Erdosteine Modulates Hepatotoxic Effect on Rats Treated with Cisplatin

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ABSTRACT

Cisplatin (CP) is a widely used anticancer drug against a broad spectrum of malignance, but at high dose, it can produce undesirable effects such as hepatotoxicity. The aim of this study was to investigate the possible protective effect of erdosteine (Erd) against CP-induced biochemical, histopathological changes and hepatotoxicity associated with oxidative stress and inflammatory changes in male albino rats. Animals were randomly divided into four equal groups of 10 rats each as follows: 1-Control group: rats received distilled water, 2- CP treated group: rats injected i.p. with CP 7.5 mg/ kg body weight, single dose, 3- Erd treated group: rats received Erd by gastric tube 100 mg/kg body weight once daily for one week, 4-CP-Erd group: rats received Erd by gastric tube 100 mg/ kg body once daily for one week before i.p. administration of CP 7.5 mg / kg body weight, single dose. The results of the present study revealed that the administration of CP induced oxidative stress in liver tissues notified by a significant decrease in superoxide dismutase (SOD), catalase (CAT), and glutathione transferase (GST) and reduced glutathione (GSH) content accompanied by a significant increase of thiobarbituric acid reactive substance (TBARS) levels. Concomitant alteration of liver function was denoted by elevated serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities. The administration of CP induced also significantly increases of serum tumor necrosis factor alpha (TNF-α), and Interleukin-1β (IL-1β) levels. Histopathological investigation showed degenerative changes, dilated blood vessels, necrosis and apoptosis. Erd treatment before CP administration has significantly attenuated oxidative stress, improved liver function and decreased TNF-α and IL-1β levels compared to their respective values in the CP group. The amelioration of biochemical variations was associated with an improvement in hepatic tissues architecture. It is concluded that Erd could alleviate CP-induced biochemical and histopathological alteration that can lead to hepatotoxicity.

Keywords: Hepatotoxic, Erdosteine, Cisplatin, Inflammation, Rats.

INTRODUCTION

Cis-Diaminedichloroplatinum (II), commonly known as cisplatin has been established as a potent chemotherapeutic agent used to treat a variety of cancers such as ovarian, bladder, testicular, head and neck, and uterine cervix carcinomas (1&2). This drug has several toxic effects that interfere with its therapeutic efficiency, namely nephrotoxicity and hepatotoxicity (3), but the chief dose-limiting one is nephrotoxicity and hepatotoxicity (4).

CP induced liver injury includes functional and structural mitochondrial damage, apoptosis, and perturbation in Ca²⁺ homeostasis (5&6). The interrelated mechanisms of CP actions to induce the nephrotoxicity includes apoptosis (7), and inflammatory mechanism (8), production of TNF-α by renal
parenchymal cells (9), and generation of reactive oxygen species (ROS) such as superoxide anion, hydrogen radical, hydrogen peroxide, singlet oxygen and nitric oxide (10).

A homocysteine derivative Erdosteine (Erd) is known to have protective role in the release of free oxygen radicals beside its mucolytic and mucomodulator properties (11,12). Yildirim et al. (2003) studied the effect of oral Erd on tissue malondialdehyde (MDA), nitric oxide (NO) levels, CAT, glutathione peroxidase (GSH-PX) and SOD activities in the CP model of acute renal failure in rats and found that a single dose of CP caused kidney damage manifested by kidney histological damage as well as increase in plasma creatinine and blood urea nitrogen (BUN) levels. Treatment with Erd attenuated the biochemical variations and provided a histologically-proven protection against CP-induced acute renal failure (13). The present study aimed to study the effect of CP on liver toxicity and also evaluate the role of Erd in CP-induced experimental hepatic toxicity in male albino rats.

MATERIALS and METHODS

Animals

Adult male Swiss Albino rats (110-130 g) were obtained from the Egyptian Organization for Biological Products and Vaccines, Giza, Egypt. The animals were kept under good ventilation and illumination conditions and received standard diet and water. The animals were treated according to the treatment protocol that was approved by the animal care committee of the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt.

