Research Article

Diacerein Ameliorates Liver Ischemia Reperfusion Insult in Rats

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Abstract. Ischemia reperfusion injury (IR) is an unavoidable problem that frequently occurs during resection of liver tumors or liver transplantation. Diacerein is a chondroprotective agent used clinically for treatment of osteoarthritis. It exhibits anti-inflammatory, anti-apoptotic and antioxidant properties. So, in the current study, we hypothesized that diacerein may attenuate the partial warm hepatic IR injury in rats. Also, the study aimed to elucidate the possible underlying mechanisms. Rats were randomly divided into: sham, IR, and two diacerein pretreated groups. Rats were treated with diacerein 5mg/kg/day and 10mg/kg/day orally for 14 days and then, partial hepatic ischemia was performed for 30 min followed by reperfusion for 6 h. Liver function markers were determined. Markers of oxidative stress, apoptosis and inflammation were measured. Hematoxylin and eosin stained sections of liver tissue were examined as well as immunohistochemical stained sections for detection of nuclear factor kappa B-p65 (NF-κB-p65) expression. IR insult deteriorated the liver function and evoked inflammation, apoptosis and oxidative stress as well as histopathological derangements. Pretreatment with diacerein 10mg/kg improved the deteriorated liver function and the histopathological changes as well as attenuating the oxidative stress, apoptosis and inflammatory processes. Diacerein attenuates partial IR induced liver damages through anti-inflammatory, anti-apoptotic actions and inhibition of oxidative stress.

Keywords: Diacerein, Malondialdehyde, peroxynitrite, caspase 3, ischemia reperfusion

1. Introduction

Ischemia reperfusion injury (IR) is an unavoidable critical problem frequently occurs during excision of liver tumors, liver transplantation, hemorrhagic or endotoxin shock [1]. During resection of liver tumors, clamping of the hepatic pedicle is mandatory to minimize blood loss with unavoidable reperfusion. During liver transplantation, ischemia reperfusion injury takes place leading to graft dysfunction [2].

Warm ischemic injury of liver (37°C) includes temporary interruption of blood supply as during liver surgery and it affects hepatocytes and endothelial cells. However, the cold type of ischemia (4°C) is used to keep organ frozen prior to transplantation and it mainly affects the sinusoidal endothelial cells [3].

Reperfusion triggers destructive effects where the activated Kupffer cells (KCs) release pro-inflammatory cytokines as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-1 (IL-1) which induces neutrophil recruitment with subsequent reactive oxygen species (ROS) production [4].
Nuclear factor kappa B (NF-κB) plays a cardinal role in liver IR insult [5]. During oxidative stress, the activated NF-κB stimulates the synthesis of inducible nitric oxide synthase (iNOS), peroxynitrite, cytokines (TNF-α, IL-1β and IL-6) and adhesion molecules. TNF-α activates several apoptotic proteins, Cytochrome-c release with DNA fragmentation [6].

Frequent therapeutic strategies have been performed and none could prevent mortality associated with IR [7]. Thus, emergence of alternative options for prevention and treatment of liver IR insult is mandatory to improve patient survival.

Diacerein is an anthraquinone derivative entirely metabolized into active metabolite called rhein which possesses anti-inflammatory, anti-apoptotic and antioxidant activity [8]. Diacerein is a potent IL-1β inhibitor with chondroprotective effect widely used clinically for treatment of osteoarthritis [6]. Diacerein inhibits the ability of nitric oxide (NO) to induce peroxynitrite production and stocktickerDNA damage [9]. Diacerein inhibits cyclooxygenase (stocktickerCOX-2) gene transcription via inactivating c-Jun N-terminal kinases (c-JNK) signaling pathway thus suppressing inflammation and apoptosis [10].

Rhein exhibited a hepatoprotective potential since it improved the liver function deteriorated by carbon tetrachloride in rats [11]. However, some osteoarthritic patients reported elevated liver enzymes with diacerein. The safety profile of diacerein is established and its merit remained positive in treatment of osteoarthritis [12].

So, in the present study, we hypothesized that diacerein may have a beneficial effect against the hazardous effects of partial hepatic IR in rats and we investigated the possible underlying mechanisms.

