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Research Article
Effect of Rutin on Cisplatin-induced Small Intestine (Jejunum) Damage in Rats

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Abstract

Background and Objective: Cisplatin is an antineoplastic, platinum derivative used in the treatment of various cancers. Mucositis is a notable side effect of cisplatin treatment. Rutin (vitamin P1) is a drug with antioxidant, anticancer, antidiabetic and antimicrobial properties. The aim of this study was to biochemically, histopathologically and immunohistochemically investigate the effect of rutin on cisplatin-induced mucositis of the small intestine (jejunum) in rats. **Materials and Methods:** Twenty four rats were divided into four groups with six animals in each group: healthy group (HG), cisplatin-only group (CCG), 50 mg kg^{-1} of rutin plus cisplatin group (RG-50) and 100 mg kg^{-1} of rutin plus cisplatin group (RG-100). Rutin or distilled water were administered via an oral gavage. One hour after the administration of rutin or distilled water, the CCG, RG-50 and RG-100 were intraperitoneally injected with 5 mg kg^{-1} of cisplatin once every 2 for 8 days. At the end of this period, all the animals were sacrificed using a high-dose anesthetic and their small intestines (jejunum) were removed for biochemical and histopathological procedures. Differences between the groups were analyzed using a one-way analysis of variance, followed by Dunnett's multiple comparisons test. **Results:** The levels of oxidants increased in all the cisplatin-treated groups, whereas those of antioxidants decreased. Rutin administered at a dose of 100 but not 50 mg kg^{-1} reduced oxidant levels and increased antioxidant levels to those of healthy tissues. Histopathologically, tissue damage was observed in jejunal tissue of the rats administered only cisplatin, whereas treatment with 100 mg kg^{-1} of rutin prevented cisplatin-induced histopathological damage. In the group administered 50 mg kg^{-1} of rutin, jejunal tissue showed a near-normal appearance, except for mildly dilated congested blood vessels. As shown immunohistochemically, rutin prevented cisplatin-induced jejunal damage more effectively when administered at a dose of 100 mg kg^{-1} than 50 mg kg^{-1}. **Conclusion:** Rutin may be useful in the prevention of cisplatin-induced jejunal mucositis.

Key words: Cisplatin, rutin, toxicity, oxidative stress, intestinal damage, mucositis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cisplatin (cis-diamminedichloroplatinum II) is an antineoplastic agent that includes platinum (heavy metal) derivate and is used in the treatment of head, neck, lung, testicular and ovarian cancers[1,2]. The chemotherapeutic effect of cisplatin increases with an increasing dosage but an increased dosage causes adverse effects, such as nephrotoxicity, autotoxicity, neurotoxicity, hepatotoxicity, nausea, vomiting and in 67% of cases, diarrhea, thus restricting its clinical use[3,4]. Mucositis is a notable side effect of cisplatin[5]. Although the mechanism of cisplatin toxicity is not fully understood, oxidative stress plays an important role in its development[6]. Cisplatin induces the production of reactive oxygen species (ROS), thus causing DNA damage, followed by secretion of proinflammatory cytokines, such as tumor necrosis factor-alpha and interleukin-1 beta from endothelial epithelial cells[7,8]. As a result, tissue damage and ulceration develop and mucosa becomes vulnerable to bacterial contamination[9]. Various treatments, such as palliative approaches, mucosal protective agents, topical antimicrobial agents and analgesics are frequently used in the prevention and treatment of mucositis but none of these are 100% effective[10]. Research aimed at reducing the effect of cisplatin toxicity on intestinal tissue is ongoing. According to a study, antioxidant treatment maybe useful in cisplatin-induced small intestinal toxicity[4].

Vitamin P1, also known as rutin is a flavonoid and vital component of nutrition. It is abundant in plants, such as passion flower, buckwheat, tea and apple[11]. It has many properties, such as antioxidant, anticancer, antidiabetic, antiulcer and tissue renewal[12-14]. These beneficial properties of rutin suggest that it may be effective against cisplatin-induced jejunal damage. In the literature, no information was found on the protective effect of rutin against cisplatin-induced jejunal mucositis. The aim of this study was to biochemically, histopathologically and immunohistochemically investigate the effect of rutin on cisplatin-induced mucositis in rats.

