Dioscin relieves cisplatin-induced intestinal toxicity by mitigating oxidative stress and inflammation

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Abstract

Cisplatin is the most widely prescribed drug in chemotherapy, but its gastrointestinal toxicity reduces therapeutic efficacy. Oxidative stress and inflammation are considered to be the main pathogenesis of cisplatin-induced intestinal toxicity. Dioscin is a steroidal saponin with potential anti-cancer, antioxidant, and anti-inflammatory activities. In this study, we established a rat model of intestinal injury by tail vein injection of cisplatin, and intragastrically administered dioscin to evaluate its effect on intestinal injury. Biochemical markers, western blotting, qRT-PCR and histopathological staining were used to analyze intestinal injury according to various molecular mechanisms. The results revealed that dioscin significantly inhibited cisplatin-induced intestinal mucosal damage and decreased DAO levels in rats. Furthermore, dioscin activated the Nrf2/HO-1 pathway to increase the level of antioxidant enzymes and reduce the levels of MDA and H_2O_2. In addition, dioscin pretreatment significantly reduced ileum epithelial NLRP3 inflammasome formation and decreased the levels of inflammatory factors compared with the cisplatin group. In terms of mechanisms, dioscin reversed cisplatin-induced up-regulation of MAPKs and up-regulated p-PI3K and p-AKT levels. Meanwhile, dioscin potently promoted Wnt3A/β-catenin signaling to relieve cisplatin-induced proliferation inhibition. In conclusion, our study suggests that dioscin could ameliorate the cisplatin-induced intestinal toxicity by reducing oxidative stress and inflammation.

Keywords: Dioscin; Cisplatin; oxidative stress; inflammation; Nrf2/HO-1 pathway; NLRP3 inflammasome

1. Introduction

Cisplatin(*cis*-diamminedichloroplatinum, Cis) is a heavy metal complex with a strong anticancer effect, a synergistic effect with various antitumor drugs and no cross-resistance, which makes it one of the most widely used chemotherapy drugs in clinical practice[1]. However, Cis and its metabolites can cause serious damage to the intestinal mucosa, which makes almost all patients usually experience different degrees of gastrointestinal side effects and seriously affects the treatment process of patients[2, 3]. The clinical limitations of Cis motivate researcher to create plenty of Cis analogs[4]. But most of the platinum compounds failed to show substantial advantage over Cis, which remains the first-line drug for the treatment of prostate cancer, testicular cancer, ovarian cancer, and lung cancer[2]. Therefore, it has become an urgent problem to find a combination strategy to alleviate intestinal damage caused by Cis.

Oxidative stress is known to be an important factor in promoting Cis-induced intestinal injury by increasing the accumulation of intracellular reactive oxygen species (ROS)[5, 6]. Excessive ROS induces massive production of inflammatory cytokines by activating MAPK signaling molecules[7]. These inflammatory mediators and oxidative stress can cause intestinal epithelial cell shedding and crypt ablation[5]. Furthermore, ROS can activate the NLRP3 inflammasome danger signal, exacerbating the subsequent inflammatory cascade and cellular damage[8]. Nrf2 (nuclear factor erythroid 2-related factor 2) acts as a sensor of oxidative or electrophilic stress and is an important regulator of antioxidant activity[9]. And Nrf2-induced HO-1(heme oxygenase 1) prevents cytotoxicity in various oxidative stress and inflammatory responses[10]. Therefore, activation of Nrf2 is considered to be an important molecular target for remission of chemotherapy-induced intestinal toxicity.

