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Protective effect of rutin against cisplatin-induced nephrotoxicity in rats

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Abstract

Objective: To study the nephroprotector activity of the bioflavonoid, rutin (50 and 100 mg/kg, p.o.) on cisplatin-induced nephrotoxicity (6mg/kg, i.p.) in albino rats. Materials and Methods: Nephroprotector activity of rutin was assessed by estimating the levels of blood urea nitrogen, serum creatinine, serum total proteins, urinary protein, creatinine clearance, urine to serum creatinine ratio and lipid peroxidation in kidney. Results: Cisplatin elevated the serum markers level, increased the protein excretion in urine, reduced the creatinine clearance, urine to serum creatinine ratio and renal malondialdehyde level was increased. Animals which received rutin, in curative and prophylactic groups significantly reversed the effects induced by cisplatin. Conclusion: From the results it is revealed that rutin effectively reduced the cisplatin-induced renal toxicity in albino rats.

Keywords: Cisplatin, Nephrotoxicity, Rutin, Nephroprotector activity.

1. Introduction

Cisplatin is a potent anticancer agent with an efficacy against a wide variety of tumors [1-3]. The major dose limiting side effect of cisplatin is nephrotoxicity, which is localized to proximal tubule. The precise mechanism of cisplatin-induced nephrotoxicity has not been elucidated, but studies suggest that oxygen free radicals play an important role [4-8]. Hydration with chloride solution and administration of diuretics like mannitol and furosemide have proven both experimentally and clinically in decreasing cisplatin- induced renal toxicity [9]. On the other hand, sulfhydryl-containing agents such as diethyl dithiocarbamate and sodum-thiosulfate blocked cisplatin mediated renal injury [10,11]. Flavonoids are potent antioxidants and are known to modulate the activities of various enzyme systems due to their interaction with various biomolecules. Quercetin and venoruton were shown to decrease renal injury produced by cisplatin [12,13]. Rutin is a flavonoidal glycoside widely distributed in plant kingdom and contains quercetin as its aglycon part and rutinose as glycon part. A detailed review of literature of rutin revealed antioxidant activity along with

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antiinflammatory, antiulcer, antiviral, antineoplastic, aldose reductase inhibitory and CNS activities [14-19]. Previous reports evidenced that antioxidants and free radical scavengers effectively reduced cisplatin-induced lipid peroxidation and nephrotoxicity [20, 21]. The present study was undertaken to explore the ability of the flavonoidal glycoside, rutin to prevent cisplatin mediated renal injury in rats.

2. Materials and Methods

2.1. Chemicals

Rutin, cisplatin were purchased from Sigma Chemical Company (St. Louis, MO, USA.). Biochemical parameters were estimated by using commercial kits (Ranbaxy Diagnostics, New Delhi, India). All other chemicals used were of analytical grade (S.D. fine or Merck, India). Cisplatin was prepared in distilled water to give 1mg/ml solution. Rutin was suspended in 2% gum acacia to give 10mg/ml solution.

2.2. Animals

Wistar strain albino rats of 8-10 weeks of age, weighing 150-200g were used for the study. They were maintained on a standard diet (Gold Mohar,Bangalore) and drinking water was given *ad libitum*. They were housed in polypropylene cages and were acclimatized to laboratory environment for about a week. The study was conducted after obtaining Institutional ethical committee clearance. Animals were divided in to 8 groups (n=6) and put on the following treatment schedule (Table-1). To induce nephrotoxicity in rats, cisplatin dose selected was 6 mg/kg, intraperitoneally, single dose. Rutin was administered orally by gastric intubation.