MATERIALS AND METHODS

Animals: In the study, 24 male albino Wistar rats weighing 230-240 g obtained from Atatürk University Medical Experimental Practice and Research Center were used. Prior to the experiment, the rats were maintained in the Pharmacology Laboratory in groups at normal room temperature (22°C), with access to food and water *ad libitum*. Ethics committee approval was obtained from Atatürk University animal experiments local ethics committee (8-24-2017, 7/102).

Chemicals: Sodium thiopental used in the experiment was purchased from IE Ulagay (Istanbul, Turkey), rutin was purchased from Solgar (N.J., USA) and cisplatin was purchased from Liba (Istanbul, Turkey).

Experimental design: The rats were divided into a healthy group (HG), cisplatin-only group (CCG), 50 mg kg^{-1} of rutin plus cisplatin group (RG-50) and 100 mg kg^{-1} of rutin plus cisplatin group (RG-100). Rutin or distilled water were administered via an oral gavage. One hour after the administration of rutin or distilled water, the CCG, RG-50 and RG-100 were intraperitoneally injected with 5 mg kg^{-1} of cisplatin once every 2 for 8 days. At the end of this period, all the animals were sacrificed using a high-dose anesthetic and their small intestines (jejunum) were removed. Malondialdehyde (MDA), nitric oxide (NO), total glutathione (tGSH), glutathione S-transferase (GST), glutathione peroxidase (GPO) and catalase (CAT) levels of small intestinal tissues were measured. All experimental results were evaluated comparatively.

Bioassays

Sample preparation: Tissue (25 mg weighed) was homogenized using a solution of 1.15% KCl (Merck, Germany). The homogenate was centrifuged at 4,000 rpm for 30 min at +4°C. The supernatants were then used for NO and MDA measurements. Tissues (25 mg) taken for tGSH analysis were washed with isotonic sodium chloride (I.E. Ulagay, Turkey). Phosphate buffer solution (0.213 g of $NaH_2PO_4.2H_2O$,[Merck, Germany]+ 1.563 g of $Na_2HPO_4.2H_2O$ [Merck, Germany]+ 0.038 g of EDTA [Sigma-Aldrich, Germany]+100 mL of dH_2O, pH = 7.4]) was then added to give a total volume of 2 mL. Subsequently, the tissue was homogenized in an icy environment. Subsequently, the tissues were centrifuged at 1,000 rpm for 15 min at +4°C. The supernatant was used as the sample for analysis. The protein concentration of the supernatant was measured using the method described by Bradford[15].

Measurement of lipid peroxidation: According to the lipid peroxidation measurement method described by

Ohkawa *et al*., MDA forms a pink-colored complex with thiobarbituric acid at 95°C and this complex can be measured using spectrophotometry at a wavelength of[16] 532 nm. A standard curve was obtained using 1,1,3,3-tetramethoxypropane (Sigma-Aldrich, Germany).

Measurement of NO activity: The NO levels of the tissues were measured as total nitrite plus nitrate levels using Griess reagent in a two-step process, as previously described[17]. Griess reagent consists of 1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid (Sigma-Aldrich, Germany). A standard curve was obtained using KNO_3 (Merck, Germany).

Measurement of tGSH level: According to the method defined by Sedlak and Lindsay[18], (5,5′-dithiobis (2- nitrobenzoic acid) (DTNB) disulfide is chromogenic in the medium and DTNB is reduced easily by sulfhydryl groups. The yellow color produced during the reduction is measured by spectrophotometry[18] at 412 nm. A standard curve was obtained using GSSG (Sigma- Aldrich, Germany).

Measurement of GST and GPO activity: The GST activity was determined by the method described by Habig and Jakoby[19]. Briefly, GST activity was assayed spectrophotometrically at 340 nm in a 4 mL cuvette containing 0.1 M PBS (pH 6.5), 30 mM GSH, 30 mM 1-chloro-2,6-dinitrobenzene and tissue homogenate. The GPO activity was determined by the method described by Lawrence and Burk[20].

