>
<td>20.09±2.25</td>
<td>35.18±1.30 *</td>
<td>–15.33% #</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>75.11% @</td>
</tr>
</tbody>
</table>

Values are represented as mean ±SD
* Significant p-value (p<0.05) comparing corresponding values in groups (Vb) and (I).
** Significant p-value (p<0.05) comparing corresponding values in subgroups (Vb) and (II).
º Insignificant p-value (p>0.05) comparing corresponding values in groups (Vb) and (I).
# % change between corresponding values in subgroup (Vb) compared to group (I).
@ % change between corresponding values in subgroup (Vb) compared to group (II).

Table (6): Comparison between the effect of Allopurinol (Allo), ischemic preconditioning (IPC) and combined allopurinol/ischemic preconditioning (Allo+IPC) on serum alanine aminotransferase (ALT in U/L), liver malondialdehyde (MDA in nmol/mg protein) and reduced form of glutathione (GSH in nmol/mg protein) in hepatic I/R rats.

<table>
<thead>
<tr>
<th></th>
<th>Group III (Allo+I/R)</th>
<th>Subgroup IVa (IPC+I/R)</th>
<th>Subgroup Va (Allo+IPC+I/R)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ALT (U/L)</td>
<td>63.37±5.2</td>
<td>66.62±4.92</td>
<td>51.53±6.41 **</td>
<td>–18.68% #</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>–22.65% @</td>
</tr>
<tr>
<td>MDA (nmol/mg ptn)</td>
<td>7.33±1.64</td>
<td>7.93±1.11</td>
<td>5.53±0.53 **</td>
<td>–24.56% #</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>–30.26% @</td>
</tr>
<tr>
<td>GSH (nmol/mg ptn)</td>
<td>29.61±1.86</td>
<td>28.25±2.71</td>
<td>35.53±1.51 **</td>
<td>19.99% #</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25.77% @</td>
</tr>
</tbody>
</table>

Values are represented as mean ±SD
* Significant p-value (p<0.05) comparing corresponding values in groups (Va) and (III).
** Significant p-value (p<0.05) comparing corresponding values in subgroups (Va) and (IVa).
# % change between corresponding values in subgroup (Va) compared to group (III).
@ % change between corresponding values in subgroup (Va) compared to subgroup (IVa).

Table (7): Comparison between the effect of Allopurinol (Allo), ischemic postconditioning (IPO) and combined allopurinol/ischemic postconditioning (Allo+IPO) on serum alanine aminotransferase (ALT in U/L), liver malondialdehyde (MDA in nmol/mg protein) and reduced form of glutathione (GSH in nmol/mg protein) in hepatic I/R rats.

<table>
<thead>
<tr>
<th></th>
<th>Group III (Allo+I/R)</th>
<th>Subgroup IVb (Allo+IR+IPO)</th>
<th>Subgroup Vb (Allo+IR+IPO)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ALT (U/L)</td>
<td>63.37±5.2</td>
<td>67.18±5.03</td>
<td>52.01±5.87 **</td>
<td>–17.93% #</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>–22.58% @</td>
</tr>
<tr>
<td>MDA (nmol/mg ptn)</td>
<td>7.33±1.64</td>
<td>7.37±0.79</td>
<td>5.25±0.46 **</td>
<td>–28.38% #</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>–28.77% @</td>
</tr>
<tr>
<td>GSH (nmol/mg ptn)</td>
<td>29.61±1.86</td>
<td>28.45±3.17</td>
<td>35.18±1.30 **</td>
<td>18.81% #</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23.66% @</td>
</tr>
</tbody>
</table>

Values are represented as mean ±SD
* Significant p-value (p<0.05) comparing corresponding values in groups (Vb) and (III).
** Significant p-value (p<0.05) comparing corresponding values in subgroups (Vb) and (IVb).
# % change between corresponding values in subgroup (Vb) compared to group (III).
@ % change between corresponding values in subgroup (Vb) compared to subgroup (IVb).
Histopathological assessment of liver sections obtained from different studied groups and stained with Hematoxinil & Eosin (HE):

**Sham operated control group (I):** Sections examined showed normal histological structure of liver tissue with preserved architecture. No evidence of congestion, necrosis or lymphocytic infiltration (Fig. 1).