2. Material and Methods

2.1. Animals

Adult male albino rats (220–250 g) of age 7-9 weeks were purchased from the Center of Experimental Animals, Faculty of Veterinary Medicine, Zagazig University. One week prior to experimentation, rats were left for acclimatization under specific pathogen-free conditions, with 12-h light/dark cycles at temperature of 23 ± 2°C. Rats were kept on a standard pellet diet and allowed tap water ad libitum. The experiments were conducted at Department of Pharmacology, Faculty of Medicine, Zagazig University.

The study protocol was approved by the Institutional Review Board, Faculty of Medicine, Zagazig University, Egypt (ZU-IRB# 4352) and also in accordance the National Institutes of Health guide for care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

2.2. Drugs and chemicals

Diacerein powder (TRB Pharma, São Paulo, Brazil), carboxy methylcellulose (1%) and pento-barbital sodium (EIPICO, 10th of Ramadan, Egypt).
2.3. Surgical procedures

Under complete aseptic conditions, the rats were anesthetized with pentobarbital sodium (40 mg/kg, intraperitoneally). A midline incision was performed and the hepatic pedicle which supplies the left and median liver lobes (70% of liver mass) was occluded with a bulldog clamp. The pale semblance of the clamped liver lobes is a sign to confirm the occurrence of ischemia; 30 min later, the clamp was removed to allow reperfusion. Sham operated animals underwent the same procedure and anesthesia without vascular occlusion. Laparotomy was closed with silk 3/0 after removal of the clamp. After 6 h of reperfusion, the rats were sacrificed and the liver and serum samples were collected for biochemical and histological assays [13].

2.4. Experimental design

Thirty two adult male albino rats were randomly assigned into four equal groups (n=8). Sham-operated group; IR group: Animals received carboxy methylcellulose (1%) (vehicle of diacerein) in a volume of 2 ml/kg body weight by oral gavage daily for 14 days and then subjected to 30 min of partial hepatic ischemia and were sacrificed after 6 h of reperfusion. Diacerein large dose pretreated group (Diacerein-LD): rats received diacerein in a dose of 10 mg/kg/day orally [14] for 14 days before induction of partial hepatic IR. Diacerein small dose pretreated group (Diacerein-SD): rats received diacerein in a dose of 5 mg/kg/day orally for 14 days before induction of partial hepatic IR. The doses were selected according to a Pharmacokinetic formula for humans and rats since the human dose of diacerein is 50 mg twice/day.

2.5. Blood and tissue preparation

At the end of the reperfusion period, all rats were sacrificed. Immediately, blood was collected from the retro orbital vein and serum was separated by centrifugation at 604 g for 10 min. Serum was used for estimation of levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) enzyme activity.

The liver lobes were excised. Each lobe was divided into two equal parts; one part was preserved in 10% formalin for histopathological and immunohistochemical examination, the second part was homogenized in ice cold saline and divided into fractions for biochemical estimation of levels of malondialdehyde (MDA), myeloperoxidase (MPO), reduced glutathione (GSH), nitrotyrosine, caspase-3 and cytochrome c as well as IL-1β, TNF-α and IL-6 levels then immediately frozen at −80°C.

2.6. Assay of serum AST, ALT and LDH activities

Serum AST and ALT activities were measured using an Automated Chemical Analyzer (Bayer Advia #1650; Leverkusen, Germany). Serum LDH activity was assayed by colorimetric enzymatic assay using commercial kits purchased from Spinreact (Gerona, Spain).
2.7. Assay of hepatic MPO content

MPO enzyme-linked immunosorbent assay (ELISA) kit (Catalog Number: MBS724567, MyBiosource. Inc, San Deigo, USA) is a solid-phase ELISA designed for the quantitative determination of rat MPO.

2.8. Assay of hepatic oxidative stress and anti-oxidant markers

MDA level in hepatic homogenates was measured using the Thiobarbituric Acid Reactive Substances (TBARS) Assay Kit (Cell Biolabs, Inc., San Diego, USA).