Dioscin(Dio)，a naturally derived triterpenoid saponin with a variety of biological activities, could be isolated from various kinds of vegetables and herbs(most of which belong to the family of *Dioscoreaceae*). In some developing countries, edible rhizomes of some *Dioscorea* species also serve as starchy staple food[11]. Dio has been used in traditional Chinese medicine for decades, and drugs with Dio as the main component have also been gradually approved to enter the pharmaceutical market in other countries, which is enough to elucidate the safety of Dio for therapeutic use [11, 12]. In recent years, the inhibitory effects of Dio on oxidative stress and inflammation can also be seen in a variety of disease models, such as hepatic fibrosis, I/R injuries,

cardiac hypertrophy, and drugs-induced toxicity[13-17]. Furthermore, Dio has been demonstrated to exhibit potent antitumor activity in a variety of human cancer types, and its feasibility for application in cancer therapy is being developed[12]. Importantly, Dio has a protective effect on liver and kidney damages induced by a variety of chemotherapeutic drugs[18-20]. Nevertheless, it remains unknown whether the antioxidant potential of Dio exerts a protective effect in preventing chemotherapy-induced intestinal injury. Hence, in current work, the protective effect of Dio against intestinal damage caused by Cis through the activation of Nrf2/HO-1 pathway and consequent inhibition of NLRP3 inflammasome activation was investigated.

2 Materials and methods

2.1 Chemicals and reagents

Dioscin ($C_{45}H_{72}O_{16}$, MW: 869.05, CAS: 19057-60-4, HPLC purity ≥ 98%) was obtained from DESITE Biological Technology Co., Ltd. (Chengdu, China). Cisplatin was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Carboxymethyl cellulose sodium (CMC-Na) was purchased from JinPin Chemical technology Co., Ltd. (Shanghai, China). All the Quantitative polymerase chain reaction (qPCR) reagents were from Roche Group (Basel, Switzerland), Reverse Transcriptase kit was from Takara Biotechnology (Shiga, Japan). The commercial assay kits of ROS, MDA, H_2O_2, SOD, CAT, GSH T-AOC and dye kits hematoxylin and eosin (H&E) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). BCA protein concentration assay kit and enhanced chemiluminescent (ECL) reagent were purchased from Beyotime Biotechnology Co., Ltd. (Shanghai, China). Carnoy's Fluid were purchased from LEAGENE Biological Technology (Beijing, China). The enzyme-linked immunosorbent assay (ELISA) kit of DAO was obtained from JINGMEI Bioengineering Co., Ltd. (Jiangsu, China). Primary antibodies for PI3K, p-PI3K, AKT, p-AKT were purchased from ABclonal Technology Corporation (Wuhan, China). The remaining antibodies were purchased from Wanlei Biotechnology (Shenyang, China). Goat serum, 3,3′-diaminobenzidine tetrahydrochloride and 4, 6-diamino-2-phenylindole (DAPI) were purchased from Solarbio Science & Technology Corporation (Beijing, China). All other chemicals and reagents, unless indicated, were provided by Beijing Chemical Factory (Beijing, China).

2.2 Animals

Female Wistar rats (6 weeks old), weighing 176–200 g, were offered by CHANGSHENG Experimental Animals Co. Ltd (Changchun, Jilin province, China), and randomly divided into four groups of twelve animals each. rats were housed at controlled temperature (25 ± 2 °C) and humidity (60 ± 10%) with 12 h light/dark cycle with food and water ad libitum. All animal experiment was reviewed and approved by Animal Experimental Committee of Northeast Agricultural University.

2.3 Experimental protocol

As shown in Fig.1A, after 1 week of acclimatization, rats were randomly divided into 3 groups as follows:

(1) Control + vehicle (Con) group (n = 6);

(2) Cisplatin + vehicle (Cis) group (n = 6);

(3) Cisplatin + Dioscin (Cis+Dio) group (n = 6);

Rats then received for 10 consecutive days intragastric administration of vehicle (0.5% CMC-Na) or Dio (60 mg/kg/day). A single injection into a tail vein of cisplatin (6 mg/kg) or stroke-physiological saline solution (SPSS) was administered after the 7th day of administration. In current study, the dose of either Cis or Dio was cited from the previous published literature[18, 21-23]. Seventy-two hours after administration of cisplatin, their blood was collected from the abdominal vein under sodium pentobarbital anesthesia, and rats were euthanized using CO_2. Then, we collected ileum samples.