Measurement of CAT activity: The CAT activity was determined by measuring the decrease in absorbance at 240 nm during the transformation[21] of H_2O_2 to H_2O.

Histopathological evaluation: Jejunal rat tissues were fixed for 24 h in 10% formalin solution. After routine tissue processing, sections 4 µm thick were cut from paraffin blocks and stained with hematoxylin and eosin. All sections were examined under a light microscope (Olympus BX 52, Tokyo, Japan) by two pathologists who were blinded to the treatment protocol.

Immunohistochemical procedures: For immunohistochemical staining, primary antibodies of Caspase-3 antibody (Santa Cruz Biotechnology, TX, Cell Signaling Technology, Inc.,MA) were used. The sections were stained using a fully automated immunohistochemistry device (Leica Bond-Max, LeicaBiosystems, Melbourne, Australia). After IHC processing, the sections were dehydrated through a graded series of ethanol to xylene and placed in a mounting medium (Entellan, Merck Millipore, Darmstadt, Germany). From the rat jejunal samples incubated in 10% formalin solution for immunohistochemical processing, sections 4 µm thick were cut on a positively charged microscope slide. The results of the analysis were evaluated under an Olympus BX51 microscope based on caspase-3 staining of jejunal tissue using a specific grading system. In this evaluation, diffuseness and intensity were considered separately. Diffuseness represented areas where dye was found and intensity represented the intensity of coloration. For diffuseness, grade I represented coloration in less than 10% of cells, grade II represented coloration in10-50% of cells and grade III represented coloration in more than 50% of cells. For coloration of cells, grade I represented mild, grade II represented intermediate and grade III represented intense.

Statistical analysis: The data from individual groups were presented as Mean±standard error (x±SEM). Differences between groups were analyzed using a one-way analysis of variance test, followed by *post hoc* Dunnett's multiple comparisons test. All statistical analyses were performed using the IBM SPSS 20 (IBM Corp. Released 2011 IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp) and $p<0.05$ was considered significant.

RESULTS

Effect of rutin on cisplatin-induced lipid peroxidation: The MDA levels were significantly higher in the CCG than HG ($p<0.01$). Upon rutin use, MDA levels significantly decreased in RG-50 ($p<0.05$) and RG-100 ($p<0.01$) compared to the CCG (Fig. 1a).

Effect of rutin on jejunal NO activity: The NO levels were significantly lower in the CCG than HG ($p<0.01$). Rutin use caused a significant increase in NO levels in the RG-50 ($p<0.05$) and RG-100 ($p<0.01$) as compared with that in the CCG (Fig. 1b).

Effect of rutin on cisplatin-induced reductions in jejunal GSH activity: The GSH levels were significantly lower in the CCG than HG ($p<0.001$). GSH levels were significantly increased in the RG-50 ($p<0.05$) and RG-100 ($p<0.01$) as compared with those in the CCG (Fig. 1c).

Effect of rutin supplementation and cisplatin on jejunal antioxidant enzyme activities: The CAT activity was significantly decreased ($p<0.001$) in the CCG as compared