GSH content in liver homogenate was determined by Quantitative Sandwich ELISA Kit (Catalog No. MBS046356, MyBiosource. Inc, San Deigo, USA).

Nitrotyrosine formation is a footprint of peroxynitrite production. The hepatic nitrotyrosine levels were assayed using the quantitative sandwich enzyme immunoassay method (Rat 3-Nitrotyrosine ELISA Kit, catalog No. MBS732683, MyBiosource. Inc, San Deigo, USA).

2.9. Assay of caspase-3 concentrations in liver homogenates

Hepatic caspase-3 concentrations were assayed by using Quantitative Sandwich ELISA kit (Catalog No. MBS018987, MyBiosource. Inc, San Deigo, USA).

2.10. Assay of cytochrome c levels of liver homogenates

Cytochrome c was determined by quantitative sandwich immunosorbent Elisa technique (Catalog No. MBS727663, MyBiosource Inc, San Deigo, USA).

2.11. Assay of tumor necrosis factor-alpha (TNF-α) concentrations in liver homogenates

For the quantitative detection of rat TNF-α concentration in tissue homogenates, Sandwich ELISA Kit (Catalog No.MBS282960, MyBiosource. Inc, San Deigo, USA) was used.

2.12. Assay of IL-6 concentrations in liver homogenates

The hepatic IL-6 concentrations were measured by using Sandwich enzyme immunoassay kit (Catalog Number: MBS355410, MyBiosource, Inc, San Deigo, USA).

2.13. Assay of IL-1β concentration in liver homogenates

The hepatic IL-1β concentration was measured by using sandwich ELISA kit (Catalog Number: ab100768; Abcam).
Table 1: Scoring features of liver injury following hepatic ischemia-reperfusion.

<table>
<thead>
<tr>
<th>Score</th>
<th>Congestion</th>
<th>Vacuolization</th>
<th>Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>Minimal</td>
<td>Minimal</td>
<td>Single-cell necrosis</td>
</tr>
<tr>
<td>2</td>
<td>Mild</td>
<td>Mild</td>
<td>&lt; 30%</td>
</tr>
<tr>
<td>3</td>
<td>Moderate</td>
<td>Moderate</td>
<td>30-60%</td>
</tr>
<tr>
<td>4</td>
<td>Severe</td>
<td>Severe</td>
<td>&gt;60%</td>
</tr>
</tbody>
</table>

2.14. Histology

Liver specimens were fixed in 10% neutral-buffered formalin solution and then, embedded in paraffin. Sections were cut at 4 μm and stained with hematoxylin and eosin (H&E) and examined under light microscope blindly by a single pathologist to elucidate the liver injury.

The extent of liver injury was semiquantitatively assessed according to Suzuki scoring criteria [15]. Congestion, inflammatory cell infiltration, cell swelling and vacuolisation were evaluated as follow: none = 0, minimal = 1, mild = 2, moderate = 3 and severe = 4. Scoring of Liver necrosis was as follow: none = 0, single cell necrosis = 1, up to 30% lobular necrosis = 2, up to 60% lobular necrosis = 3 and more than 60% lobular necrosis = 4 (Table 1).

2.15. Immunohistochemical staining of NF-κB-p65

Immunostained sections for detection of NF-κB-p65 expression in liver tissue were prepared according to the method described by Butler et al. (2002). All slides were evaluated in a blind fashion. Immunoreactivity (cytoplasmic staining) was graded semi quantitatively and each specimen was assigned a score on a scale from 0 to 3, designated as 0 (negative), 1 (weakly positive cells), 2 (multifocal aggregates of uniformly staining cells), and 3 (diffuse positive staining throughout the cells), the extent score (1, 1-10%; 2, 10-50%; and 3, 50-100%). An overall score was calculated in which extent of positive cells was multiplied by the intensity of the staining (overall score= extent X intensity) yielding a 9-point score ranging from 0 (no staining) to 9 (extensive, strong staining) for each case [16].