**Effect of I/R on rat liver histopathology:** Sections examined showed marked central, periportal & sinusoidal congestion together with marked lymphocytic infiltration, hepatocyte vacuolization & glycogen depletion (Fig. 2).

**Effect of Allopurinol, ischemic preconditioning (IPC), or ischemic post-conditioning (IPO) on I/R rat liver histopathology:** Sections examined showed moderate attenuation of histopathological changes seen in I/R group (II) with moderate sinusoidal & central venous congestion, mild portal lymphocytic infiltration, mild vacuolization & glycogen depletion (Figs. 3-5).

**Combined effect of Allopurinol+IPC or Allopurinol+IPO on I/R rat liver histopathology:** Sections examined showed marked attenuation of histopathological changes seen in I/R group (II) with minimal sinusoidal & central venous congestion and mild portal lymphocytic infiltration (Figs. 6,7).

---

![Fig. (1): Hematoxylin & eosin stained sections of normal liver in sham-operated rats showed normal histological structure of liver tissue with preserved architecture.](image1)

![Fig. (2): Hematoxylin & eosin stained sections of liver from hepatic ischemia reperfusion group showed marked central, periportal & sinusoidal congestion together with marked lymphocytic infiltration, hepatocyte vacuolization & glycogen depletion.](image2)

![Fig. (3): Hematoxylin & eosin stained sections of liver from allopurinol-treated (Allo+I/R) group showed moderate sinusoidal & central venous congestion, mild portal lymphocytic infiltration, mild vacuolization & glycogen depletion.](image3)

![Fig. (4): Hematoxylin & eosin stained sections of liver from ischemic preconditioning (IPC+I/R) group showed moderate sinusoidal & central venous congestion, mild portal lymphocytic infiltration, mild vacuolization & glycogen depletion.](image4)
Fig. (5): Hematoxylin & eosin stained sections of liver from ischemic postconditioning (I/R+IPO) group showed moderate sinusoidal & central venous congestion, mild portal lymphocytic infiltration, mild vacuolization & glycogen depletion.

Fig. (6): Hematoxylin & eosin stained sections of liver from combined allopurinol and ischemic preconditioning (Allo+IPC+I/R) group showed minimal sinusoidal & central venous congestion and mild portal lymphocytic infiltration.

Fig. (7): Hematoxylin & eosin stained sections of liver from combined allopurinol and ischemic postconditioning (Allo+I/R+IPO) group showed minimal sinusoidal & central venous congestion and mild portal.

Discussion

The results of the present study show that allopurinol pretreatment, ischemic preconditioning (IPC) or postconditioning (IPO) protects the liver function and structure against hepatic ischemia/reperfusion (IR) injury. Moreover, there is additive protective effect on combining allopurinol treatment with either IPC or IPO. This study reports statistical significant ($p<0.05$) reduction of serum alanine aminotransferase (ALT) and hepatic malondialdehyde (MDA) with concomitant statistical significant ($p<0.05$) increase in hepatic reduced form of glutathione (GSH) on comparing either Allo+IR, IPC+IR, IR+IPO, Allo+IPC+IR or Allo+IR+IPO to the hepatic IR group.

It was suggested that the production of ROS during I/R of the liver is a major pathophysiological component of acute liver failure in I/R situation. Various methods have been attempted to decrease I/R injury. Among the therapeutic strategies currently tested, ischemic preconditioning (IPC) has been investigated as a surgical tool for many years [14]. The benefit of IPC is restricted, however, by injured liver and prolonged ischemic time (>60min). Therefore, IPC was considered to have unclear effects in transplantation and there is currently no evidence to support or refute the use of IPC in donor liver retrievals [15]. Among other approaches, researchers investigated the application of ischemic postconditioning (IPO). They suggested that IPO could alter the hepatic hemodynamics and stimulate endogenous mechanisms that attenuate the reperfusion injury [13].

Pharmacological interventions that mimic IPC effects have the greatest potential to eliminate the post-ischemic oxidant stress and reduce the level of hepatic I/R injury. The inhibition of xanthine oxidase by allopurinol was reported to decrease the level of ROS production and reduce the hepatic injury associated with liver transplantation [11].

