Chrysin abrogates cisplatin-induced oxidative stress, p53 expression, goblet cell disintegration and apoptotic responses in the jejunum of Wistar rats

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(Submitted 7 September 2011 – Final revision received 9 November 2011 – Accepted 10 November 2011 – First published online 6 February 2012)

Abstract

Cisplatin (cis-diaminedichloroplatinum (II) (CDDP)) is a commonly used chemotherapeutic drug for the treatment of numerous forms of cancer, but it has pronounced adverse effects, namely nephrotoxicity, ototoxicity, neurotoxicity, hepatotoxicity, diarrhoea and nausea. CDDP-induced emesis and diarrhoea are also marked toxicities that may be due to intestinal injury. Chrysin (5,7-dihydroxyflavone), a natural flavone commonly found in many plants, possesses multiple biological activities, such as antioxidant and anti-inflammatory properties. In the present study, we investigated the protective effect of chrysin against CDDP-induced jejunal toxicity. The plausible mechanism of CDDP-induced jejunal toxicity includes oxidative stress, p53 and apoptosis via up-regulating the expression of caspase-6 and -3. Chrysin was administered to Wistar rats orally in maize oil. A single intraperitoneal injection of CDDP was given and the animals were killed after 24 h of CDDP injection. Chrysin ameliorated CDDP-induced lipid peroxidation, increase in xanthine oxidase activity, glutathione depletion, decrease in antioxidant (catalase, glutathione reductase, glutathione peroxidase and glucose-6-phosphate dehydrogenase) and phase-II detoxifying (glutathione-S-transferase and quinone reductase) enzyme activities. Chrysin attenuated CDDP-induced goblet cell disintegration, enhanced expression of p53 and apoptotic tissue damage. Histological findings further substantiated the protective effects of chrysin against CDDP-induced damage in the jejenum. The results of the present study demonstrate that oxidative stress and apoptosis are closely associated with CDDP-induced toxicity and chrysin shows the protective efficacy against CDDP-induced jejunal toxicity possibly via attenuating the oxidative stress and apoptotic tissue damage.

Key words: Cisplatin: Jejunum toxicity: Oxidative stress: p53: Caspases: Goblet cells

Cisplatin (cis-diaminedichloroplatinum (II) (CDDP); Fig. 1) is a commonly used chemotherapeutic drug for the treatment of various forms of cancer1–3. The chemotherapeutic efficacy of CDDP is increased by increasing the dose, but it is usually accompanied by severe adverse effects including nephrotoxicity, ototoxicity, neurotoxicity, hepatotoxicity, nausea and emesis, with 67% of patients experiencing diarrhoea4–8. The cytotoxic effects of anti-neoplastic drugs are not specific in action against tumour cells but also damage normal rapidly proliferating cells, namely intestinal epithelial cells9. The exact mechanism of CDDP toxicity is not fully understood, but the plausible mechanism may involve oxidative stress10 which is due to the devastating production of reactive oxygen species (ROS), e.g. the superoxide anion (O2·−), H2O2, hydroxyl radical (OH), etc. by CDDP11, and consequently these ROS may further interact with DNA, lipids and proteins12. CDDP can act on the sulphhydril (―SH) groups of cellular proteins13, but DNA is the main cellular target of CDDP that may lead to DNA damage induced by ROS and platinum–DNA (Pt–DNA) adduct formation, thus hampering the cell division or DNA synthesis and its repair mechanism which leads to apoptotic cell death14,15.

Increasing amounts of evidence suggest that the natural compounds with antioxidant properties subside CDDP toxicity16–20. Therefore, chemotherapy treatment with compounds having antioxidant properties may augment the efficiency of antineoplastic drugs and also may decrease the systemic toxicity induced by chemotherapy21. There is
Chrysin modulates cisplatin toxicity

also no report or finding to date that antioxidants impede conventional cancer therapeutics in vivo; moreover, antioxidants and chemotherapy may augment the efficacy of the treatment\(^\text{11}\). Thus, there is a need to explore the natural compound that can effectively diminish the CDDP-induced toxicity to improve its chemotherapeutic efficacy via decreasing the chemoresistance and increasing the chemosensitisation of CDDP. Flavonoids are naturally occurring polyphenols that possess various pharmacological properties and therapeutic applications. This is attributed possibly due to the phenolic structures which have antioxidant and free-radical-scavenging properties\(^\text{22}\).