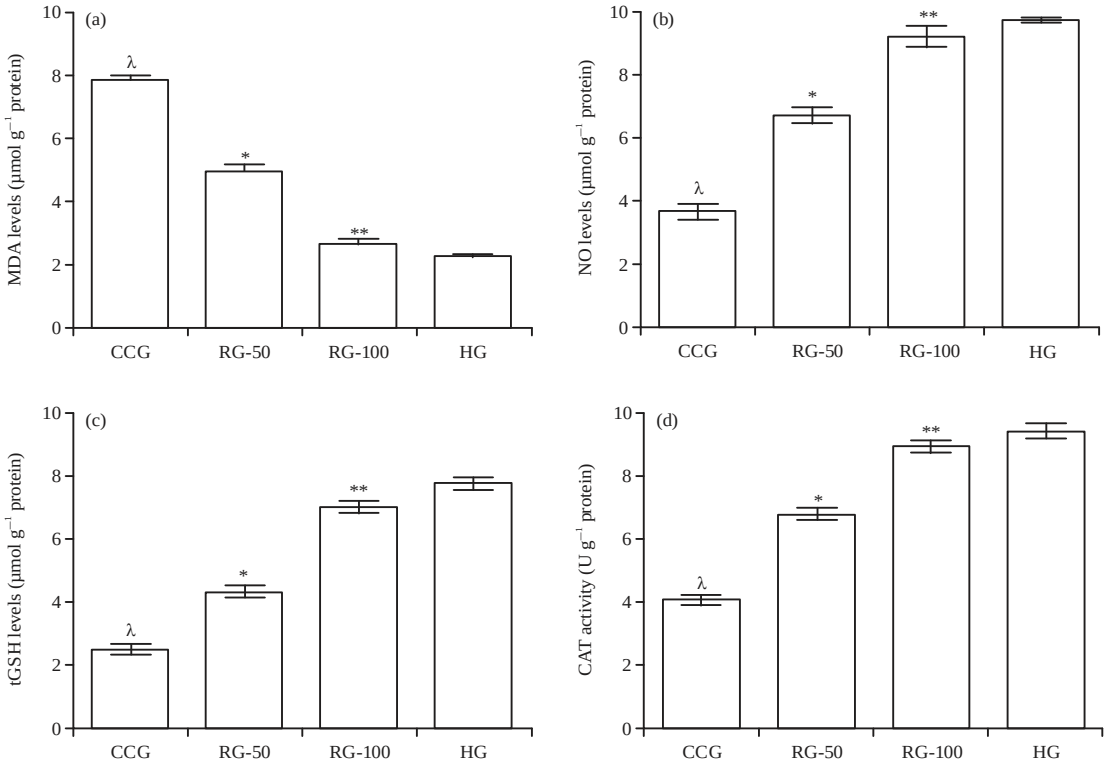

Fig. 1(a-d): Effects of rutin on (a) MDA, (b) NO, (c) GSH levels and (d) CAT activity in the jejunal tissues of rats administered with cisplatin.

Bars are mean±SD. ($^{\lambda}$p<0.01, according to HG group, *p<0.05, according to CCG group,**p<0.01, according to CCG group)

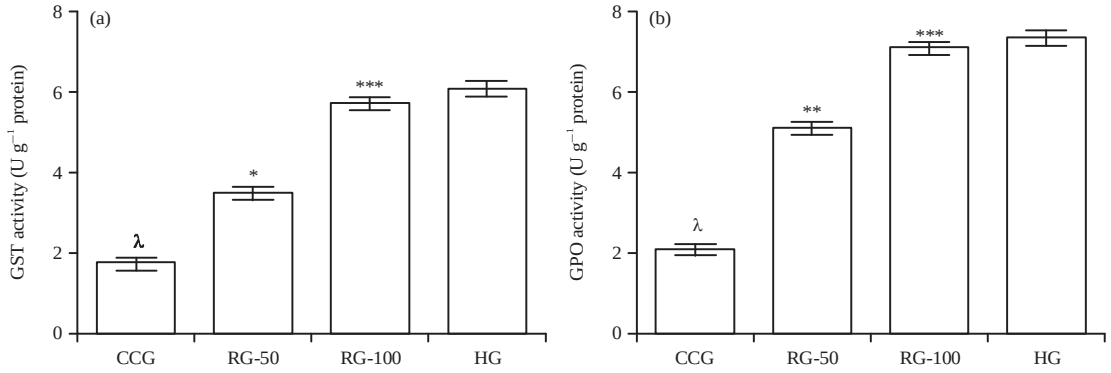

Fig. 2(a-b): Effects of rutin and cisplatin on the activities of GST(A), GPO(B) in rat jejunum

Bars are Mean±SD. ($^{\lambda}$p<0.01, according to HG group, *p<0.05, according to CCG group, **p<0.01, according to CCG group, ***p<0.001, according to CCG group)

with that in the HG. Rutin pretreatment at a dose of 50 mg significantly augmented CAT activity (p<0·05) in the RG-50 compared to the CCG. The higher dose of rutin (100 mg) also significantly increased CAT activity (p<0.01) in the RG-100 as compared with that in the CCG (Fig. 1d).

