Protective antioxidant effects of grape seed extract in a cisplatin-induced hepatotoxicity model in rabbits

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SUMMARY

The present study was designed to investigate the protective effects of grape seed extract (GSE) on the liver oxidant/antioxidant status in cisplatin treated rabbits. For that, 18 male New Zealand rabbits were randomly allotted in 3 equal groups: in the control group, rabbits were injected with 0.9% saline whereas in the 2 other groups, they were intraperitoneally injected with cisplatin (5 mg/kg) (group CP) and in the 3rd group (group CP/GSE), they have also received GSE (250 mg/kg/day) by gavage for 6 days before and after cisplatin injection. The AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatases) and GGT (γ-glutamyl transpeptidase) activities were determined in serum samples whereas the oxidant/antioxidant status (based on measurement of tissue MDA (malondialdehyde) and GSH content as well as glutathione peroxidase and catalase activities) and histopathological changes were explored in liver. In the cisplatin group, severe histopathological alterations, mainly mononuclear infiltration, hepatocyte degeneration and necrosis, were associated to marked increases in serum activities of the liver enzymes and to tissue MDA accumulation coupled to reduction in antioxidant systems. In rabbits co-treated with GSE, liver injury was considerably attenuated, the antioxidant systems were quite preserved despite a weak and significant increase in MDA content compared to the healthy controls but serum enzyme activities were similar to the control values. These results evidence that oxidative stress is involved in cisplatin induced hepatotoxicity and that antioxidant compounds included in grape seed extract can strongly alleviate cisplatin-mediated liver injury in rabbits.

Keywords: Cisplatin, rabbit, hepatotoxicity, grape seed extract, oxidative stress, antioxidants.

RÉSUMÉ

L’objectif de cette étude a été de rechercher les effets protecteurs de l’extrait de pépins de raisins dans un modèle d’hépatotoxicité induite par le cisplatine chez le lapin.

Dans le groupe traité seulement par le cisplatine, de sévères lésions histologiques, essentiellement des infiltrations mononucléées et des images de dégénérescence hépatocytaire et de nécrose, ont été constatées et ont été associées à de fortes augmentations des activités enzymatiques sériques, à l’accumulation intrahépatique du MDA et à une réduction des systèmes antioxydants. Chez les lapins cotraités par l’extrait de pépins de raisins, les lésions hépatiques ont été nettement diminuées, les systèmes antioxydants ont globalement été bien préservés en dépit d’une faible mais significative augmentation des teneurs hépatiques en MDA par rapport aux contrôles mais les activités enzymatiques sériques étaient similaires à celles observées chez les témoins. Ces résultats démontrent l’implication du stress oxydatif dans l’hépatotoxicité du cisplatine et que des composés antioxydants contenus dans l’extrait de pépins de raisins diminuent fortement les lésions hépatiques induites par le cisplatine chez le lapin.

Mots clés : Cisplatine, lapin, hépatotoxicité, extrait de pépins de raisins, stress oxydatif, antioxydants.

Introduction

Cisplatin (cDDP, cis-diaminedichloroplatinum II) is currently one of the most important cytostatic agents in the treatment of a wide range of solid tumours, including cancers of the ovary, testis, bladder, head and neck, lung, cervix and endometrium [10, 14, 35]. The clinical side effects of cisplatin include nausea, vomiting, myelosuppression, allergic reaction, nephrotoxicity, ototoxicity, neurotoxicity and bone marrow suppression [16, 39]. Cisplatin hepatotoxicity is observed during aggressive treatment protocols in which higher doses than required for effective tumour suppression were used but liver injury is also encountered during low-dose repeated cisplatin therapy [26, 33]. Hepatotoxicity is the less well-known aspect of cisplatin treatment, and there is little information about the underlying mechanism. The major alterations reported in cisplatin toxicity [51] are the occurrence of oxidative stress linked to the accumulation of reactive oxygen species (ROS) [12] and decreased efficiency of some antioxidant defense systems including antioxidant enzymes [37] and non-enzymatic antioxidants.
such as reduced glutathione (GSH). In addition, functional and structural mitochondrial damage, apoptosis, perturbation in Ca\(^{2+}\) homeostasis [21, 30], involvement of pro-inflammatory genes such as cox-2 and inducible nitric oxide synthase (iNOS) may play some important roles in the mechanism of cisplatin hepatotoxicity [23]. Prevention of cisplatin side effects is one of the major clinical issues, and in this respect the use of free radical scavengers has been recently explored [13].