2.16. Statistical analysis

Statistical differences between groups were computed by one-way analysis of variance (ANOVA) followed by the Tukey post hoc test for multiple comparison. In all cases, $P$ value $<$ 0.05 was considered to be statistically significant. Data were expressed as means ± S.E.M. The results were analyzed using the SPSS Software version 17, SPSS Inc., Chicago, USA.
3. Results

3.1. Effect of diacerein on the serum levels of AST, ALT and LDH

IR group showed significant (p < 0.05) increase in serum levels of AST, ALT, and LDH (from 22 ± 1.23, 15 ± 0.92, and 80 ± 3.24, respectively in sham group to 130 ± 9.51, 71 ± 3.22, and 189 ± 8.61, respectively). Diacerein-SD group showed non-significant decrease in AST, ALT, and LDH serum levels compared with IR group. However, Diacerein-LD group showed significant (p < 0.05) decrease in serum levels of the aforementioned parameters (to 36 ± 1.32, 29 ± 1.23, and 90 ± 3.24, respectively, compared with IR group) (Figure 1a). Diacerein-LD significantly (p < 0.05) lowered serum levels of AST, ALT and LDH compared with Diacerein-SD (Figure 1a).

3.2. Effect of diacerein on hepatic levels of TNF-α, IL-1β and IL-6

The hepatic levels of TNF-α, IL-1β and IL-6 were significantly (p < 0.05) increased in IR group (from 12.46 ± 1.2, 32.19 ± 1.6 and 22.18 ± 1.8 respectively, in sham group to 46.21 ± 1.4, 102.21 ± 4.5 and 72.31 ± 2.06 respectively). The previously mentioned parameters showed non-significant decrease in Diacerein-SD group in respect to IR group. On contrary, Diacerein-LD group showed significant (p < 0.05) decrease in hepatic levels of TNF-α, IL-1β and IL-6 (from 46.21 ± 1.4, 102.21 ± 4.5 and 72.31 ± 2.06 respectively in IR group to 19.32 ± 0.8, 40.62 ± 1.63 and 30.12 ± 1.2, respectively). Diacerein-LD significantly (p < 0.05) lowered hepatic levels of TNF-α, IL-1β and IL-6 compared with Diacerein-SD (Figure 1b).

3.3. Effect of diacerein on hepatic lipid peroxidation, reduced GSH and nitrotyrosine levels

Hepatic IR injury induced lipid peroxidation evidenced by significant (p < 0.05) increase in hepatic MDA level in respect to sham group. Pretreatment with 5mg/kg diacerein showed non-significant decrease in hepatic MDA levels compared with IR group while diacerein 10mg/kg succeeded to decrease hepatic MDA levels significantly (p < 0.05) compared with both IR and Diacerein-SD groups (Table 2).

The hepatic GSH levels were significantly (p < 0.05) decreased by IR injury compared with sham group. Diacerein-SD group showed non-significant increase in hepatic GSH levels compared with IR group. Diacerein-LD showed significant (p < 0.05) increase in hepatic GSH levels in relation to both IR and Diacerein-SD groups (Table 2).

The hepatic nitrotyrosine levels were significantly (p < 0.05) increased in IR group compared with sham group. Diacerein-SD produced non-significant decrease in hepatic nitrotyrosine levels compared with IR group. However, Diacerein-LD showed significant (p < 0.05) decrease in hepatic nitrotyrosine levels in respect to both Diacerein-SD and IR groups (Table 2).
Figure 1: Effect of pretreatment with diacerein (5, 10mg/kg) on (a): serum levels of AST, ALT and LDH. (b): tissue levels of TNF-α, IL-6 and IL-1β in rats subjected to IR liver injury. Data represent means ± S.E.M. *p<0.05 compared with sham group, # non-significant difference compared with IR group, $p<0.05$ compared with IR group.