Effect of rutin supplementation and cisplatin on jejunal GST and GPO activity: The GST and GPO activity (both p<0.001) significantly decreased in the CCG as compared with that in the HG. The rutin group, which was administered 50 mg of rutin, had significantly higher GST (p<0.05) and GPO (p<0.01) levels as compared with those in the CCG. Moreover, the RG-100, which was administered 100 mg of rutin, had significantly higher GST (p<0.001) and GPO (p<0.001) as compared with that in the CCG (Fig. 2a,b).

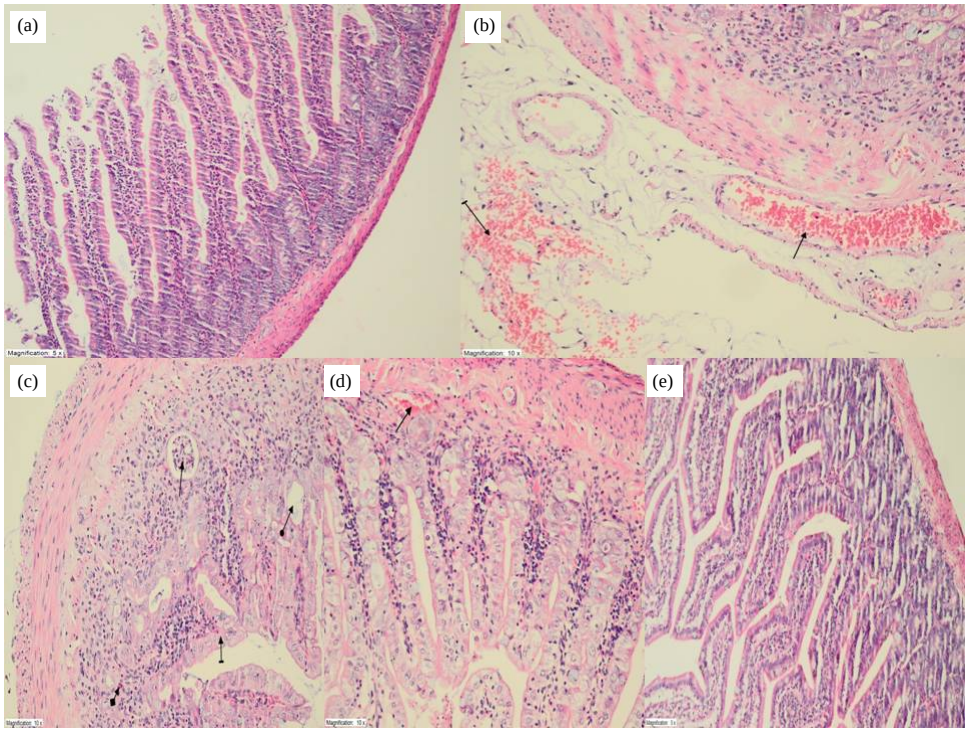

Fig. 3(a-e): Histopathological findings of the jejunum tissues, (a) Normal healthy jejunum of HG group(HEX100), (b) Dilated congested blood vessel (solid arrow) and hemorrhage (dashed arrow) in the serosa of the jejunum in CCG group, (c) Cryptitis in the jejunum (solid arrow), damage in crypt structure (round arrow), flattening and atrophy in the villus epithelium, atrophy (dashed arrow) and mixed inflammatory cell infiltration accompanied by PMNL in the mucosa (square arrow) of CCG group (HEX200), (d) Jejunum tissue of RG-50 group has near-normal appearance except the dilated congested blood vessel (HEX200) and (e) Jejunal tissue has near-normal tissue appearance in RG-100 group (HEX100)