Grape seed extract is a natural plant constituent that contains lipids, proteins, carbohydrates and polyphenols. Proanthocyanidins, high molecular weight polymers comprised dimers or trimers of (+)-catechin and (−)-epicatechin [15], are the most abundant phenol compounds in grape seeds and the grape seed proanthocyanidin extract (GSPE) exhibits more powerful antioxidant effects than other well-known antioxidants such as vitamins C and E and gallic acid [4, 24]. In addition to free radical scavenging and antioxidant activity, proanthocyanidins exert vasodilatory, anticarcinogenic, anti-allergic, anti-inflammatory, antibacterial, cardioprotective, immune-stimulating, anti-viral and oestrogenic activities [49]. Particularly, they inhibit phospholipase A2, cyclooxygenase and lipoxygenase enzymes [34, 38]. The chemical proanthocyanidin properties, specifically the antioxidant activity, result from the phenol structure allowing the availability of the phenol hydrogens and singlet oxygen quenching [11, 34]. Proanthocyanidins have also been shown to inhibit lipid peroxidation, platelet aggregation, capillary permeability and fragility [24, 49]. Furthermore, proanthocyanidins were proved to strongly inhibit xanthine oxidase, highly involved in production of free radicals. They can also chelate free iron molecules, and inhibit iron-induced lipid peroxidation [15, 24].

The purpose of the present study was to evaluate the oxidative stress occurrence in cisplatin mediated hepatotoxicity and to investigate possible protective effect of grape seed extract on cisplatin-induced liver damage in rabbits.

**Material and Methods**

**CHEMICALS**

Cisplatin (50 mg/100 ml) was purchased from Ebewe Pharma (Unterach-Austria). GSPE (batch no. AV 9090940) was kindly provided by mikrogen Pharmaceuticals, (Istanbul, Turkey).

**ANIMALS AND EXPERIMENTAL PROCEDURE**

A total of 18 healthy male New Zealand white rabbits, weighing 2.5-3.0 kg, were used throughout this study. The animals purchased from the Veterinary Control and Research Institute, Elazig, Turkey, were kept under standard laboratory conditions (12 hour light/12 hour dark and 24 ± 3°C) and fed with standard commercial rabbit chow (pellet form, in the sack, Elazig Food Company). Food and water were provided ad libitum. The experimental protocol was approved by the Veterinary Control and Research Institute Ethics Committee.

The rabbits were randomly divided into 3 equal groups; the first group served as control and received a single intraperitoneal dose of 0.9% saline. Rabbits from the second group were intraperitoneally injected with a single 5 mg/kg body weight cisplatin dose. The cDDP dosage was selected on the basis of its effectiveness in inducing hepatotoxicity [31, 48]. The third group of rabbits was treated with GSE (proanthocyanidins dissolved in water) by oral gavage at the dose of 250 mg/kg body weight [24] for 6 consecutive days before and 6 consecutive days after the cisplatin injection.

Blood samples were collected from all animals by puncture of the jugular vein into sterile microtubes after cisplatin and GSE treatments. After clotting at room temperature for 1 hour, samples were centrifuged at 700g for 10 minutes at room temperature and sera were carefully harvested and stored at -20°C until analysed. Then, all rabbits were decapitated under slight ether anaesthesia and liver samples were immediately collected for biochemical and histopathological examinations.

**BIOCHEMICAL ANALYSES**

The liver tissues were homogenized in glass-glass homogenizer with a buffer containing 1.5% potassium chloride to obtain 1:10 (w/v) whole homogenates. The concentrations of malondialdehyde, reflecting the lipid peroxidation intensity [33], were directly measured in the homogenates using the thiobarbituric-acid reaction described by PLACER et al. [32] and values were expressed as nmol/g tissue. After homogenate centrifugation (2800g, 20 minutes, 4°C), the glutathione concentrations and the activities of catalase and glutathione peroxidase were immediately determined in the supernatants. The glutathione contents in liver were measured at 412 nm using the method of SEDLAK and LINDSAY [42] and expressed as nmol/g of liver tissue. The glutathione peroxidase activity was determined according to the method of LAWRENCE and BURK [25] and expressed as U/g protein for liver tissue. The liver tissue catalase activity was determined by measuring the decomposition of hydrogen peroxide at 240 nm, according to the method of AEI [1] and expressed as katal/g protein. In parallel, the protein concentration was also measured in the supernatants by the method of LOWRY et al. [27].

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatases (ALP) and γ-glutamyl transferase (GGT) activities were measured using auto analyzer (Olympus AU 600, Japan).