3.4. Effect of diacerein on hepatic neutrophil infiltration

Hepatic neutrophil infiltration was assayed by MPO activity. MPO activity significantly (p < 0.05) increased in livers of IR group as compared with livers from the sham group. Diacerein-SD group showed non-significant difference from IR group while Diacerein-LD group showed significant (p < 0.05) decrease in MPO activity compared with both IR and Diacerein-SD groups (Table 2).
Table 2: Effect of diacerein (5, 10mg/kg) pretreatment on the hepatic MPO activity, MDA, GSH and nitrotyrosine levels in IR liver injury in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MPO (ng/g tissue)</th>
<th>MDA (nmol/g tissue)</th>
<th>GSH (nmol/g tissue)</th>
<th>Nitrotyrosine (ng/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>1.77 ± 0.9</td>
<td>4.56 ± 0.28</td>
<td>6.83 ± 0.63</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>IR</td>
<td>7.34 ± 0.37*</td>
<td>41.64 ± 1.76*</td>
<td>3.39 ± 0.28*</td>
<td>1.69 ± 0.13*</td>
</tr>
<tr>
<td>Diacerein-SD</td>
<td>6.92 ± 0.46$</td>
<td>37.36 ± 1.34$</td>
<td>3.98 ± 0.39*</td>
<td>1.29 ± 0.14$</td>
</tr>
<tr>
<td>Diacerein-LD</td>
<td>2.35 ± 0.19$</td>
<td>8.23 ± 0.91$</td>
<td>5.98 ± 0.47$</td>
<td>0.62 ± 0.07$</td>
</tr>
</tbody>
</table>

Data represent mean ± S.E.M.

*p<0.05 compared with sham group, *non-significant difference compared with IR group, $p<0.05$ compared with IR group.

Table 3: The effect of diacerein (5, 10mg/kg) pretreatment on hepatic caspase-3 and cytochrome-c levels in IR liver injury in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Caspase-3 (ng/g tissue)</th>
<th>Cytochrome-c (ng/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>2.45 ± 0.13</td>
<td>69 ± 1.02</td>
</tr>
<tr>
<td>IR</td>
<td>18.47 ± 0.8*</td>
<td>179 ± 2.54*</td>
</tr>
<tr>
<td>Diacerein-SD</td>
<td>17.99 ± 0.7$</td>
<td>172 ± 1.67$</td>
</tr>
<tr>
<td>Diacerein-LD</td>
<td>4.65 ± 0.8$</td>
<td>73 ± 2.35$</td>
</tr>
</tbody>
</table>

Data represent mean ± S.E.M.

*p<0.05 compared with sham group, $ non-significant difference compared with IR group, $p < 0.05$ compared with IR group.

3.5. Anti-apoptotic effect of diacerein

IR injury significantly (p < 0.05) increased the hepatic caspase-3 and cytochrome c levels in respect to sham group. Pretreatment with 10mg/kg diacerein significantly (p < 0.05) decreased hepatic levels of caspase-3 and cytochrome c in comparison with both IR group and diacerein 5mg/kg pretreated rats (Table 3).

3.6. Liver histology

By light microscopy, the hepatocytes and sinusoidal endothelial cells in sham rats exhibited no apparent degeneration or necrosis, and the central venous and periportal structures are well defined (Figure 2a). In the IR group, hepatocytes showed swelling, congestion, enlarged sinusoids (black arrows), necrosis (red arrows), inflammatory cell infiltration (blue arrow), and detectable liver structural damage (Figure 2b). Diacerein-SD group showed non-significant change in respect to IR group (Figure 2c) however, the observed liver injury was improved in the Diacerein-LD group compared with both diacerein-SD and IR groups (Figure 2d).

The liver damage scores in the IR group were significantly increased compared with the sham group (p < 0.05) and were reduced in the diacerein-LD pretreated group compared with both IR and Diacerein-SD groups (p < 0.05) (Table 4).
**Figure 2:** Representative HE stained sections photomicrographs (magnification 400x) (a) sham group: normal liver tissue structure. (b) IR group: showed severe injury with cell swelling (black arrow), disruption, degeneration and necrosis (red arrow) with aggregates of inflammatory cells (blue arrow) compared with the sham group. (c) Diacerein-SD group showed cell disruption (black arrows), inflammation (white arrows), necrosis with degenerative changes (red arrows). (d) Diacerein-LD group showed minimal features of cell injury such as cell swelling.