2.3. Assessment of renal function

Blood was collected on days mentioned in Table-1 for estimation of blood urea nitrogen (BUN: Di acetyl monooxime method) [22], Serum creatinine (SC: Alkaline Picrate method) [22], Serum total proteins (S_{TP}: Biuert method) [22] by use of commercial kits (Ranbaxy Diagnostics, New Delhi, India). On day15 (Gr-IV, Gr-V_a and Gr-V_b) or 16 (Gr-II,Gr-III_a and Gr-III_b) urine was collected from each animal for six hours (initiated at 8 am) by use of metabolic cages and analysed for urinary creatinine, urinary total proteins (Sulphosalicylic acid method) [22], urine to serum creatinine ratio and creatinine clearance. Creatinine clearance was calculated by using formula:

Creatinine clearance = urinary creatinine X urinary volume h^{-1}/ serum creatinine

2.4. Lipid Peroxidation (LPO) in kidney

Lipid peroxidation was evaluated as the malondialdehyde (MDA) production as described by Heath and Beacker [23]. The animals were sacrificed by decapitation on day 15 or 16. The kidneys were dissected out, pressed in between blotted paper and both the kidneys were weighed and homogenized in 1.5% KCl with the help of Teflon homogenizer to get 20% of homogenate. To 1 ml of homogenate, 2.5 ml of trichloroaceticacid (20%) was added and centrifuged at 3500 rpm for 10 min. The resulting pellet was dissolved in 2.5 ml of 0.05 M H_2SO_4, and then 3 ml of thiobarbituric acid was added and incubated in water bath at 37°C for 30 minutes. The contents were extracted into 4 ml of n-butanol and the absorbance was measured spectrophotometrically at 530 nm against blank.

2.5. In vitro Studies

Effect of rutin on cisplatin-induced toxicity in rat renal cortical slices :

Renal cortical slices were prepared according to the method of Inselman [24]. Immediately

after the rats had been decapitated, the kidneys were removed and renal cortical slices were prepared using razor blade to achieve thickness of about 0.3 to 0.5 mm. 300 mg/ sample incubated, being shaken in oxygen-saturated solution with the following composition (in mM): 0.074 PAH, 96.7 NaCl, 7.4 sodium phosphate buffer (pH 7.4), 40.0 KCl, 7.4 $CaCl_2$ and 10.0 lactic acid, in a sample volume of 4.0 ml with cisplatin in the presence or absence of rutin for 120 min at 37°C. In control experiments, slices were incubated under identical conditions without cisplatin. At the end of the incubations renal cortical slices were removed from medium blotted on filterpaper, weighed and preceded for estimation of malondialdehyde (MDA), glutathione (GSH), uptake of p-aminohippurate (PAH). 100 mg of renal cortical slices from each sample were used to determine the amount of MDA as indicator of lipid peroxidation as described by Beuge and Aust [25]. Cortical slices were homogenized using Teflon homogenizer (Remi Motors, Mumbai) for 2 min in 5 ml of sodium phosphate buffer (PH 7.4) at 2°C, centrifuged 1000 g for 10 minutes at 2°C. To 1 ml of supernatant, 1 ml of thiobarbituric acid reagent (TBA) containing 0.375% TBA, 15% trichloroacetic acid (TCA) and 0.25N HCl was added. Samples were boiled for 15 min, cooled and centrifuged. Absorbance of the supernatant was spectrophotometrically measured at 532 nm. The MDA, concentration was calculated using molar extinction coefficient of MDA $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ and expressed as nmoles/ g of tissue as reported. Another 100 mg of cortical slices from each sample was used to estimate the glutathione (GSH) by the method of Seldak and Lindsay [26]. Homogenate was prepared in 20 mM EDTA solution. To 2.5 ml homogenate TCA (2.5 ml of 10%) was added.

Table 1. Treatment Schedule- Rutin.

Group	Treatment	Day of biochemical estimations	Purpose
I	Gum acacia	15, 16	Normal Control
II	Cisplatin 1st day, Gum acacia 6th to15th day	16	To act as curative- control(CC)
III_a	Cisplatin 1st day, Rutin (50 mg/kg) 6th to15th day	16	To assess curative effect
III_b	Cisplatin 1st day, Rutin (100 mg/kg) 6th to15th day	16	To assess curative effect
IV	Gum acacia 1st to10th day, Cisplatin 11th day.	15	To serve as prophylactic- control (PC)
V_a	Rutin (50 mg/kg) 1st to 10th day, Cisplatin 11th day	15	To assess prophylactic effect
V_b	Rutin (100 mg/kg) 1st to 10th day, Cisplatin 11th day	15	To assess prophylactic effect
VI	Rutin (100 mg/kg) 1st to 10th day	11	- -

Blood and urine were collected from animals of group-I, II, III_a, III_b on day 5 to check the induction of renal toxicity.