**HISTOPATHOLOGICAL EXAMINATION**

At the end of the experiment, necropsy of the rabbits was performed and liver tissue samples were fixed in 10% buffered neutral formalin. Paraform-embedded blocks were routinely processed and 5 μm thick sections were stained with haematoxylin-eosin and examined under an optical microscope (Olympus BX51) and 10 microscopic fields were examined at 20 X magnification regarding perportal cell infiltration, necrosis, sinusoidal congestion, acute cell swelling, hepatic cord disorganisation, hepatic steatosis and capsule fibrosis. Every fields were evaluated as severe (+++), moderate (++), mild (+) and none (-).
ANTIOXIDANT EFFECTS OF GRAPE SEED EXTRACT IN CISPLATINE-INDUCED HEPATOTOXICITY IN RABBITS

STATISTICAL ANALYSIS

Data are presented as mean ± standard error of means (SEM). One-way analysis of variance and post hoc Duncan’s test were used to determine the differences between groups in terms of all studied parameters using the SPSS/PC computer program (version 12.0; SPSS, Chicago, IL, USA). The Kruskal Wallis test was used for the comparison between the control and experimental groups for histopathological examination. Differences were considered as significant when P value was less than 0.05.

Results

EFFECTS OF CISPLATIN AND GSE TREATMENT ON THE SERUM ACTIVITIES OF LIVER ENZYMES

As reported in Table I, the serum AST, ALT, ALP and GGT activities were dramatically increased in rabbits from the cisplatin group compared to the not treated controls (P < 0.001). By contrast, when animals received the oral GSE treatment, the enzyme activities remained similar to the control values.

EFFECTS OF CISPLATIN AND GSE TREATMENT ON THE LIVER OXIDANT/ANTIOXIDANT EQUILIBRIUM

The antioxidant/oxidant balance in liver from rabbits treated with cisplatin alone or coupled to grape seed extract was summarized in Table II. A marked increase in MDA content (P < 0.001) was observed in the cisplatin group and was associated to a severe drop in the reduced glutathione content (P < 0.05) as well as to significant reductions of the antioxidant glutathione peroxidase (P < 0.001) and catalase (P < 0.01) activities compared to the negative control group. When rabbits were additionally administered with grape seed extract, the global antioxidant status appeared to be preserved: although the glutathione peroxidase activity was weakly but significantly depressed compared to the healthy controls (P < 0.001), the catalase activity was unchanged and the mean liver glutathione amount was significantly increased (P < 0.05). Furthermore, it was found that the mean MDA content in liver from GSE treated rabbits (CP/GSE group) was dramatically lowered compared to the animals treated with cisplatin alone although it was remained significantly higher than in the healthy controls (P < 0.001).

EFFECTS OF CISPLATIN AND GSE TREATMENT ON LIVER HISTOPATHOLOGY

The histopathological changes observed in the liver are summarized in Table III. Whereas no lesion was found in the healthy controls (figure 1C), many alterations were observed in liver from rabbits only treated with cisplatin. Among them, the most important alteration was numerous mononuclear cell infiltrations (figure 1A) leading to the hepatic cord disorganization. In addition, Kupffer cell activations in periportal areas coupled to cell hypertrophy, steatosis and acute cell bloating as well as necrotic areas were also frequently observed.

Congested sinusoids and capsule fibrosis were also often seen in this group. However, in rabbits co-treated with cisplatin

<table>
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<tr>
<th>Control</th>
<th>CP group</th>
<th>CP/GSE group</th>
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<tr>
<td>AST (U/L)</td>
<td>23.60 ± 1.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.40 ± 2.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.80 ± 2.05&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>ALT (U/L)</td>
<td>20.20 ± 0.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.80 ± 1.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.40 ± 0.50&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>ALP (U/L)</td>
<td>62.60 ± 2.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>125.00 ± 13.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.00 ± 5.36&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>GGT (U/L)</td>
<td>2.20 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.20 ± 0.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.60 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
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CP: Cisplatin; GSE: Grape seed extract; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatases; GGT: γ-glutamyl transferase.
Different superscripts <sup>a,b</sup> in the same row indicate significant difference (P < 0.001) between groups.

<table>
<thead>
<tr>
<th>Control</th>
<th>CP group</th>
<th>CP/GSE group</th>
<th>P</th>
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<tr>
<td>MDA (nmol/g tissue)</td>
<td>7.41 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.46 ± 0.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.80 ± 0.39&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>GSH (nmol/g tissue)</td>
<td>2.47 ± 0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.94 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.65 ± 0.31&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>GPx (U/g proteins)</td>
<td>27.98 ± 1.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.16 ± 0.70&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.70 ± 0.46&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CAT (katal/g proteins)</td>
<td>365.8 ± 27.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>277.4 ± 5.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>421.8 ± 6.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
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CP: Cisplatin; GSE: Grape seed extract; MDA: malondialdehyde; GSH: reduced glutathione; GPx: glutathione peroxidase; CAT: catalase.
Different superscripts <sup>a,b,c</sup> in the same row indicate significant difference (P < 0.05 or more) between groups.