Chrysin (5,7-dihydroxyflavone; Fig. 2) belongs to this category which is found in high amounts in honey and bee propolis and is also present in various plants\(^\text{23}\). It has antioxidant, anti-inflammatory, antiviral and anti-cancer properties\(^\text{23}\). Chrysin has very low oral bioavailability and after recommended oral doses of 400 mg, there were only trace amounts present in plasma, equivalent to an approximate bioavailability of 0.003–0.02 %\(^\text{24–26}\). Chrysin enhances the level of testosterone by inhibiting the aromatase enzyme which converts testosterone into oestradiol and is already available on the market as a dietary supplement in the form of capsules (500 mg/capsule; iHerb, Inc. and VitaDigest), with six capsules/d as the highest suggested dose\(^\text{25}\). The potential side effect of chrysin has not been well studied, but it has cytotoxic effects on normal trout liver cells\(^\text{27}\). These insights into chrysin may help in reducing the CDDP toxicity which may lead to improving the chemotherapeutic efficacy of CDDP.

Based on this information, the present study was intended to explore the anticipatory effects of chrysin against CDDP-induced jejunal toxicity. The aim of this study was hence to examine the prophylactic effects of chrysin against CDDP-induced oxidative stress, p53 expression, goblet cell disintegration and apoptotic responses in the jejunum of Wistar rats.

### Experimental methods

#### Chemicals

Reduced glutathione (GSH), oxidised glutathione, NADPH, NADP+, FAD, EDTA, thiobarbituric acid, pyrogallol, poly-i-lysine, xanthine, glucose-6-phosphate, bovine serum albumin, Mayer’s haematoxylin, dichlorophenolindophenol, 5,5'-dithio-bis-(2-nitrobenzoic acid), chrysin, 1-chloro-2,4-dinitrobenzene and glutathione reductase (GR) were obtained from Sigma (Sigma Chemical Company). CDDP was purchased from Dr Reddy’s. H\(_2\)O\(_2\), magnesium chloride, sulphosalicylic acid, perchloric acid, TCA, Tween-20, Folin-Ciocalteau reagent, potassium tartarate, di-sodium hydrogen phosphate, sodium di-hydrogen phosphate and sodium hydroxide were purchased from E. Merck Limited. All other chemicals and reagents were of the highest-purity grade commercially available.

#### Animals

For the experimental study, 4- to 6-week-old male albino rats (120–150 g) of the Wistar strain were obtained from the Central Animal House of Hamdard University, New Delhi, India. All procedures for using experimental animals were checked and permitted by the ‘Institutional Animal Ethical Committee’ that is fully accredited by the Committee for Purpose of Control and Supervision on Experiments on Animals Chennai, India. Approval ID/project number for this study is 740. The animals were housed in polypropylene cages in groups of four rats per cage and were kept in a room maintained at 25 ± 2°C with a 12 h light–12 h dark cycle. They were allowed to acclimatise for 1 week before the experiments and were given free access to standard laboratory animal diet and water ad libitum.

#### Treatment regimen

To study the effect of prophylactic treatment with chrysin on CDDP-induced oxidative stress and apoptotic responses in the jejunum, thirty male Wistar rats were randomly allocated to five groups of six rats each. The rats of Group I (control...
post-mitochondrial supernatant preparation and estimation of different parameters

Jejunums were removed quickly, cleaned free of irrelevant material and immediately perfused with ice-cold saline (0.85% NaCl). The jejunos (10% w/v) were homogenised in chilled phosphate buffer (0.1 M, pH 7.4) using a Potter Elvehjem homogeniser. The homogenate was filtered through muslin cloth, and centrifuged at 3000 rpm for 10 min at 4°C in a Remi Cooling Centrifuge (C-24 DL) to separate the nuclear...
debris. The aliquot so obtained was centrifuged at 12 000 rpm for 20 min at 4°C to obtain post-mitochondrial supernatant (PMS), which was used as a source of various enzymes.