Chemicals and Drugs

Cisplatin 50 mg/ 50 ml solution for infusion vials and erdosteine tablets (300 mg) were purchased from Sigma-chemical Company, Cairo, Egypt.

Experimental Design

Forty rats were divided into four groups of 10 rats each. Group I (control), rats of this group received orally an equivalent volume of distilled water (vehicle of CP and Erd) during the period of Erd and CP administration. Group II (CP) received CP at a dose of 7.5 mg/kg, injected i.p. single dose (14). Group III (Erd), rats were administrated 100 mg/kg body weight once daily Erd for one week by gastric tube (15). Group IV (CP - Erd) rats were administrated 100 mg/kg body weight once daily Erd for one week by gastric tube then one hour later after last treated animals received CP at a dose of 7.5 mg/kg injected i.p., single dose. Rats were sacrificed on the 3rd day post the last dose of CP or Erd injection.

Samples Collection

The rats were sacrificed 3 days after the injection under light ether anesthesia. Blood samples from each rat were collected by retro-orbital puncture using blood capillary tubes. Serum was obtained by centrifugation of blood samples at 3000 rpm for 10 min. Liver was rapidly separated and homogenized at 3000 rpm for 15 min. using Teflon homogenizer ( MPW-302, Poland), for biochemical analysis. Tissue specimens from liver were collected and fixed in 10% buffered formalin solution followed by dehydration, clearing and embedding in paraffin. Paraffin sections of 5-micron thickness were prepared and stained routinely with haematoxylin and eosin according to Bancroft and Stevens, (1996) and examined microscopically (16).

Estimation of Biochemical Parameters

The activity of ALT, aspartate aminotransferase AST was assayed according to the method of Reitman and Frankel, (1957) (17), ALP activity was assayed depending on the method of Bowers and Mc Comb, (1966) (18). Liver TBARS concentration was determined as described by Nichans and Samuelson, (1968) (19). The activities of liver SOD, catalase CAT, and GST and GSH content were
assayed according to the methods described by Kakkar and Vishwanath, (1984) (20); Calibre, (1985) (21); Bentler and Kelly, (1963) (22); and Beutler, (1986) (23), respectively. Detection of serum TNF-α, and IL-1β were performed by ELISA technique (BioSource International, Camarillo, CA, USA) according to the manufacturer’s instructions.

**Statistical Analysis**

Data were analyzed using one way analysis of variance (ANOVA) followed by LSD as is post hoc test. The results obtained were expressed by mean ± standard deviation of the mean. Differences were considered significant at P ≤ 0.05 (23).

**RESULTS**

As presented in table 1, the activity of serum ALT, AST, and ALP and the level of TNF-α and IL-1β significantly increased in the CP treated group compared to control group. Erd- CP treated group manifested a significant decrease in the liver function and inflammatory markers compared to their relative values in the CP treated group. Rats treated with Erd showed non-significant changes compared to the values of control group.

Table (1): The activity of serum ALT, AST, ALP, and TNF-α and IL-1β levels in the different groups

<table>
<thead>
<tr>
<th>Rat Groups</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>TNF-α (pg/mL)</th>
<th>IL-1β (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21.27±1.34</td>
<td>32.77±1.07</td>
<td>70.91±1.58</td>
<td>33.48±1.45</td>
<td>13.74±1.20</td>
</tr>
<tr>
<td>Erd</td>
<td>20.47±1.21</td>
<td>31.97±1.21</td>
<td>69.40±1.775</td>
<td>32.71±1.51</td>
<td>13.02±1.41</td>
</tr>
<tr>
<td>CP</td>
<td>55.32±3.42</td>
<td>60.33±2.25</td>
<td>115.07±4.38</td>
<td>59.89±2.56</td>
<td>29.96±2.04</td>
</tr>
<tr>
<td>CP- Erd</td>
<td>33.41±2.11</td>
<td>43.28±2.37</td>
<td>86.28±2.73</td>
<td>42.56±2.32</td>
<td>19.74±1.53</td>
</tr>
</tbody>
</table>

Values are expressed as Means ± Standard Deviation (n=10).