2.4 Histopathological examination

After euthanasia, Ileum tissues (7-15 cm segment before the cecum) were collected and fixed in 4% paraformaldehyde (PFA) or 24 h, embedded in paraffin. Sections of 4 μm were stained with conventional hematoxylin & Eosin (H&E). A blinded and randomized histopathological analysis by experienced histopathologists to assess the severity of mucositis using the intestinal pathology scoring system of Galeazzi et al [24].

2.5. DAO assay

Diamine oxidase (DAO) levels in serum can be used to determine the degree of intestinal villi injury. According to the manufacturer's instructions provided by the DAO ELISA kit, added standard reagents, test samples, and reagents to the test plate in order. After a series of reactions, the OD value was read by a microplate reader (Bio-Rad, California, USA) to calculate the DAO

content in serum.

2.6Measurement of ROS levels

According to the instructions of Reactive Oxygen Species (ROS) Assay Kit (fluorescence chemistry), the ileum tissue was rinsed with PBS, cut into small pieces, and prepared into single cells by enzymatic digestion. Take part of the cell suspension to measure the protein concentration with BCA protein concentration kit. DCFH-DA was added to the remaining cell suspension. After incubation and washing, the fluorescence value of the sample was detected by a fluorescence microplate reader (Bio-Rad, California, USA) at an excitation wavelength of 485 nm and an emission wavelength of 525 nm. Results were calculated and expressed as fluorescence value/ng of protein.

2.7 Measurement of oxidant status and antioxidant enzymes.

Levels of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and activities of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), total antioxidant capacity (T-AOC) in rat ileum tissue homogenates were determined using commercial kits. The absorbance values were measured using a microplate spectrophotometer (Bio-Rad, California, USA) according to the manufacturer's instructions.

2.8 Immunofluorescence staining

The 3μm thick paraffin sections were deparaffined, rehydrated, and prepared for immunofluorescence assays according to a standard protocol. After incubating with the anti-NLRP3 antibodies for 24 h at 4 °C, the sections were washed with PBS and then were treated with secondary antibodies. After being washed with PBS 3 times, the sections were incubated with secondary antibody, then the sections were washed with PBS 3 times again. DAPI was used to label nucleus. Fluorescence images were acquired with a Nikon Eclipse Ni inverted microscope (TE2000; Nikon, Tokyo, Japan).

2.9 Immunohistochemical staining.

After the paraffin sections were deparaffinized, the rat ileum tissues were incubated with 3% hydrogen peroxide solution for 25 minutes. After rinsing the tissues with PBS, the tissues were blocked with 10% normal goat serum at room temperature for 1 hour, and then incubated with the primary antibody (Ki67, 1:500; Wanlei Biotechnology Corporation, Shenyang, China) at 4°C overnight. Subsequently, an anti-rabbit IgG antibody (diluted 1:500) was used to react with the

tissues at room temperature for 2 hours. The tissues were then reacted with diaminobenzidine for 10 minutes. Finally, images were obtained through microscope. The mean integral optical density of positive cells was then determined using Image-Pro Plus (IPP) image software.

2.10 qRT-PCR analysis.

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) was used to detect the mRNA abundance of NLRP3, ASC, Caspase1, WNT3a, β-catenin and c-Myc. Total RNA was isolated from the frozen samples. Available concentration and purity of mRNA were retranscripted to cDNA strand using TransScript reverse transcriptase kits following the manufacturer's instructions. All the primer sequences listed in Tab.1 were specifically validated and conformed to the normal distribution. SYBR Green master mix and the primers were used to amplify cDNA. The obtained amplification data were analyzed using GAPDH as internal parameter by the $2^{-\Delta\Delta Ct}$ method. [Table 1 near here]