Measurement of lipid peroxidation
The assay for membrane lipid peroxidation (LPO) was done by the method of Wright et al.\(^{280}\) with some modifications. The reaction mixture in a total volume of 3.0 ml consisted of 1.0 ml tissue homogenate, 0.05 ml TRIS-HCl (0.1 M, pH 7.6), 0.05 ml EDTA (0.5 mM), 0.05 mM MgCl\(_2\), 0.1 ml NADPH (0.1 mM) and 0.1 ml of 10% PMS in a total volume of 2.0 ml. The enzyme activity was assessed at 25°C by measuring the disappearance of NADPH at 340 nm and was calculated as nmol NADPH oxidised/min per mg protein using a molar extinction coefficient of 6.22 \(\times 10^3/M\) per cm.

Measurement of xanthine oxidase activity
The assay mixture consisted of 2.0 ml Tris–HCl buffer (0.05 M, pH 7.6), 0.05 ml GR (1 IU/ml), 0.05 ml GSH (1 mM), 0.1 ml NADPH (0.1 mM) and 0.01 ml H\(_2\)O\(_2\) (0.25 mM) and 0.1% 1-chloro-2,4-dinitrobenzene (10 mM) and 0.2 ml of cytosolic fraction in a total volume of 3.0 ml. The changes in absorbance were recorded at 340 nm and the enzyme activity was calculated as \(\mu\)mol 1-chloro-2,4-dinitrobenzene conjugated/minute/mg protein using a molar extinction coefficient of 13.6 \(\times 10^3/M\) per cm.

Measurement of glucose-6-phosphate dehydrogenase activity
The activity of glucose-6-phosphate dehydrogenase (G6PD) was determined by the method of Zaheer et al.\(^{34}\). The reaction mixture consisted of 0.3 ml Tris–HCl buffer (0.05 M, pH 7.6), 0.1 ml NADP (0.1 mM), 0.1 ml glucose-6-phosphate (0.8 mM), 0.1 ml MgCl\(_2\) (8 mM), 0.3 ml PMS (10%) and 2.1 ml distilled water in a total volume of 3 ml. The changes in absorbance were recorded at 340 nm and the enzyme activity was calculated as nmol NADPH oxidised/min per mg protein using a molar extinction coefficient of 6.22 \(\times 10^3/M\) per cm.

Measurement of glutathione-S-transferase activity
The glutathione-S-transferase (GST) activity was measured by the method of Habig et al.\(^{52}\). The reaction mixture consisted of 2.4 ml phosphate buffer (0.1 M, pH 7.5), 0.2 ml GSH (1.0 mM), 0.2 ml 1-chloro-2,4-dinitrobenzene (1.0 mM) and 0.2 ml of cytosolic fraction in a total volume of 3.0 ml. The changes in absorbance were recorded at 340 nm and the enzyme activity was calculated as \(\mu\)mol 1-chloro-2,4-dinitrobenzene conjugated/formed/minute/mg protein using a molar extinction coefficient of 9.6 \(\times 10^3/M\) per cm.

Measurement of superoxide dismutase activity
The superoxide dismutase (SOD) activity was measured by the method of Marklund & Marklund\(^{55}\). The reaction mixture consisted of 2.875 ml Tris–HCl buffer (50 m\(_7\), pH 8.2), pyrogallol (24 m\(_7\) in 10 m\(_7\) HCl) and 100 \(\mu\)l PMS in a total volume of 3 ml. The enzyme activity was measured at 420 nm and was expressed as units/mg protein. Here, one unit of enzyme is defined as the enzyme activity that inhibits the auto-oxidation of pyrogallol by 50%.