Histopathological findings: Figure 3a showed the absence of pathological findings in jejunal tissue of the HG. However, damage in villus epithelial cells and dilated congested capillaries were observed in jejunal tissue of the CCG (Fig. 3b). Figure 3c showed near-total necrosis in villus structures, damage in villus epithelial cells and mucosal crypt damage, in addition to mixed inflammatory cell infiltration accompanied by polymorphonuclear leukocytes and eosinophils, in jejunal tissue of cisplatin-treated groups. As shown in Fig. 3d, mild irregularities in villus structures and dilated congested capillaries in mucosa were observed in jejunal tissue of the RG-50. In contrast, jejunal tissue in the RG-100 had a near-normal tissue appearance (Fig. 3e).

Immunohistochemical results: In the HG, diffuseness and intensity of coloring of jejunal tissue by caspase-3 regarded as grade I diffuseness and intensity (+) were observed (Fig. 4a). As shown in Fig. 4b, diffuseness in jejunal tissue in the CCG was classed as grade III, intensity (+++). Jejunal tissue in the RG-50 had diffuseness grade II, intensity (++) as shown in Fig. 4c. Jejunal tissue in the RG-100 had diffuseness grade I, intensity (+)(Fig. 4d).

DISCUSSION

In this study, the protective effect of rutin against cisplatin-induced jejunal mucositis in rats was investigated. The results showed that rutin treatment prior to cisplatin exposure provided protection against cisplatin-induced jejunal toxicity. The findings of this study demonstrated that rutin provides protection against cisplatin-induced jejunal toxicity, possibly by preventing oxidative stress and apoptotic tissue damage. Many studies on cisplatin, the primary drug used in chemotherapy have been performed with the aim of enhancing its effectiveness and reducing its toxicity[2,4,6]. Despite these studies, the rate of mucositis after cisplatin treatment remains high. In response to cisplatin treatment, ROS levels increase and antioxidant defense system reserves decrease. The ROS induces oxidative stress and triggers