2.1 Western blotting analysis.

Total protein was extracted from the ileum by Tissue or Cell Total Protein Extraction Kit, respectively. Proteins concentration were determined by BCA protein assay reagent. Proteins (30–50 μg per lane, depending on protein of interest) were separated using sodium dodecyl sulfate-polyacrylamide gels electrophoresis which were electro-transferred onto PVDF membranes. Then, the PVDF membranes were incubated with Nrf2, HO-1, Keap1, p62, COX2, iNOS, IFNγ, TGF-β1, JNK, p-JNK, ERK, p-ERK, p-P38, P38, NLRP3, ASC, Caspase1, PI3K, p-PI3K, AKT, p-AKT, WNT3a, β-catenin, c-Myc, β-tubulin, β-actin antibody, respectively. Subsequently, the PVDF membranes were incubated with an appropriate secondary antibody. Finally, protein bands were detected using ECL reagent. The protein grey intensity was quantified by NIH ImageJ software and normalized using β-tubulin and β-actin protein levels.

2.12 Statistical analysis

All statistical analyses were performed with SPSS software (SPSS Inc., Chicago, IL). And the data were analyzed using the one-way Analysis of Variance (ANOVA) and were reported as mean ± standard deviation (M±SD). The date was plotted using GraphPad Prism 7 (GraphPad Software, La Jolla, CA, United States). $p < 0.05$ and $p < 0.01$ were considered statistically significant.

3. Results

3.1 Dio ameliorated Cis-induced intestinal injury

We first investigated the effect of pretreatment with Dio on Cis-induced intestinal toxicity by administering Dio to rats for 10 consecutive days and injecting Cis on the seventh day (Fig. 1A). After collecting blood from rats, the DAO content in serum was measured to assess the intestinal protective potential of Dio. Cis injection dramatically increased serum DAO level, indicating severe intestinal damage and successful establishment of animal model ($p < 0.01$). Compared with the Cis model group, Dio administration showed protective effect ($p < 0.01$) (Fig. 1B). To further evaluate whether Dio retained intestinal function, histopathological examinations were performed using H&E staining to evaluate ileum structures in the Wistar rats. In the ileum of rats in the Con group, intestinal villi were stretched and crypt morphology was normal. However, in the Cis group, the ileal villi were broken, red blood cells and inflammatory cells were increased, and goblet cells were decreased. Notably, preadministration of Dio reduced intestinal villus fragmentation and inflammatory cell pleocytosis. Surprisingly, Dio promoted goblet cell increase in intestinal crypts (Fig.1C). The histologicail damage scores in the Cis group were significantly higher than those in the control groups ($p < 0.01$), while the Cis+Dio group had lower histologicail damage scores than the Cis group ($p < 0.05$) (Fig. 1D). [Figure 1 near here]

3.2 Dio induced Nrf2-HO-1 pathway activation and ameliorated Cis-induced intestinal oxidative stress

To confirm the ileum protective activity of Dio against Cis-induced oxidative stress, we examined several some oxidant status (ROS, MDA, H_2O_2) and the activities of relative antioxidant enzymes (SOD, CAT, GSH and T-AOC) in all groups of ileums. As shown in Fig. 2A-G, compared with the Con group, Cis treatment resulted in a prominent increase in ROS, MDA and H_2O_2 levels, while increasing the levels of SOD, CAT, GSH and T-AOC ($p < 0.01$). In contrast, Dio pretreatment dramatically inhibited the overproduction of ROS, MDA and H_2O_2 ($p < 0.01$) and restored the antioxidant state, which was manifested by an increase in SOD, CAT, GSH and T-AOC levels ($p < 0.05$). Then, we determined the protein levels of Nrf2 pathway-related proteins, including HO-1, Keap1 and p62. Cis treatment led to the down-regulation of Nrf2, HO-1, and p62 proteins, but a significantly up-regulation was observed in rats orally administered Dio. Additionally, Keap1, the suppressor of Nrf2, was significantly increased in the Cis group (Fig. 2H-L). [Figure 2 near here]