Measurement of catalase activity
The catalase (CAT) activity was measured by the method of Claiborne\(^{36}\). In brief, the assay mixture consisted of 2.0 ml phosphate buffer (0.1 M, pH 7.5), 0.95 ml H\(_2\)O\(_2\) (0.019 M) and 0.05 ml of PMS (10%) in a final volume of 3.0 ml. Changes in absorbance were recorded at 240 nm. The CAT activity was calculated in terms of mmol H\(_2\)O\(_2\) consumed/minute/mg protein.
Measurement of quinone reductase activity

The quinone reductase (QR) activity was determined by the method of Benson et al. (37). The 3 ml reaction mixture consisted of 2.13 ml Tris-HCl buffer (25 mM, pH 7.4), 0.7 ml bovine serum albumin, 0.1 ml FAD, 0.02 ml NADPH (0.1 m) and 50 μl PMS (10%). The reduction of dichlorophenolindophenol was recorded colorimetrically at 600 nm and the enzyme activity was calculated as μmol of dichlorophenolindophenol reduced/min per mg protein using a molar extinction coefficient of 2.1 × 10^4 M cm^-1.

Immunohistochemical staining for detection of p53

Sections of 4 μm were cut from formalin-fixed, paraffin-embedded tissue blocks and mounted on poly-l-lysine-coated microscopic slides. The paraffinised sections were dewaxed in xylene and rehydrated through graded series of ethanol to water followed by antigen retrieval in sodium citrate buffer (10 mM, pH 6.0). The sections were then allowed to cool for 15 min and washed three times with Tris-buffered saline (TBS) for 5 min each. The slides were then incubated in 3% H2O2 in methanol for 10 min to reduce the endogenous peroxidase activity and then subjected to power block (UltraVision Plus Detection System; Thermo Scientific) for 10 min to block non-specific binding. After rinsing the sections in TBS, the slides were incubated overnight at 4°C with primary antibody inside a humidified chamber and then were washed in TBS. The sections were incubated with biotinylated goat anti-polyvalent secondary antibody (UltraVision Plus Detection System) for 20 min and then were rinsed in TBS. The sections were again incubated with streptavidin peroxidase plus (UltraVision Plus Detection System) for 30 min. Following this, the sections were washed in TBS and developed with 3,3’-diaminobenzidine solution (UltraVision Plus Detection System) until they became brown. The sections were then counterstained with Mayer’s haematoxylin, mounted by using mounting media and then visualised under a light microscope (Olympus BX51). The primary antibody used was rabbit anti-p53 (dilution 1:100, Santa Cruz).

Measurement of caspase-6 and -3 activities

Caspase-6 and -3 activities were measured with an ‘Invitrogen Caspase Colorimetric Protease Assay Sampler Kit’ (supplied with VEID (for caspase-6) and DEVD (for caspase-3) substrates) according to the manufacturer’s instructions.

Staining for goblet cells analysis

The jejunal sections of 4 μm were cut from formalin-fixed, paraffin-embedded tissue blocks and mounted on poly-l-lysine-coated microscopic slides. The paraffinised sections were dewaxed in xylene and rehydrated through graded series of ethanol to water. The sections were stained with 1% Alcian blue (pH 2.5) in 3% acetic acid solution for 30 min and then rinsed for 1 min in 3% acetic acid solution to prevent non-specific staining. The slides were then washed in distilled water and the sections were counterstained with neutral red (0.5% aqueous solution) for 20 s, dehydrated in alcohol and mounted by using mounting media. Following this, the slides were evaluated under the light microscope (Olympus BX51).

Histology

The jejunum was excised, flushed with saline, cut open longitudinally along the main axis, and then again washed with saline. These jejunal sections were fixed in 10% buffered formalin for at least 24 h and after fixation, the specimens were dehydrated in ascending grades of ethanol, cleared in benzene, and embedded in paraffin wax. Blocks were made and 5 μm-thick sections were cut from the jejunum. The paraffin-embedded jejunal tissue sections were deparaffinised using xylene and ethanol. The slides were washed with PBS and permeabilised with permeabilisation solution (0.1% Triton X-100). These sections were then stained with haematoxylin and eosin and were observed under a light microscope at 40X magnification to investigate the histoarchitecture of jejunal mucosa.

Measurement of protein

The protein concentration in all samples was determined by the method of Lowry et al. (38) using bovine serum albumin as the standard.