Table I: Effects of cisplatin (5 mg/kg, intraperitoneally) and grape seed extract (GSE, per os, 250 mg/kg for 6 days before and 6 days after cisplatin injection) treatments on serum activities of liver enzymes in rabbits. Results are expressed as mean ± standard error of the mean (SEM).

Table II: Effects of cisplatin (5 mg/kg, intraperitoneally) and grape seed extract (GSE, per os, 250 mg/kg for 6 days before and 6 days after cisplatin injection) treatments on antioxidant / oxidant equilibrium in liver homogenates in rabbits. Results are expressed as mean ± standard error of the mean (SEM).
and grape seed extract, the liver histological alterations were less intense compared to cisplatin-treated rabbits (figure 1B); although the intensity of the sinusoid congestion and acute hepatocyte degeneration remained similar, hepatic cord disorganization and periportal infiltrations were significantly less marked (P < 0.001), necrotic areas were more scarcely seen through the parenchyma (P < 0.001) and capsule fibrosis was nearly absent (P < 0.01). Surprisingly, a high degree of liver steatosis was reported in this group and was significantly higher than in the cisplatin group (P < 0.001).

### Discussion

Anticarcinogenic drugs used extensively against various cancer types during treatment largely interfere with physiological homeostasis of some organs. Cisplatin, one of the most efficient anticarcinogens, was reported to cause toxicity in such organs as livers, hearts and kidneys [16, 46, 47]. In the present study, the application of single 5 mg/kg dose of cisplatin was determined to cause liver toxicity and this fact is supported with biochemical and histopathological findings (strong increases in serum activities of liver AST, ALT, ALP and GGT enzymes and important mononuclear infiltration in periportal areas coupled to necrotic lesions and hepatocyte degeneration, respectively). Cisplatin-induced liver toxicity is characterized by mild or moderate rise in transaminases [17]. Transaminases are the most sensitive biomarkers that reflect cellular damage and toxicity because they are localized mainly in the cytoplasm and are released after cellular damages [36, 44]. In this way, it was reported that serum AST and ALT activities change in liver diseases and myocardial infarct [47]. MANSOUR et al. [28] reported that cisplatin treatment in rats caused significant changes in circulating AST and ALT activities due to hepatocyte damage. KART et al. [19] determined in a similar study conducted on rabbits that AST and ALT activities increased in the group treated with cisplatin. Moreover, the cisplatin hepatotoxicity was obviously evidenced by histopathological examination which revealed severe mononuclear infiltration in periportal areas coupled to the Kupffer cell activations and acute hepatocyte degeneration associated to necrotic changes.

On the other hand, it was argued that oxidative stress and reactive oxygen types contributed to the formation of cisplatin-induced liver toxicity [35]. PARTIBHA et al. [33] reported that extensive usage of chemotherapy considerably reduced antioxidant enzymes but increased the amount of free radicals. In some studies, it was argued that decreases in GSH content and in catalase, glutathione peroxidase and glutathione transferase activities coupled to an increase in MDA concentrations are the criteria for determining tissue damage and oxidative stress occurrence [2, 29, 43]. It is already known that cisplatin enables the formation of free oxygen radicals such as hydrogen peroxide, superoxide anions and hydroxyl radicals [9, 16, 18, 49]. Hydroxyl radical, in turn, by isolating a hydrogen atom from membrane lipids allows starting of lipid peroxidation [40].

MDA is the best indicator for lipid peroxidation. An increase in the liver MDA content may lead to tissue damage [33]. In the present study, it was determined that MDA significantly accumulated in liver from cisplatin-treated rabbits compared to healthy controls. In agreement, it was previously reported that MDA content increased in the liver of rabbits experimentally intoxicated with cisplatin [19]. In the same way, SAYED [41] demonstrated MDA accumulation in kidneys from rats experimentally intoxicated with cisplatin.

GSH is one of the compounds required for various cell functions, leading to preservation of cellular integrity and cell protection against free radicals and inherent oxidative damage [6]. GSH, an essential part of the non-enzymatic antioxidant system, plays an important role in the elimination of cisplatin [16]. There are numerous studies which document that cisplatin application decreases the tissue GSH content [19, 36, 41]. In the present study, cisplatin significantly decreased liver GSH content compared to the control group. PARTIBHA et al. [33] reported that cisplatin application leads to an increase in the MDA amount in rat livers and a decrease in the GSH content. It was argued that GSH consumption increased lipid peroxidation and excessive lipid peroxidation in turn accelerated GSH consumption [20].