3.3 Dio inhibits NLRP3 inflammasome activation and inflammatory factor expression in Cis-induced intestinal injury.

To assess the possible role of Dio-induced Nrf2 activation on the protective effect of NLRP3 inflammasome activation, we examined the effects of NLRP3 inflammasome proteins and inflammatory factors in the rats' intestine. Immunofluorescent staining of ileum sections was performed. As shown in Figure 3A, Cis treatment significantly increased NLRP3 expression in intestinal villi. However, Dio treatment ameliorated NLRP3 expression compared with that of Cis treated rats. By further evaluating the inflammatory response of renal tissue (Fig 3B–J), we found that cisplatin significantly increased expression of NLRP3, ASC, caspase-1, COX2, iNOS and IFNγ, which were all downregulated by Dio ($p < 0.05$ and $p < 0.01$). And Dio protreatment also significantly increased the expression of anti-inflammatory cytokine TGF-β1 (Fig 3K)($p < 0.01$). [Figure 3 near here]

3.4 Dio induced antioxidant and anti-inflammatory defense through PI3K/AKT and MAPK signaling pathways

As shown in Figure 3A, We observed that p-PI3K and p-AKT expression were down-regulated after Cis treatment ($p < 0.01$), whereas Dio administration promoted phosphorylation of both ($p < 0.01$) (Fig 4A-C). In addition, the phosphorylated levels of ERK1/2, JNK and P38 proteins in the Cis group was significantly larger than in the CON group (Fig. 6B, $p < 0.01$). Nevertheless, the aforementioned changes caused by Cis were markedly relieved by Dio pretreatment compared with the Cis groups ($p < 0.01$). These data demonstrate that PI3K/AKT and MAPK pathways may play a specific role in Dio-induced antioxidant and anti-inflammatory defense in rat. [Figure 4 near here]

3.5 Dio promoted Wnt3A/β-catenin signaling to relieve Cis-induced proliferation inhibition.

Immunostaining of Ki67, a cellular marker of proliferation, was performed to assess whether Dio induces proliferation of intestinal cells in Cis treated rats (Fig. 5A). Compared with the Cis group, the proliferation zone in Dio+Cis rats moved progressively upward in the crypts toward the crypt-villus junction. The means integral optical density of Ki67 positive cells were clearly reduced in intestinal crypts in the Cis group compared with the Con group ($p < 0.01$) (Fig. 5B).

However, pretreation with Dio significantly alleviated the inhibition of proliferation caused by Cis ($p < 0.01$). To investigate whether Dio treatment affects the signaling pathway for intestinal regeneration, protein and mRNA levels of Wnt3A, β-catenin and c-Myc in ileum were measured and found to be reduced after cisplatin injection. However, compared with the Cis group, the mRNA and protein levels of Wnt3A, β-catenin and c-Myc were significantly up-regulated in the Dio group ($p < 0.01$) (Fig. 5C-I). [Figure 5 near here]

4. Discussion

Chemotherapy-induced mucositis is a common and serious side effect of chemotherapy, which seriously affects the quality of life among cancer patients and even reduce their compliance with chemotherapy [25]. Cisplatin, a drug commonly used in chemotherapy regimens for human cancer, can cross-link with DNA and thus block cancer cell transcription and replication [26]. Cis-induced intestinal toxicity has been attributed to various events which are, in turn, linked with ROS mediated oxidative stress and inflammation [5, 6]. Therefore, screening natural antioxidants from botanicals could serve as a novel strategy to prevent gastrointestinal dysfunction associated with Cis-based regimens. Dioscin is a natural product, and it has been shown to protect against a variety of organ injuries by modulating multiple signaling pathways to modulate oxidative stress [19, 20, 27]. We therefore investigated for the first time the effect of Dio on Cis-induced intestinal oxidative damage in rats and tested whether Dio could be used as a possible natural agent for the treatment of chemotherapy-induced intestinal toxicity.