Statistical analysis

The data from individual groups were presented as the means and standard deviations. Differences between groups were analysed using ANOVA followed by Dunnett’s multiple comparison test and the minimum criterion for statistical significance was set at P<0.05 for all comparisons.

Results

Effect of prophylactic treatment of chrysin against cisplatin-induced lipid peroxidation

The level of MDA was significantly enhanced (P<0.01) in Group II as compared to Group I. Chrysin pretreatment significantly decreased the level of MDA in Group III (P<0.05) and Group IV (P<0.01), respectively, as compared to Group II. No significant difference was found in the level of MDA between Group I and Group V (Fig. 2(a)).

Effect of chrysin pretreatment and cisplatin on the xanthine oxidase activity in jejunum

The activity of XO was significantly increased (P<0.001) in Group II as compared to Group I. Chrysin pretreatment significantly decreased the activity of XO in Group III (P<0.001) and Group IV (P<0.001) as compared to Group II. Group V exhibited no significant change in the activity of XO as compared to Group I (Fig. 2(b)).
**Effect of prophylactic treatment of chrysin against cisplatin-induced reduced glutathione depletion in the jejunum**

The level of GSH was depleted significantly (P<0.001) in the CDDP-treated group (Group II) as compared to the control group (Group I). Chrysin pretreatment showed a significant increase in the level of GSH in Group III (P<0.05) and Group IV (P<0.05) when compared with Group II. No significant difference was found in the level of GSH between Group I and Group V (Fig. 2(c)).

**Effect of chrysin supplementation and cisplatin on the activities of glutathione-dependent enzymes in the jejunum**

CDDP treatment caused a significant decrease in the activities of GPx (P<0.001), GST (P<0.001), GR (P<0.001) and G6PD (P<0.001) in Group II as compared to Group I. Chrysin supplementation at the dose of 25 mg/kg b.wt. significantly increased the activity of GST only (P<0.05) but not other enzymes in Group III as compared to Group II. But the higher dose of chrysin (50 mg/kg b.wt.) also showed significant increase in the activities of CAT (P<0.05), QR (P<0.01) and SOD (P<0.001) in Group IV as compared to Group II. The higher dose of chrysin (50 mg/kg b.wt.) also showed significant increase in the activities of CAT (P<0.05), QR (P<0.001) and SOD (P<0.001) in Group IV as compared to Group II. However, the activities of these enzymes in Group V did not change significantly as compared to Group I (Table 1).

**Effect of chrysin pretreatment and cisplatin on the expression of p53 in the jejunum**

The jejunal sections of the CDDP-treated group (Group II) have more p53 immunopositive staining (arrows) as indicated by brown colour as compared to the control group (Group I), while pretreatment with chrysin in Groups III and IV reduced p53 immunostaining as compared to Group II. However, there were no significant differences in the immunostaining in Group V as compared to Group I. For immunohistochemical analyses, brown colour indicates specific immunostaining of p53 and light-blue colour indicates haematoxylin staining. Original magnification, 40 x (Fig. 3).

**Table 1.** Effects of chrysin and cisplatin (CDDP) on the activities of glutathione peroxidase (GPx), glutathione-S-transferase (GST) and glutathione reductase (GR) in rat jejunum

(Mean values and standard deviations)

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>GPx Mean</th>
<th>SD</th>
<th>GST Mean</th>
<th>SD</th>
<th>GR Mean</th>
<th>SD</th>
<th>G6PD Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (vehicle-treated control)</td>
<td>1.89</td>
<td>0.14</td>
<td>1.46</td>
<td>0.23</td>
<td>793.96</td>
<td>115.17</td>
<td>208.44</td>
<td>30.69</td>
</tr>
<tr>
<td>II (CDDP only)</td>
<td>1.13***</td>
<td>0.12</td>
<td>0.54***</td>
<td>0.19</td>
<td>355.2***</td>
<td>84.22</td>
<td>102.93***</td>
<td>18.88</td>
</tr>
<tr>
<td>III (CDDP + chrysin D1)</td>
<td>1.22</td>
<td>0.15</td>
<td>0.94†</td>
<td>0.14</td>
<td>435.45</td>
<td>40.07</td>
<td>131.94</td>
<td>21.24</td>
</tr>
<tr>
<td>IV (CDDP + chrysin D2)</td>
<td>1.44††</td>
<td>0.09</td>
<td>1.00†</td>
<td>0.11</td>
<td>676.41††</td>
<td>55.66</td>
<td>197.23††</td>
<td>23.36</td>
</tr>
<tr>
<td>V (chrysin D2 only)</td>
<td>1.85</td>
<td>0.15</td>
<td>1.33</td>
<td>0.37</td>
<td>785.91</td>
<td>54.69</td>
<td>200.94</td>
<td>42.06</td>
</tr>
</tbody>
</table>