**Table 4:** Effect of diacerein (5, 10mg/kg) pretreatment on Suzuki score of hepatic histopathological changes in IR liver injury in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sham</th>
<th>IR</th>
<th>Diacerein-SD</th>
<th>Diacerein-LD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suzuki score</td>
<td>0.15 ± 0.08</td>
<td>4.45 ± 0.15*</td>
<td>4.01 ± 0.14</td>
<td>0.22 ± 0.13</td>
</tr>
</tbody>
</table>

Data represent mean ± S.E.M.

* p<0.05 compared sham group, ̂ non-significant difference from IR, ¥ p<0.05 compared with IR group.

### 3.7. The expression level of NF-κB-p65 in liver tissue

The expression levels of NF-κB-p65 in the liver samples of IR group were evaluated by immunohistochemical analysis. IR induced significant increase in NF-κB (p65) hepatic expression levels evidenced by intense brown staining (Figure 3B) relative to the staining seen in tissue samples from sham rats (Figure 3a). Diacerein 10 mg/kg pretreated rats had significant reductions in NF-κB-p65 expression compared to both IR and diacerein 5 mg/kg pretreated rats (Figure 3c, d, e).
Figure 3: Representative NF-κB (p65) immunohistochemistry photomicrographs of different groups (magnification 400x). (a) Sham group showed negative expression of NFκp-p65. (b) IR group showed strong positive expression (black arrows). (c) Diacerein-SD group showed strong positive staining (black arrows). (d) Diacerein-LD group showed minimal staining for protein expression. The staining when quantified. Data shown in (e) is mean ±S.E.M. * p < 0.05 compared with sham group, # non-significant difference from IR group, $ p < 0.05$ compared with IR group.

4. Discussion

IR is a common problem occurs in patients undergo liver surgery. IR insult elicits ROS generation, microvascular changes, activation of inflammatory mediators, apoptosis and necrosis with subsequent hepatocellular dysfunction [17].

The current study demonstrated that diacerein in a dose of 10mg/kg lowered the serum liver enzymes level, inhibited inflammatory cytokines production, enhanced antioxidant defense,
suppressed apoptosis, improved histological derangements and liver injury score worsened by IR injury.

IR injury disturbs the hepatocellular membrane permeability causing hepatic enzymes leakage into the circulation [18]. The current study demonstrated elevated serum ALT, AST and LDH levels which were lowered by diacerein pretreatment indicating that diacerein conserves the structural integrity of the hepatocellular membrane and contributes, in part, to ameliorate the ischemic insult.

In agreement with this finding Zhao and his colleagues (2011) reported that rhein ameliorated acetaminophen induced hepatotoxicity in rats. Treatment with both rhein and acetaminophen greatly decreased the serum ALT, AST and total bilirubin levels in respect to acetaminophen alone moreover, normalized the toxic histopathological derangements.

In the same context, Sheng et al. (2011) reported that rhein reversed steatohepatitis, and normalized ALT levels in high fat diet fed mice. On contrary, Zheng et al. (2006) reported that some osteoarthritic patients encountered mild to moderate elevation in hepatic enzymes with diacerein therapy without increase in bilirubin. The mechanism underlying this effect is not fully recognized however, idiosyncrasy is proposed [19].

IR injury triggers excessive ROS production which induces hepatocellular injury and death through DNA oxidation, fragmentation and lipids peroxidation. Free radicals stimulate cell membrane lipid peroxidation with subsequent increase in MDA levels [20]. GSH neutralizes and scavenges free-radical species. Reduction of GSH concentrations in rat hepatocytes increased their susceptibility to anoxia and oxidative damage [21].

The current results showed decreased hepatic GSH content with increased MDA level by IR injury. Fukai et al. (2005) reported that IR triggered oxidative stress that lowered GSH content and reduced the antioxidant activity. Correspondingly, the elevated MDA level reflects Kupffer cell activation and ROS generation with subsequent lipid peroxidation, disruption of membranes and cell lysis.

The current study showed that diacerein increased hepatic GSH content but decreased MDA level. Coincided with that, Refaie and El-Hussieny [22] found that diacerein reduced uterine MDA level and increased SOD activity in estradiol benzoate-induced endometrial hyperplasia and atypia in rats with improvement of estradiol benzoate induced histopathological damages.