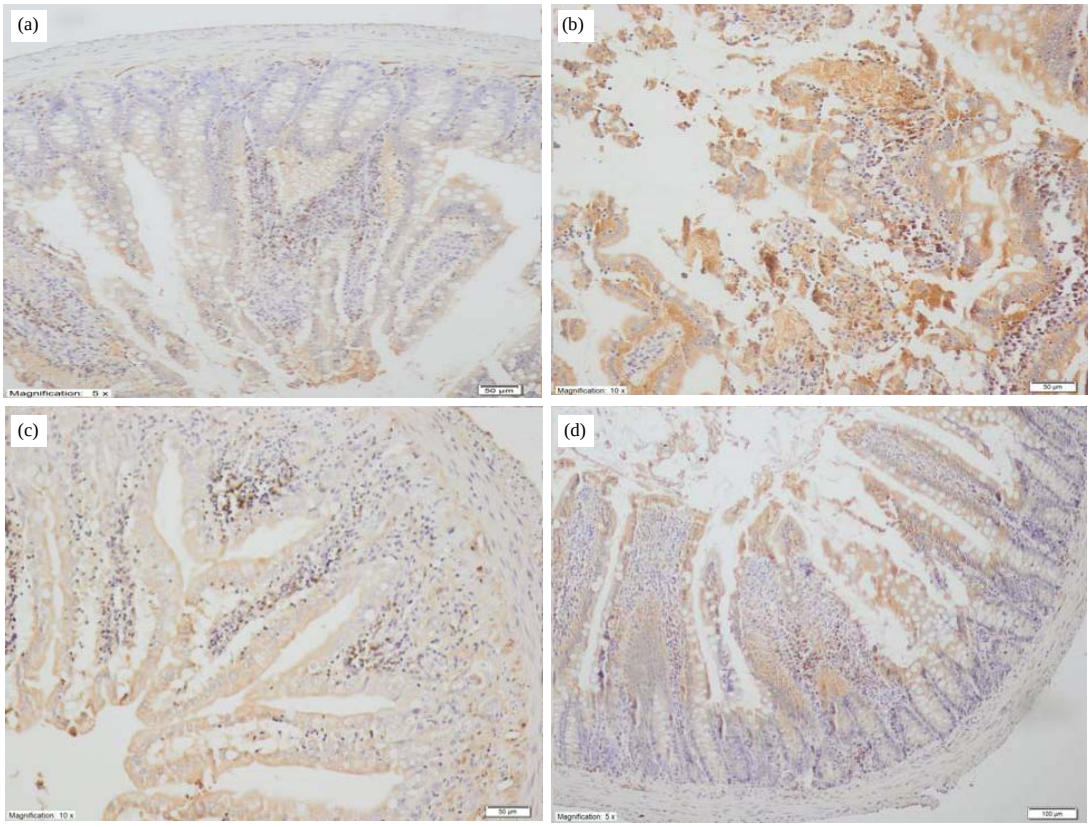

Fig. 4(a-d): Immunohistochemical evaluation of diffuseness and intensity of coloring jejunum tissues by caspase-3, (a) HG group's jejunal tissue, diffuseness grade I, intensity(+) (HEX 100), (b) CCG group, diffuseness grade III, intensity (+++) (HEX200), (c) RG-50 group, diffuseness grade II, intensity (++) (HEX200) and (d) RG-100 group; diffuseness grade I, intensity (+)(HEX 200)

apoptosis, which contributes to the active oxygen small intestinal toxicity[6,22-24]. An experimental study showed that the use of natural compounds with antioxidant, antiinflammatory and antiapoptotic potential, together with chemotherapeutics, increased the efficiency of chemotherapeutics and decreased systemic toxicity induced by chemotherapy[24]. Researches also demonstrated that rutin has important scavenging properties on oxidizing species, such as OH radicals, superoxide radicals and peroxyl radicals[25]. Also, rutin was superior to other flavonoids that act as pro-oxidizing agents and catalyze the production of oxygen radicals[26]. Thus, rutin appears to have potential as a nontoxic and non-oxidizable treatment agent.

Based on the biochemical results of the present study, upon cisplatin exposure, the level of MDA, which is an oxidizing molecule, increased in rat jejunum, whereas the levels of antioxidants, such as NO, GSH, GST, GPO and CAT decreased. Moreover, cisplatin caused jejunal cryptitis, crypt structure damage, villus epithelium flattening and atrophy, serosal blood vessel dilatation and mixed inflammatory cell infiltration. Rutin treatment prevented the increase in oxidants, decrease in antioxidants and histopathological jejunal tissue damage observed in the group treated only with cisplatin. Lipid peroxidation is an indicator of oxidative stress. In this study, the level of MDA, which is an end product of lipid peroxidation, was significantly elevated after cisplatin treatment in CCG. Moreover, upon oral administration of rutin prior to treatment, the cisplatin-induced increase in MDA levels significantly decreased. No studies in the literature appear to have investigated the effect of rutin on cisplatin-induced jejunal toxicity. However, one study examined the effect of rutin on methotrexate (MTX)-induced colon damage[27]. The authors showed that MTX increased MDA, an oxidation marker, in colon tissue and that intraperitoneal administration of rutin at doses of 50 and 100 mg kg^{-1} significantly inhibited MTX-induced increases in MDA[27]. In another study, rutin protected jejunal tissue from oxidative damage induced by radiation[28]. Furthermore, a combination of podophyllotoxin and rutin prevented radiation-induced gastrointestinal damage[29].