G6PD, glucose-6-phosphate dehydrogenase.
*** Mean value was significantly different from that of Group I (P<0.001).
Mean value was significantly different from that of Group II: † P<0.05, †† P<0.01, ††† P<0.001.

**Table 2.** Effects of chrysin and cisplatin (CDDP) on the activities of catalase (CAT), glucose-6-phosphate dehydrogenase and quinone reductase (QR) in rat jejunum

(Mean values and standard deviations)

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>CAT Mean</th>
<th>SD</th>
<th>QR Mean</th>
<th>SD</th>
<th>SOD Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (vehicle-treated control)</td>
<td>240.42</td>
<td>39.19</td>
<td>3.7</td>
<td>0.83</td>
<td>23.6</td>
<td>0.57</td>
</tr>
<tr>
<td>II (CDDP only)</td>
<td>169.73†</td>
<td>29.3</td>
<td>1.06***</td>
<td>0.31</td>
<td>29.87***</td>
<td>0.79</td>
</tr>
<tr>
<td>III (CDDP + chrysin D1)</td>
<td>248.89†</td>
<td>36.9</td>
<td>2.83††</td>
<td>1.09</td>
<td>26.02††</td>
<td>0.61</td>
</tr>
<tr>
<td>IV (CDDP + chrysin D2)</td>
<td>251.93††</td>
<td>49.4</td>
<td>3.15†††</td>
<td>0.58</td>
<td>24.31†††</td>
<td>0.72</td>
</tr>
<tr>
<td>V (chrysin D2 only)</td>
<td>225.45</td>
<td>38.94</td>
<td>3.67</td>
<td>0.8</td>
<td>22.87</td>
<td>0.68</td>
</tr>
</tbody>
</table>

SOD, superoxide dismutase.
Mean value was significantly different from that of Group I: † P<0.05, †† P<0.001.
Mean value was significantly different from that of Group II: † P<0.05, †† P<0.01, ††† P<0.001.
Effect of chrysin pretreatment and cisplatin on the activities of caspase-6 and -3 in the jejunum

The CDDP-treated group (Group II) exhibited significant elevation in the activities of caspase-6 ($P < 0.01$) and caspase-3 ($P < 0.01$) as compared to Group I. The higher dose of chrysin (50 mg/kg b.wt.) significantly attenuated the activities of caspase-6 ($P < 0.05$) and caspase-3 ($P < 0.01$) in Group IV as compared to Group II. However, there is no significant difference between the activities of caspase-6 and -3 in Group V as compared to Group I (Fig. 2(d) and (e)).

Effect of chrysin pretreatment against cisplatin-induced goblet cell disintegration in the jejunum

The jejunal sections of the CDDP-treated group (Group II) showed distorted crypts of Lieberkuhn, the presence of mucus at the apical surfaces of the sections (shown by arrow) and goblet cells disintegration, whereas there was no distortion of crypts of Lieberkuhn, the absence of mucus at the apical surfaces and no disintegration of goblet cells in the control group (Group I). In Groups III and IV, chrysin supplementation at both the doses (50 and 100 mg/kg b.wt.)
showed protection against CDDP-induced distorted crypts of Lieberkuhn, the presence of mucus at the apical surfaces of the sections and goblet cells disintegration as compared to Group II (Fig. 4).