Glutathione peroxidase (GSH-Px) is a GSH-dependent endogenous antioxidant enzyme which disrupts the lipid peroxidation chain by reducing hydrogen peroxide and lipid peroxides into alcohols and therefore, plays an important role in the preservation of cellular and subcellular cell membranes against lipid peroxidation [20]. It was reported that the

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Table III: Effects of cisplatin (5 mg/kg, intraperitoneally) and grape seed extract (GSE, per os, 250 mg/kg for 6 days before and 6 days after cisplatin injection) treatments on liver histology in rabbits. Results are expressed as mean ± standard error of the mean (SEM).

<table>
<thead>
<tr>
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<th>Control</th>
<th>CP group</th>
<th>CP/GSE group</th>
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<tr>
<td>Periportal cell infiltration</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.00 ± 0.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.33 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.001</td>
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<tr>
<td>Necrosis</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.17 ± 0.31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.17 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.001</td>
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<td>Sinusoidal congestion</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.17 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.001</td>
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<tr>
<td>Acute cell swelling</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.50 ± 0.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00 ± 0.45&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>&lt; 0.05</td>
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<td>Hepatic cord disorganisation</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.33 ± 0.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.83 ± 0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.001</td>
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<td>Hepatic steatosis</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.17 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.17 ± 0.31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt; 0.001</td>
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<tr>
<td>Capsular fibrosis</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.17 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt; 0.01</td>
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CP: Cisplatin; GSE: Grape seed extract. Different superscripts<sup>a,b,c</sup> in the same row indicate significant difference (P < 0.05 or more) between groups.
decrease in GSH-Px activities in the cisplatin-induced damages are due to the fact that cisplatin reduces substrate usage. In accordance, the liver glutathione peroxidase activity was found to be markedly lowered in cisplatin treated rabbits in the current study. It is thought that the decrease in the enzyme activity in the cisplatin group may be related to decline in the liver GSH content.

Catalase (CAT) is an enzyme that turns hydrogen peroxide into water and molecular oxygen [45]. In the present study, a significant decrease in the catalase activity was observed in the cisplatin group as previously reported [50].

Although the mechanism of cisplatin-induced toxicity is not entirely elucidated, the occurrence of an oxidative stress is highly suspected leading to investigations of the protective effects of various antioxidants against the side effects of cisplatin [3, 17]. Various agents, including flavonoids found especially in fruits and vegetables, were claimed to prevent or reduce the side effects of most of the chemotherapeutic agents. Flavonoids have antioxidant, free radical repellent, chelating properties and may modify various enzyme activities [22]. For example, grape seed extract contains strong free radical repellent phenols such as procyanidin and proanthocyanidin [8].

Rabbit co-treatment with grape seed extract has significantly alleviated hepatotoxicity in the present study since histopathological changes were markedly less pronounced compared to animals treated with cisplatin alone and serum activities of liver enzymes (AST, ALT, ALP and GGT) were similar to the control values. BAGCHI et al. [7] have also reported that grape seed proanthocyanidin extract (GSPE) treatment significantly lessened the cisplatin-induced liver damage. Furthermore, although significant compared to the healthy controls, the MDA accumulation in liver was remarkably attenuated in animals receiving grape seed extract whereas the liver glutathione content and the catalase activity were preserved, even slightly increased, and the glutathione activity remained unaltered compared to the not treated rabbits. These results were in agreement with the preventive effects of proanthocyanidin application on MDA accumulation in kidneys in models of cisplatin induced nephrotoxicity in rats [36, 41]. It was also previously reported that the glutathione peroxidase activities were similar in cisplatin exposed rats treated with grape seed extract and in healthy controls [5, 36, 50]. Similar beneficial effects of grape seed extract on catalase activity were also reported [50]. Moreover, the most important liver steatosis observed in rabbits co-treated with cisplatin and grape seed extract would be linked to a reduced lipid peroxidation and utilisation.

To conclude, cisplatin which is a chemotherapeutic drug used in the treatment of various cancer types was determined to cause liver toxicity and to weaken the antioxidant defence systems in liver tissue and to increase oxidative stress. It was also determined that grape seed extract co-treatment was markedly efficient in reducing ROS accumulation and strengthening endogenous antioxidant systems leading to a considerable attenuation of the hepatotoxicity as evidenced by histopathological and biochemical examinations. It was concluded that GSE application can be considered as a supportive adjuvant therapy able to reduce or prevent the hepatic effects of chemotherapeutic drugs such as cisplatin.

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