GSH is a low-molecular weight tripeptide-containing cellular antioxidant, which is involved in the detoxification of ROS produced by chemotherapeutic agents, thus maintaining cellular homeostasis crucial for proper functioning of cells[30]. The GSH forms a conjugate with electrophiles, such as cisplatin, which triggers ROS production and protects lipid membranes from peroxidation[31]. In this study, GSH levels decreased upon cisplatin administration as compared with that in the healthy group. The GSH levels were restored in the groups treated with 50 and 100 mg kg^{-1} of rutin prior to cisplatin treatment, although the degree was dose dependent. There are no studies in the literature have investigated the effect of rutin on cisplatin-induced decreases in jejunal tGSH. However, a previous study reported that endogenous antioxidants, such as GSH, appeared to be elevated by MTX and decreased by intraperitoneal administration of rutin at doses[27] of 50 and 100 mg kg^{-1}. In addition, in this study oral administration of rutin significantly inhibited cisplatin-induced decreases in GST and GPO activities in jejunal tissue. These findings support the idea that rutin prevents the reduction in GSH induced by cisplatin. Previous research reported that phase-II detoxifying enzymes, such as GST, catalyzed the conjugation between GSH and cisplatin and thus GST activity decreased after cisplatin treatment[32]. In this study, rutin treatment significantly decreased GST levels. Moreover, the activities of antioxidant enzymes, such as GPO, CAT and NO, decreased in the cisplatin group , whereas they increased significantly in the groups treated with rutin before cisplatin administration. Although no previous studies have investigated the effect of rutin on the reduction of cisplatin-induced increases in CAT activity in jejunal tissue, previous research reported that rutin inhibited decreased CAT activity caused by MTX[27].

Caspases are cysteine-dependent enzymes and are activated upon oxidative stress[33]. Caspase-3 plays an important role in regulating nuclear apoptosis, in addition to chromatin condensation. Caspase-3, an indicator of apoptosis, plays an essential role in processes associated with cellular damage and the formation of apoptotic bodies[33]. Previous research reported that caspase-3 was upregulated in rats treated with cisplatin[33-35]. In this study, caspase-3 expression significantly increased in the group treated only with cisplatin as compared with that in the healthy group. Pretreatment with rutin significantly reduced caspase-3 expression.

In this study, rutin treatment provided protection against the damage induced by cisplatin and prevented the disruption of the oxidant/antioxidant equilibrium in jejunal tissue of rats. In addition, rutin ameliorated histopathological disruption in jejunal tissue induced by cisplatin treatment. These results provide evidence of the protective effects of rutin against cisplatin-induced toxicity. To the best of our knowledge, there are no studies in the literature on the protective effect of rutin against cisplatin-induced jejunal mucositis.

CONCLUSION

The results of the present study indicated that oxidative stress and apoptosis were closely associated with cisplatin-induced toxicity. In this regard, rutin appeared to provide protection against cisplatin-induced jejunal toxicity via the attenuation of oxidative stress and apoptotic tissue damage. Rutin may be useful in the prevention of cisplatin-induced intestinal mucositis. The anticancer activity of cisplatin increases in accordance with increments in the dose but high doses cannot be administered in the clinical setting because they lead to small bowel damage. Rutin treatment during cisplatin chemotherapy may help to overcome this problem. The protection of rutin against cisplatin-induced small intestinal toxicity can be explored as a protective effect against the toxic effects of other chemotherapeutic drugs. In the present study, the protective effects of rutin against cisplatin-induced intestinal toxicity were better at a higher dose (100-50 mg kg^{-1}).

SIGNIFICANCE STATEMENT

This study discover the protective effect of rutin against cisplatin-induced oxidative damage that can be beneficial for preventing mucosal damage in patients using cisplatin. As is known, cisplatin causes oxidative damage in many tissues and organs, such as the gastrointestinal tract[3,4]. Although the protective effect of rutin against cisplatin-induced oxidative damage has been studied previously, this is the first study to investigate the protective effect of the drug against cisplatin-induced jejunal oxidative damage. The results revealed that rutin provided protection against cisplatin-induced decreases in the activity of jejunal antioxidant enzymes, including CAT. In the present study, rutin inhibited cisplatin-induced reductions in tGSH, GST and GPO levels. The findings indicate that rutin may be useful in reducing cisplatin-induced jejunal damage. Furthermore, rutin treatment could prevent treatment interruptions caused by intestinal damage. This study will help the researcher to uncover the critical areas of oxidative intestinal damage caused by cisplatin that many researchers were not able to explore. Thus a new theory on protective effects of rutin on cisplatin induced intestinal damage may be arrived at.

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