Effects of chrysin pretreatment and cisplatin on histology of the jejunum

The haematoxylin and eosin-stained sections exhibited normal histoarchitecture with mild inflammatory cells infiltration in the control group (Group I), while the CDDP-treated groups showed distorted mucosal glandular architecture, villous atrophy, crypt ablation with intense inflammatory cell infiltration in the mucosal and submucosal layers. In Groups III and IV, chrysin significantly attenuated the CDDP-induced histopathological changes at both the doses (50 and 100 mg/kg b.wt.). There is no significant difference in the histological changes in Group V as compared to Group I (Fig. 5).

Discussion

In the present study, we have observed that pretreatment with chrysin showed protection against CDDP-induced jejunal toxicity. CDDP-induced diarrhoea and apoptosis in the...
intestinal epithelial cells are the pitfalls of this chemotherapeutic drug\(^\text{17}\). The upsurge for the finding of dietary antioxidants that can effectively protect against CDDP-induced gastrointestinal toxicity is gaining much attention. In the present study, we have observed the protective effects of chrysin against CDDP-induced jejunal toxicity. The data of the present study showed that pretreatment with chrysin resulted in the protection against CDDP-induced jejunal toxicity by amelioration of oxidative stress and apoptotic tissue damage.

CDDP results in the generation of ROS, namely the superoxide anion (\(O_2^-\)), \(H_2O_2\), hydroxyl radical (OH), etc., which are known to induce oxidative stress. XO is an enzyme that reduces oxygen (\(O_2\)) to the superoxide anion radical (\(O_2^-\)) and consequently produces oxidative stress\(^\text{39}\). The present study exhibited that the activity of XO enhanced after CDDP

FIG. 5. Effects of chrysin and cisplatin (CDDP) on the histoarchitecture of the jejunum. Photomicrographs of jejunal sections depicting (a) vehicle-treated control group (Group I), (b) CDDP-treated group (7.5 mg/kg body weight (b.wt.); Group II), (c) dose 1 of chrysin (25 mg/kg b.wt.) + CDDP (Group III), (d) dose 2 of chrysin (50 mg/kg b.wt.) + CDDP (Group IV) and (e) only dose 2 of chrysin (50 mg/kg b.wt.; Group V). The haematoxylin and eosin-stained sections exhibited normal histoarchitecture with mild inflammatory cells infiltration in the control group (Group I), while the CDDP-treated group showed distorted mucosal glandular architecture (shown by arrow heads), villous atrophy (shown by bold arrows), and crypt ablation with intense inflammatory cells infiltration in the mucosal and submucosal layers (shown by arrows). Pretreatment with the higher dose of chrysin (50 mg/kg b.wt.) significantly attenuated the CDDP-induced histopathological changes in Group IV as compared to Group II, while the lower dose of chrysin (25 mg/kg b.wt.) showed less protection as compared to the higher dose. There was no significant difference between the histology of Group V and Group I. Insets on the right panel show a magnified view (40 \(\times\) magnifications) of the insets showed on the left panel (10 \(\times\) magnifications) (a colour version of this figure can be found online at journals.cambridge.org/bjn).
treatment, while chrysin significantly attenuated its activity; and these ROS may play a key role in the initiation of LPO (11,12) (Fig. 6).

LPO is a marker of oxidative stress; and remarkable elevation in the level of MDA, a LPO product, was observed after treatment with CDDP (9,17,19,40). In the present study, it was demonstrated that pretreatment with chrysin significantly attenuated CDDP-induced MDA level.

Besides LPO, the level of GSH also depleted following CDDP treatment. GSH is a low-molecular-weight tripeptide, a cellular antioxidant (41). It protects the peroxidation of lipid membrane by conjugating with the electrophile such as CDDP, which leads to the production of ROS and thus the intracellular level of GSH depleted in GSH–CDDP conjugation reaction (42). This conjugation of GSH via the sulphydryl (–SH) group to electrophile is catalysed by a phase-II detoxifying enzyme, i.e. GST, and thus the activity of GST decreased after CDDP treatment (43). In the present study, it was observed that chrysin supplementation significantly attenuated the GSH level and the activity of GST (Fig. 6).