Peroxynitrite plays an important role in pathophysiology of IR injury. Peroxynitrite, the reaction product of nitric oxide (NO) and superoxide, triggers cytotoxic processes such as lipid peroxidation, suppression of mitochondrial respiration, glutathione depletion, hang up of membrane pumps, DNA destruction and cellular energy consumption [23].

The current results revealed increased hepatic nitrotyrosine level in IR rats however, diacerein could decrease the elevated hepatic nitrotyrosine level. In agreement with that Martel-Pelletier and Pelletier (2010) reported that diacerein ameliorated doxorubicin induced nephrotoxicity through inhibiting total nitrites (NO₂⁻) producing pathway and subsequent peroxynitrite formation. Interleukin 1β is a very potent stimulator of NO. Diacerein markedly decreased interleukin 1β-induced NO production [24].

Moreover, the activated NF-κB induces the synthesis of iNOS with subsequent production of NO [25]. Inhibition of NF-κB-p65 by diacerein as detected immunoohistochemically in the current study contributes to attenuate peroxynitrite production. Under normal circumstances,
NF-κB with its inhibitory protein IκB exists in the cytoplasm. Oxidative stress breaks down IκB, giving the chance for NF-κB to translocate to the nucleus. The activated NF-κB induces iNOS, cytokines (TNF-α) and adhesion molecules [26].

NF-κB induces a proinflammatory cascade including production of IL-1β, IL-6, and TNF-α. NF-κB in turn activates KCs. ROS can induce inflammatory pathways that lead to neutrophil accumulation in the hepatocytes with upregulation of NF-κB and TNF-α formation. The current histopathological findings showed that diacerein reduced neutrophil infiltration and hepatic NF-κB-p65 expression. In harmony with our results, Alvarez-Soria et al. (2008) found that diacerein inhibited IL-1β stimulated NF-κB activation in synoviocytes and chondrocytes. Diacerein Inhibited IL-1β-induced activation of DNA binding of NF-κB and mitogen activated kinases [27].

Diacerein suppresses NO production induced by IL-1 and inhibits NO induced peroxynitrite generation, DNA fragmentation and inactivation of antioxidant enzymes [28].

MPO activity reflects tissue neutrophils infiltration. MPO converts hydroperoxides into free radicals, initiating lipid peroxidation [29].

The present study demonstrated increased hepatic MPO activity by IR injury and Diacerein reduced this activity. The ability of diacerein to reduce MPO activity was illustrated by Saleh et al. (1999) who reported that diacerein reduced MPO levels in pleural exudate in carrageenan induced pleurisy in mice. Additionally, diacerein inhibited leukocyte and neutrophil migration.

During reperfusion, the activated KCs release proinflammatory mediators (TNF-α, IL-6, IL-1, and prostaglandins) and ROS. In the present study, TNF-α, IL-1β and IL-6 hepatic levels were elevated by IR injury. Diacerein decreased TNF-α, IL-6 and IL-1β levels [30]. These results coincided with Pasin et al. (2010) who reported that diacerein decreased TNF-α and IL-1β levels in peritoneal fluid and prevents Baker’s yeast-induced fever in rats.

During IR injury, TNF-α activates several apoptotic proteins with release of Cytochrome-C to the cytosol to format the apoptosome initiating the apoptotic degradation phase [31]. The current results revealed increased hepatic cytochrome c and caspase-3 levels in IR rats which were reduced with diacerein pre-treatment. This is in agreement with Pelletier et al. (2003) who reported that diacerein reduces chondrocyte cytochrome c, caspase-3 in osteoarthritic cartilage.

On conclusion, diacerein in a dose of 10mg/kg decreased serum liver enzymes, inhibited oxidative stress, apoptosis and inflammatory cytokines production. Thus, the antioxidant, anti-inflammatory and anti-apoptotic actions exhibited by diacerein could antagonize the signaling mechanisms underlying the liver IR insults.

Acknowledgement

The author is great full to Dr. Kamal Elqashishi, Professor of pathology, Faculty of Medicine, Zagazig University for performing the histological analysis for this study.

Competing Interests

The author declares no competing interests.
References