First, we found that Dio normalizes the functional integrity of intestinal mucosa. DAO is an intracellular enzyme in the intestinal epithelium[28]. The content of DAO in blood is a sensitive indicator for the early diagnosis of intestinal mucosal injury. Our results showed that the activity of DAO in serum of rats in Cis group was significantly increased. This is consistent with previous data[3, 29]. Whereas pretreatment with Dio significantly reduced DAO into the blood. We also observed significant ileum epithelium hemorrhage, villus breakage, crypt distortion, and reduction of goblet cells in the cisplatin group. While the degradation of this intestinal tissue was improved by Dio treatment. It is worth mentioning that we observed a significant increase in goblet cells between intestinal epithelial cells in the Cis+Dio group. The hydrated mucin secreted by goblet cells could act as a mucus barrier[30], which also serves as a demonstration of Dio's enteroprotective potential.

Major Cis-induced reactive oxygen species include superoxide anion (O_2^-), hydroxyl radical (•OH), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and peroxyl radicals. These radicals cause extensive mucosal damage by directly reacting with cellular proteins, lipids and DNA[31]. However, small intestine responds to Cis treatment by altering the activities and levels of these enzymatic and non-enzymatic antioxidants respectively[6]. In this paper, Dio inhibited oxidative damages of intestinal caused by Cis by increasing SOD, CAT, GSH and T-AOC levels, as well as decreasing ROS， MDA and H_2O_2 levels. The present data confirmed that the activities of Dio against Cis-induced intestinal damages might be achieved by suppressing oxidative stress. The Nrf2/Keap1 axis plays a crucial role in the development of the gastrointestinal tract and the prevention of inflammatory bowel disease[32]. Our data show that Dio may prevent Cis-induced oxidative damage by promoting p62-associated Keap1 (Kelch-like ECH-associated protein1) degradation and Nrf2 activation. HO-1, an antioxidant enzyme regulated by Nrf2 activation, is essential for redox homeostasis by preventing ROS production[33]. Activation of the Nrf2/HO-1 pathway provides an endogenous defense system to reduce inflammation and oxidative stress by stimulating the expression of antioxidant proteins, making it a promising therapeutic mechanism to suppress inflammation[34]. These results illustrate that the antioxidant potential of Dio can play a role in preventing Cis-induced intestinal toxicity.

The NLRP3 inflammasome, consisting of the NLR protein and the adaptor protein ASC (apoptotic speck-like protein containing a caspase recruitment domain), is vital component of the innate immune system. In recent years, the crosstalk between Nrf2 and inflammasome has been unveiled, and Nrf2 activation represses NLRP3 inflammasome and inflammation[35, 36]. And Nrf2 activation was also found to be accompanied by NLRP3 inflammasome inhibition in the study of inflammatory bowel disease[37-39], which is consistent with the results of in our study. NLRP3 silencing also effectively reduces the secretion of inflammatory factors. These results show that Dio may inhibit NLRP3 inflammasome to protect against Cis via Nrf2, indicating that Nrf2-NLRP3-caspase-1 axis may underlie the favorable effects of Dio in Cis treatment.

In order to further explore the antioxidant and protective effects of Dio in chemotherapy-induced mucositis, we investigated the PI3K/AKT and MAPK pathways, which are upstream regulators of Nrf2 in the intestine[29, 32]. Phosphorylation of PI3K and AKT promotes the release of Nrf2, which subsequently initiates an antioxidant cascade. In the current study, Dio pre-