Table 2. Effect of rutin on cisplatin-induced renal damage.

Group	Treatment regimen	BUN (mg/dl)	SC (mg/dl)	S_{TP} (g/dl)
I	Vehicle	24.4 ± 0.8	0.5 ± 0.08	6.1 ± 0.9
II	Cisplatin + vehicle (Curative control)	48.2 ± 1.4[a]	1.2 ± 0.1[a]	7.4 ± 0.4[a]
III_a	Cisplatin + rutin, 50 mg/kg	39.9 ± 0.8[b]	1.1 ± 0.1[b]	7.2 ± 0.3[b]
III_b	Cisplatin + Rutin, 100 mg/kg	23.7 ± 2.8[a,b]	0.9 ± 0.1[a,b]	6.1 ± 0.2[a,b]
IV	Vehicle + Cisplatin (Preventive control)	104.0 ± 9.5[a]	2.5 ± 0.6[a]	10.7 ± 1.0[a]
V_a	Cisplatin + rutin, 50 mg/kg	80 ± 6.7[c]	2.3 ± 0.5[c]	9.9 ± 0.9[c]
V_b	Cisplatin + rutin, 100 mg/kg	77.2 ± 8.2[c]	2.2 ± 0.6[c]	9.5 ± 1.3[c]

Values were expressed as mean ± s.e.m. n=6. [a]P< 0.05 compared to group-I: [b]P<0.05 compared to group-II: [c]P<0.05 compared to group-IV (one-way ANOVA followed by student's Newman – Keuls post hoc test). Cisplatin was administered i.p single dose on day 1 in groups II, III_a, III_b animals and on day 11 in Gr - IV, V_a, V_b animals. Rutin was given orally for ten days (Gr-III_a, III_b animals from day 6 to 15 and Gr - IV, V animals from day 1 to 10). On day 15 (Gr-I, IV, V_a and V_b animals), 16 (Gr-I, II, III_a and III_b animals) blood and urine was collected to measure the BUN,SC,STP in rats.

Table 3. Effect of rutin on cisplatin-induced changes in renal. Functional parameters in rats

Group	Treatment regimen	U_{cr} / S_{cr}	Cl_{Cr} (ml/h/100gBd.Wt)	U_p (mg/24hr)
I	Vehicle	15.2 ± 0.7	17.0 ± 0.8	6.1 ± 0.3
II	Cisplatin + vehicle (Curative control)	7.1 ± 0.3[a]	9.9 ± 0.1[a]	16.1 ± 0.6[a]
III_a	Cisplatin + rutin, 50 mg/kg	12.8 ± 0.7[b]	12.0 ± 0.9[b]	8.9 ± 0.1[b]
III_b	Cisplatin + Rutin, 100 mg/kg	13.4 ± 0.4[a,b]	15.0 ± 0.6[a,b]	7.9 ± 0.4[a,b]
IV	Vehicle + Cisplatin (Preventive control)	6.1 ± 0.9[a]	6.0 ± 0.3[a]	17.1 ± 1.0[a]
V_a	Cisplatin + rutin, 50 mg/kg	6.6 ± 0.5[c]	7.2 ± 0.5[c]	14.3 ± 0.8[c]
V_b	Cisplatin + rutin, 100 mg/kg	7.1 ± 0.4[c]	8.3 ± 1.1[c]	12.2 ± 1.0[c]

Values were expressed as mean ± s.e.m. n=6. [a]P< 0.05 compared to group-I: [b]P<0.05 compared to group-II: [c]P<0.05 compared to group-IV.
The rutin was given p.o. from day1to day10 (Gr-V_a, V_b), from day 6 to day 15 (Gr-III_a and Gr-III_b), cisplatin was administered i.p. (6 mg kg-1 on day on day 1 (Gr-II, Gr-III_a and Gr-III_b) and 11 (Gr-IV Gr- V_a and Vb), Urine was collected on day nd on day 15 (Groups-I, II, III_a and III_b), or 16 (Gr-IV Gr- V_a and V_b) from all groups for measurement of above parameters.