Moreover, it was observed that the activities of antioxidant enzymes, namely SOD, CAT, GPx, GR and G6PD and a phase-II detoxifying enzyme, namely QR, were diminished in the CDDP-treated group, whereas pretreatment with chrysin significantly attenuated the activities of these antioxidant and phase-II detoxifying enzymes. QR is a phase-II enzyme involved in xenobiotic metabolism that catalyses the two-electron reduction and thus protects cells against free radicals and ROS generated by the one-electron reductions catalysed by cytochromes P450 and other enzymes (44). The diminished activities of antioxidant and phase-II detoxifying enzymes in the CDDP-treated group supported the involvement of oxidative stress in the pathophysiology of CDDP-induced jejunal toxicity (Fig. 6).

CDDP is a DNA-damaging drug and it is also known to generate ROS. These ROS are considered to be the main culprit related to the toxicity of this antineoplastic drug (45) and these ROS also promote the intracellular DNA damage, thus leading to the activation and stabilisation of the genome safeguard, i.e. p53 (46,47). p53 is a key mediator of the DNA damage response.
response and is suggested to have an essential role in CDDP toxicity\(^{(48)}\). p53 is a tumour-suppressor protein and also acts as a transcription factor that regulates the transcription of genes involved in cell cycle, DNA repair and apoptosis\(^{(49)}\). Mdm-2 is a co-repressor of p53 and it maintains the low level of p53 via ubiquitin-mediated proteosomal degradation\(^{(50)}\). The present study showed that the CDDP-treated group has more p53 immunopositive staining as compared to the control group, while prophylactic treatment with chrysin significantly attenuated the p53 immunopositive staining. These results further supported the involvement of oxidative DNA damage caused by CDDP-generated ROS (Fig. 6).

p53 can be activated and stabilised in response to several stress signals, namely DNA damage. Upon activation, p53 induces apoptosis, which ultimately leads to the orchestration of caspases and plays a key role in the initiation and execution of cell death\(^{(47,51)}\). Caspases are cysteine-dependent enzymes and are activated by oxidative stress\(^{(51)}\). Caspase-6 is considered as an initiator caspase, while caspase-3 is the main executioner caspase because it can be activated through both intrinsic and extrinsic pathways. Activated caspase-6 leads to DNA fragmentation and cleavage of specific cellular proteins like PARP, actin and lamins during apoptosis\(^{(52)}\). The present study has demonstrated that caspase-6 and -3 activities significantly up-regulated in the CDDP-treated group and pretreatment with a higher dose of chrysin (50 mg/kg b.wt.) significantly attenuated the caspase-6 and -3 activities.

Goblet cells, the specialised exocrine cells of intestinal crypts, synthesise and secrete mucins. Mucins are high-molecular-weight, highly glycosylated proteins which form a protective layer in the form of gel in the intestinal lumen\(^{(53,54)}\). It was observed in our study that chrysin significantly attenuated the CDDP-induced distortion of the crypts of Lieberkühn and goblet cell disintegration. These results exhibited the protective effects of chrysin against CDDP-induced toxicity.

The aforementioned results further substantiated the historical data which exhibited the protective effects of chrysin against CDDP-induced distorted mucosal glandular architecture, villous atrophy, and crypt ablation with intense inflammatory cell infiltration in the mucosal and submucosal layers.

In conclusion, the results of the present study demonstrate that oxidative stress and apoptosis are closely associated with CDDP-induced toxicity and chrysin shows the protective efficacy against CDDP-induced jejunum toxicity, possibly via attenuating the oxidative stress and apoptotic tissue damage. Chrysin could be used as an adjuvant therapy with CDDP, but the exact mechanism of chrysin is not fully defined yet. Hence, further studies are warranted to elucidate the exact mechanism of action of chrysin.

**Acknowledgements**

S.S. is thankful to the University Grants Commission (New Delhi, India), UGC-Special Assistance Programme Departmental Research Support-II (UGC-SAP DRS-II) and a Research Fellowship in Sciences for Meritorious Students (RFSMS) to carry out this work. The contributions of the authors to the present study were as follows: R. K., A. Q. K., W. Q., A. L., M. T., F. A. and M. U. R. designed and conducted the experimental work. S. S. designed the experiment and wrote the manuscript. The authors declare that they have no conflicts of interest.

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