administration can promote the activation of PI3K/AKT pathway, Phosphorylation of PI3K and AKT promotes the release of Nrf2, which subsequently initiates an antioxidant cascade. In the current study, Dio pre-administration promoted the activation of the PI3K/AKT pathway, consistent with the expected results. Previous studies have found that Dio can promote the phosphorylation of PI3K and AKT in the liver through miR-125a-5p/STAT3 [40, 41]. This also further illustrates that the PI3K/AKT pathway is an important mechanism in Dio resistance to Cis-induced intestinal injury. Mitogen-activated protein kinase (MAPK) pathways including extracellular signal-regulated kinase (ERK), c-Jun NH2-terminal kinase (JNK) and P38 MAPK pathways have been reported to be involved in the antioxidant regulation of Nrf2[42]. In addition, MAPKs are classic inflammation-related signals involved in the expression of pro-inflammatory mediators[43]. According to our study, Dio significantly alleviated Cis-induced MAPKs dysregulation. Therefore, MAPKs might also play important roles in the anti-inflammatory mechanisms of Dio.

While this is the first study to demonstrate the relationship between Dioscin and oxidative stress in chemotherapy-induced intestinal toxicity, it is not surprising as Dio has been reported to be an important factor in promoting pancreatic cell and liver regeneration[44, 45]. Considering the increase of goblet cells in the Dio group in the histopathological analysis, we felt that it was necessary to explore the role of Dio in intestinal epithelialization. The present data indicate that Dio may play a role in promoting proliferative zone repair in crypts via the Wnt3a/β-catenin pathway. Of note, previous studies have demonstrated that Nrf2 affects the Wnt signaling pathway that coordinates intestinal development and epithelial cell differentiation[46]. However, whether Dio promotes intestinal epithelial regeneration through Nrf2 to prevent Cis-induced intestinal injury needs more detailed research.

5. Conclusions

In conclusion, we provide evidence that Dio has a protective effect against Cis-induced intestinal injury (Fig 6). Mechanically, Dio treatment exerts antioxidant effects by activating PI3K/AKT/Nrf2 signaling. In addition, Nrf2 activation and MAPKs inhibition also play a role in Dio resistance to Cis-induced intestinal toxicity, as shown by the reduction of NLRP3 inflammatory bodies and inflammatory factors. Meanwhile, Dio relieved Cis-induced proliferation

inhibition. Through the results of our study, Dio may be developed as a drug for clinical treatment of chemotherapy-induced intestinal injury.

Abbreviations

Dio: Dioscin; Cis, Cisplain; CMC-Na, Carboxymethyl cellulose sodium; H&E, Hematoxylin and Eosin, PI3K: Phosphoinositide 3 kinase, AKT: Protein kinase B, MDA: Malondialdehyde, H_2O_2: Hydrogen peroxide, NO: Nitric oxide, iNOS: Inducible nitric oxide synthase, SOD: Superoxide dismutase, CAT: Catalase, GSH: Glutathione, T-AOC: Total antioxidant capacity, Nrf2: Nuclear factor erythroid 2-related factor 2, qRT-PCR: Quantitative reverse transcriptase polymerase chain reaction, GAPDH: Glyceraldehyde-3-phosphate dehydrogenase, ROS: Reactive oxygen species, Wnt3A: MMTV integration site family member 3A, NLRP3: Nucleotide- binding oligomerization domain, leucine- rich repeat and pyrin domain- containing 3, ASC: Apoptotic speck-like protein containing a caspase recruitment domain, ERK: Extracellular signal-regulated kinase, JNK: c-Jun NH2-terminal kinase, HO-1: Heme oxygenase 1, MAPK: Mitogen-activated protein kinase, Keap 1: Kelch-like ECH-associated protein 1.

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Author Contributions

SJ and XL conceived the original idea and designed the experiment. SD, DL and JL carried out the sample collection and data analysis. SJ wrote the manuscript and YL reviewed the manuscript. All authors contributed to the article and approved the submitted version.

Ethics Statement

The animal procedures in this study were guided and approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University (SRM-11)

Data Availability Statement

The datasets generated for this study are available on request to the corresponding author.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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