Thus, in this study we examined the effects of ischemic pre-, post-conditioning or allopurinol (Allo) on the extent of hepatic I/R injury in a trial to evaluate their role as a protective strategy against hepatic I/R injury in rats.

Our results revealed that I/R group showed significant rise in the serum level of ALT compared to control group. As well, histopathological assessment revealed glycogen depletion, marked congestion & lymphocytic infiltration. These data denote liver injury following reperfusion.

Oxidative stress is a direct measure of tissue injury as it will greatly destroy the cells. Reducing
hydrogen peroxide by glutathione peroxidases is a mechanism of protecting cells from oxidative stress. Intracellular ROS can be measured indirectly by malondialdehyde (MDA), a byproduct of lipid peroxidation while the mitochondria contain a small number of antioxidant systems to counteract the deleterious effects of ROS, which are represented mainly by GSH. Therefore, the conditions that lower the mitochondrial GSH level might increase the level of oxidative injury associated with I/R [11,12].

In agreement with our results, Sepodes et al. [10] reported that liver ischaemia resulted in significant rise in the serum levels of ALT, AST & lactate dehydrogenase enzymes.

In addition, Lee & Lee [12] and Gedik et al. [17] reported that hepatic I/R resulted in elevation of MDA level and decline of GSH level in experimental rat livers. They suggested that pronounced oxidant stress is the major cause of the hepatic I/R injury and that excessive ROS cause tissue damage and cell death by binding and altering cellular macromolecules, including DNA, proteins and lipids, and affect their function. Therefore, ROS initiate tissue injury and stimulate a cellular cascade leading to inflammation. The inflammatory process is secondary to endothelial activation and dysfunction, adherence and activation of neutrophils and platelets, and the activation of complement and T cells. The proinflammatory process that follows results in cell death and in severe cases leads ultimately to organ failure [18].

Furthermore, Gedik et al. [17] noticed that sinusoidal endothelial cell (SEC) is the main target of injury following I/R. Its dysfunction contributes to increased expression of adhesion molecules which are critical for the recruitment and infiltration of inflammatory cells into the injured tissue.

Zhai et al. [19] proposed the involvement of Toll-like receptors (TLRs) in hepatic IR injury. TLR4 stimulation result in the activation of endothelial cells, macrophages, and maturation of dendritic cells that lead to the release of cytokines, chemokines, local activation of complement, and T cell activation. This hypothesis is partially responsible for the increased rejection rates and allograft dysfunction following transplant-related I/R injury.

It is known that XO plays a critical role in generating superoxide anions and administering allopurinol, a blocker of XO, will be expected to decrease the production of superoxide anions and in turn reduce oxidant stress [11,12]. The results of the present study are in accordance and reveal that pretreatment with allopurinol before ischemia, improve I/R injury & decrease oxidative stress as indicated by significant lower levels of serum ALT & MDA in liver tissue & higher level of GSH in liver tissue compared to I/R group. However compared to control group, serum ALT and MDA levels are still significantly higher; while GSH level is significantly lower in allopurinol group (III) representing deficient recovery.

Although histopathological changes occurring as a result of I/R injury are not completely restored after treatment with allopurinol, yet they showed less marked glycogen depletion, pericentral congestion & lymphocytic infiltration compared to I/R group.

The partial restoration of GSH level, reversed MDA content in the liver and consequently, lower levels of serum ALT and improved liver histopathology, demonstrates the protective effects of allopurinol in the hepatic I/R model. In agreement with this result, Liu et al. [11] who used allopurinol pretreatment before I/R operation in mice found significant reduction in serum ALT, TNF-a & MDA level in liver tissue & significant rise in GSH level as measured 6 & 24 hours after reperfusion compared to untreated group. Other studies reported that allopurinol have a protective effect on hepatic congestion, fatty degeneration, necrosis, apoptosis, and lesions in the central lobule [5,20].