*: Significantly different from control group. b: Significantly different from CP group

As shown in table 2, liver antioxidants SOD, CAT, and GST activities and GSH content significantly decreased while a significant increase in the concentration of lipid peroxidation markers TBARS was noticed in the CP treated group compared to the control group. The animal group treated with Erd prior to CP injection revealed a significant increase in antioxidants associated with a significant decrease in TBARS concentration compared to their respective values in the treated group. The animals treated with Erd showed non-significant changes in the level of liver antioxidants and TBARS level compared to their corresponding values in the control group.

Table (2): Liver SOD, CAT, and GST activities and GSH and TBARS contents in the different groups

<table>
<thead>
<tr>
<th>Rat Groups</th>
<th>SOD (units/mg protein)</th>
<th>CAT (units/mg protein)</th>
<th>GST (U/mg protein)</th>
<th>GSH (nmol/g tissue)</th>
<th>TBARS (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.45±1.05</td>
<td>3.34±0.98</td>
<td>0.46±0.22</td>
<td>131.49±3.10</td>
<td>1.56±0.58</td>
</tr>
<tr>
<td>Erd</td>
<td>5.67±1.14</td>
<td>3.24±1.02</td>
<td>0.43±0.17</td>
<td>128.74±4.02</td>
<td>1.44±0.69</td>
</tr>
<tr>
<td>CP</td>
<td>3.56±1.36 *</td>
<td>1.59±0.89 *</td>
<td>0.27±0.31 *</td>
<td>88.25±4.88 *</td>
<td>2.34±1.32 *</td>
</tr>
<tr>
<td>CP- Erd</td>
<td>4.85±1.21 *</td>
<td>2.68±1.21 *</td>
<td>0.38±0.26 *</td>
<td>116.29±4.28 *</td>
<td>1.65±1.04 *</td>
</tr>
</tbody>
</table>

Values are expressed as Means ± Standard Deviation (n=10).

*: Significantly different from control group. b: Significantly different from CP group
**Histopathological Findings**

In control (group 1) the hepatic cells are polygonal, vary in size, contain large, round nucleus and may occasionally be binucleate. The cells have a granular acidophilic cytoplasm (fig. 1). The liver of rats received CP (group II) showed an obvious widespread hydropic degeneration of hepatocytes owing to acute swelling which progresses to necrosis of hepatic cells through the lobule (fig. 2). Moreover some sinusoids are congested. Other cases showed necrosis and periportal leukocytic infiltration extended out from the portal tract to involve hepatic tissues (fig. 3). In Erd group, the hepatic tissues showed normal structure. On the other hand, most cases of CP and Erd group showed a normal structure of hepatic tissues and relatively well preserved architecture without degenerative changes, congested blood vessels or necrosis (fig. 4).

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**Fig. (1):** Liver of the control rat (group 1) showing a normal structure (H&E × 400).

**Fig. (2):** Liver of the CP group (group 2) showing an acute vacuolation, death of hepatic tissues around portal area (H&E × 400).

**Fig. (3):** Liver of the CP group (group 2) showing a dilated portal vein with leukocytic infiltration (H&E × 400).

**Fig. (4):** Liver of the CP and Erd group (group 4) showing a normal structure (H&E × 400).
DISCUSSION

Cisplatin is widely used, often in combination with radiation and other drugs, against malignant, solid and epithelial tumors (25). The major limitation of its use is the development of resistance by tumors (26), and the cumulative dose dependent severe hepatotoxicity and nephrotoxicity that can culminate in acute hepatic and renal failure (27&28). Despite intensive prophylactic measures, irreversible hepatic and renal damage occurs within days in approximately one-third of CP-treated patients (29&30). CP acts on cancer cells by releasing free radicals, which can make damage to liver and kidney cells. Free radicals are known to attack the highly unsaturated fatty acids of the cell membrane to induce lipid peroxidation, which is considered a key process in many pathological events and is one of the reactions induced by oxidative stress (41). Many cellular pathways have been suggested to contribute to the induction of oxidative stress and lipid peroxides. Lieber, (1997) suggested that CP induced oxidative stress synergize to produce hepatotoxicity (31). Several studies reported that CP exhibits an apoptotic effect on kidney and liver tissues (32&33).

In the current study, the administration of CP to male albino rats provoked oxidative stress in liver tissues notified by a significant decrease in SOD, CAT, and GST activities and GSH content accompanied by a significant increase of TBARS content significant increase in serum ALT, AST, and ALP activity and TNF-α and IL-1β levels compared to the control group.

Histopathological investigations of liver sections revealed an acute vaculation, death of hepatic tissues around portal area and dilated portal vein with leukocytic infiltration. The mechanisms of CP toxicity involve generation of ROS leading to oxidative stress, which is imbalance state between the formation of ROS and scavenging by antioxidants. The ROS reacts with poly unsaturated fatty acids and produce toxic aldehyde metabolites as MDA that is a principle end product of lipid peroxidation. The results verify that alterations of liver functions induced by CP are closely associated with an increase in lipid peroxidation and reactive oxygen species (ROS) in the tissues (34). Such results corroborate with results of Ashry and Elkady (35). Moreover, the results are in agreement with those previously reported by Dal Negro et al. (2008) (36), and Zhang and Lindup (1996) (37) who recorded that a single dose of CP (7.5 mg/kg) induced hepatotoxicity manifested by an increase in serum ALT and AST activities and a decrease in serum levels of NO, albumin and calcium compared to control animals. The ability of CP to cause alterations in the activity of ALT and AST could be a secondary event following CP-induced liver damage with the consequent leakage from hepatocytes (38&39&40).

The results are in agreement with the findings of Liao et al. (2008) (41) and Fasihi et al. (2012) (14) that animal treated with Erd prior to CP injection revealed a significant increase in the activity of liver SOD, CAT, GST, GSH associated with a significant decrease in TBARS concentration compared to those of the CP treated group, respectively. The antioxidant activities of Erd could be attributed to the presence of two blocked sulphydryl groups one of which, after hepatic metabolism and opening of the thiolactone ring, becomes available both for the mucolytic and free radical scavenging and antioxidant activity too. The results support that erdosteine protects the liver against free radicals induced hepatotoxicity (42) and corroborate the previous findings of Moretti and Marchieni, (2007) who reported that in acute injury induced by a variety of pharmacological or noxious agents, mediated by products of oxidative stress, Erd increases the tissue antioxidant enzyme activities such as SOD, CAT and glutathione peroxidase, and decreases the level of nitric oxide, and xanthine oxidase, which catalyze oxygen-free radical production (43).

The results obtained in the current study revealed that Erd treatment during 7 days before CP administration has significantly attenuated oxidative stress, improved liver function and decreased TNF-α and IL-1β levels compared to their relative values in the CP treated group. The biochemical amelioration was associated to improvement of the histopathological changes. The results verify that Erd may prevent inflammation by suppressing the accumulation of neutrophils, inhibition of lipid peroxidation, and chemokine production and release (44). Furthermore, the beneficial anti-inflammatory protective effects of Erd may be mediated through the inhibition of proinflammatory cytokines,
interleukin 1β (IL-1β), interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), interferon gamma (IFN-γ) and this in turn, would lead to reduced production of free radicals and subsequent damage. Yesildag et al. (2009) suggested that Erd protects against liver toxicity by inhibiting the production of ROS via the enzymatic antioxidant system. The final result is a protective effect on tissues which reduces lipid peroxidation, neutrophil infiltration or cell apoptosis mediated by noxious agents.

CONCLUSION

The present work documents that treatment with Erd offers protection from CP-induced hepatotoxicity. The deterioration of biochemical changes and histological damage in liver caused by CP are markedly improved by Erd treatment before injection of CP. These observations may be attributed to an antioxidant effect of Erd and suggest that it may be a valuable prophylactic agent against a variety of conditions and diseases.

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REFERENCES