Currently, the mechanism by which allopurinol exerts a protective benefit in ischemia reperfusion related events is not fully understood. Several researchers consider that the protective mechanism of allopurinol may not be totally related to the inhibition of ROS generation by XO. There are various theories: It may act by inhibiting the irreversible breakdown of purine substrates, inhibiting the formation of ROS, and/or protecting against damage to the mitochondrial membrane [21].

Allopurinol’s beneficial effect is indicated as the preservation of mitochondrial function by inhibiting mitochondrial pore opening and thus protecting mitochondrial membrane integrity. Support for this theory has been demonstrated by the decreased mitochondrial swelling and cell death and decreased lipid peroxidation with allopurinol pretreatment [12]. It is also suggested that allopurinol has scavenging properties of its own, either by hydroxyl radical or as an electron transfer agent [21].

On the other hand there are a few studies that have shown allopurinol has no effect on transam-
inases [22], and on oxidant stress as measured by intracellular GSH stores, biliary GSH, and mitochondrial glutamate dehydrogenase [23].

Protective strategies have been developed for protection of organs from I/R injury, which are referred to as ischemic preconditioning (IPC). This manipulation elicits organ tolerance to a subsequently longer period of ischemia [24].

In this study, rats exposed to IPC prior to I/R showed significant reduction in serum ALT & MDA level in liver tissue and significant elevation in GSH level when compared to I/R group indicating the beneficial role of IPC against I/R injury & attenuation of associated oxidative stress. As well, histopathological specimens described less marked glycogen depletion, pericentral congestion & lymphocytic infiltration compared to I/R group. However, compared to the control group, serum ALT and MDA levels were still significantly higher; while GSH level is significantly lower demonstrating partial improvement.

Various substances, such as ROS have been shown to play roles both in the development of hepatic IRI and in the protective effect of IPC. During hepatic ischemia and reperfusion, IPC protects sinusoidal endothelial cells and hepatocytes through modulation of the imbalance in the endogenous oxidant/antioxidant system [25]. Bile flow has been employed as a predictive indicator of the recovery of energy metabolism from hepatic IRI. IPC attenuates the decrease in bile flow after reperfusion following 60min of hepatic ischemia [26].

Peralta et al. [26], proposed that, in the liver NO mediates preconditioning by inhibitory actions on endothelin, activation of adenosine A2 receptors, and subsequent NO formation. NO modulates microvascular perfusion through its vasodilatory effect and through its anti-inflammatory actions, including inhibition of stellate cell activation, neutrophil adhesion, and platelet aggregation.

Navarro-Sabate et al. [27], investigated the profile of gene expression patterns related to IPC of the liver. Twenty-six genes involved in the cell cycle, in improving cell viability, in protein processing, and in signaling were found to be upregulated after IPC preceding a long period of cold ischemia.

On investigating the effect of ischemic postconditioning on hepatic I/R injury. We found that IPO significantly reduced serum ALT & MDA level & raised reduced GSH level compared to I/R group. In addition, histopathological spicemens showed less marked glycogen depletion, pericentral congestion & lymphocytic infiltration compared to I/R group. However compared to control group, serum ALT and MDA level were still significantly higher; while reduced GSH level was significantly lower in indicating also partial recovery.

This beneficial effect of postconditioning was also identified by Sun et al. [28], they related that gradual reperfusion minimizes lipid peroxidation and also depress synthesis of free oxygen radicals, preserving mitochondrial ultrastructure and function. They hypothesized that the decrease of lipid peroxidation may also be related to the protective effects of adenosine that remains inside the parenchyma, like in the heart [29].

Wang et al. [13], applied hepatic IPO procedure, and found reduced hepatic tissue damage, hepatocellular lesion, and improved survival rate after warm or cold IR. They suggested that postconditioning significantly reduced superoxide anion (O²⁻) generation associated with a reduction in plasma MDA activity. A Reduction of ROS may be achieved by increasing the endogenous antioxidant defense potential by preserving reduced glutathione levels.

Kaur et al. [30], explained the significant protective role provided by IPO against I/R injury to be closely related to the NO production following the increase in endothelial and inducible NO synthases expression and the suppression of TNF-α and macrophage inflammatory protein-2 overproduction.

Several studies have compared the effectiveness of ischemic preconditioning or postconditioning versus pharmacological strategies aimed to modulate hepatic I/R injury in normothermic ischemia and liver transplantation [31,32]. Drug therapy has distinct advantages when compared to surgical strategies since it can be applied in conditions where the surgical strategies cannot be applied (typically transplantation or hemorrhagic shock), and at the same time avoid the detrimental effects that the surgical techniques possess.

In the present work, we compared the protective effect of pharmacological prophylaxis by allopurinol with mechanical approaches (IPC or IPO) in a trial to evaluate which strategy is better to be used as a protective method against hepatic I/R injury. We found that there is no significant difference in all studied parameters comparing allopurinol treated group to groups subjected to either IPO or IPC.
Our results were in agreement with Lee & Lee [12], who reported that the increment in ALT and MDA levels was suppressed by IPC or allopurinol alone. They also found that GSH concentration decreased significantly after 5 hours of reperfusion. This decrease was markedly lower in the livers treated with IPC or allopurinol alone.

On the other hand, Fernández et al. [31] postulated that if preconditioning could be understood at the molecular level, it might be possible to develop drugs that achieve similar protection without repeated vascular clamping and prolonged surgery. They investigated whether the protective effect of IPC is related to reduced xanthine oxidase-derived oxidant stress in the liver and lung after liver transplantation. The investigators observed an increased conversion of xanthine dehydrogenase to the oxidase, which correlated with increased MDA levels in the liver and increased liver injury. IPC or a high dose of allopurinol prevented these effects and protected the liver and lung.

On the contrary, Sola et al. [33], described that allopurinol pre-treatment caused higher apoptotic activity while IPC resulted in less DNA fragmentation and diminished morphological changes during I/R injury compared to allopurinol pre-treatment group. They concluded that IPC seems to be simple suitable and powerful protective strategy against I/R injury in clinical practice. Preconditioning is easy to apply, inexpensive and does not require a drug with potential side effects [34].

In support to this strategy, our data showed that a combination of IPC+allopurinol or IPO+allopurinol is more favorable than the single application of each. This may be partially a result of better alleviation of the post-ischemic oxidative injury to the liver. The additive protective effect on hepatic I/R injury, don’t significantly depend on the applied conditioning protocol. We noticed an insignificant difference, regarding all studied parameters, on comparing allo+IPC+I/R, and allo+I/R+IPO. In addition, histopathological specimens assessment of either allo+IPC+I/R or allo+I/R+IPO revealed marked improvement of congestion & inflammatory infiltration that was obviously found in I/R group.

Moreover, IPC+allopurinol; IPO+allopurinol succeeded to improve the liver function to that degree where no significant difference was observed as regard serum ALT level when compared to control values. However, concerning their effect against oxidative stress induced by I/R, they failed to provide full protection.

Our results seemed to be consistent with Lee & Lee [12], who demonstrated that the hepatoprotective effect against a warm I/R injury was more clearly in the rats pretreated with both allopurinol and IPC rather than with either agent alone. They found that a combination of IPC and allopurinol completely inhibited the generation of H$_2$O$_2$ after reperfusion of the livers that had been subjected to a prolonged period of ischemia. They suggested that a combination of IPC and allopurinol may be sufficient to prevent subsequent oxidative stress in the hepatic tissue and increases the mitochondrial pool of GSH, and the improvement of hepatic function after I/R is more efficiently.

Several studies reported the protective role of NO in I/R. Indeed, the serum NO level increased significantly during IPC and was enhanced by allopurinol. However, the precise mechanism for this process is unclear [26].

Moreover, the hepatic ATP concentration has been used extensively in several studies as an indicator of the liver function. A Combination of IPC and allopurinol increased the hepatic ATP and ADP levels to 76% and 115% of the normal level, respectively. It is possible that a combination of IPC and allopurinol is involved in regenerating the ATP [12].

In conclusion, this study addresses the protective effect of allopurinol & different conditioning protocols on I/R injury in rat liver. Moreover it shows the possible additive protective effect of allopurinol in combination with conditioning protocols on I/R injury in rat liver. However, further larger human studies and multicenter trials are needed to confirm such an effect. Those studies should tell the cost benefit, the proper time of administration, the proper dosage, and the population who will have the best benefit.