Table 4. Effect of rutin on Lipid peroxidation in kidney

Group	Treatment regimen	MDA(nmol/mg tissue)
I	Vehicle	0.54 ± 0.09
II	Cisplatin + vehicle (Curative control)	0.89 ± 0.02[a]
III_a	Cisplatin + rutin, 50 mg/kg	0.78 ± 0.02[b]
III_b	Cisplatin + rutin, 100 mg/kg	0.64 ± 0.03[b]
IV	Vehicle + Cisplatin (Preventive control)	1.17 ± 0.07[a]
V_a	Cisplatin + rutin, 50 mg/kg	1.04 ± 0.06[c]
V_b	Cisplatin + rutin, 100 mg/kg	0.93 ± 0.11[c]

Values were expressed as mean ± s.e.m. n=6 (Student-Keuls Test was used). [a]P< 0.05 compared to group-I: [b]P<0.05 compared to group-II: [c]P<0.05 compared to group-IV.

Table 5. Effect of rutin against cisplatin-induced cytotoxicity in renal cortical slices

Drug	Concentration	GSH (n moles/g tissue)	MDH (n moles /g tissue)	PAH(slice/ medium ratio)
Control	-	108.1 ± 0.26	13.7 ± 0.2	3.7 ± 0.5
Cisplatin	2 mM	87.1 ± 0.7[a]	21.3 ± 1.9[a]	2.3 ± 0.3[a]
Cisplatin	100 µg/ml	85.2 ± 0.3[b]	14.6 ± 0.6[b]	2.6 ± 0.2[b]
(2mM) +Rutin	500 µg/ml	94.5 ± 1.0[b]	13.0 ± 0.4[b]	3.1 ± 0.2[b]
	1000 µg/ml	102.9 ± 0.9[b]	10.7 ± 0.3[b,a]	3.2 ± 0.2[b,a]

[*a]P<0.05 vs control * [b]P<0.05 vs cisplatin (one-way ANOVA followed by student's Newman–Keuls post hoc test) *Renal cortical slices were incubated with rutin in the presence or absence of cisplatin (2 mM) for 120 minutes. The rutin alone had no effect on Malondialdehyde (MDA) level, glutathione(GSH) content and p-aminohippuricacid (PAH) level. Results are expressed as mean ± SD (n=5).

After vigorous shaking, samples were centrifuged. To 0.5 ml supernatant, 1 ml of 400 mM Tris buffer (pH 8.9) and 0.1 ml of 5 mM DTNB (in methanol) was added and the absorbance was spectrophotometrically measured at 412 nm after 20min. The GSH concentration was calculated by using molar extinction coefficient 13,600 M^{-1} cm^{-1} and expressed as nmoles/g of tissue. Remaining 100 mg renal cortical slices were used for determination of PAH uptake. Renal cortical slices were homogenized in 5 ml of 3% TCA at 2°C; 2.0 ml of the corresponding medium was treated similarly. After centrifugation for 10 minutes at 1000 g, the PAH concentration of the supernatant of homogenate and the medium was measured according to Bratton and Marshal [27]. To 2.0 ml homogenate or incubation medium, 0.3 ml of 10 N H_2SO_4, 0.1 ml of 3M NaCl, 0.1 ml of 0.4% $NaNO_2$, 0.1 ml of 2% ammonium sulphamate and 0.1 ml of 0.2% napthyl ethylene diamine hydrochloride reagent were added. Each reagent was added at an interval of 5 minutes. After 60 min, absorbance of the samples were measured at 556 nm. The concentrations in the tissue and medium were calculated using PAH standard curve. The accumulation of PAH was expressed as slice/medium (S/M) ratio (concentration of PAH per g of tissue/ concentration of PAH per ml of medium)

2.6. Statistical analysis

The results are expressed as mean ± SEM and the data analysed using one way analysis of variance followed by post hoc Student-Keuls test using SPSS computer software for *in vivo* studies. Statistical significance was set at $P \leq 0.05$.

3. Results

Table-2 lists the effect of oral administration of rutin on cisplatin-induced elevation of BUN, SC and S_{TP} in rats. The levels of BUN, SC and STP were increased significantly in cisplatin treated animals (group II and group IV), when compared to normal control animals (group I). The extent of the elevation was reduced significantly in animals which received rutin in curative groups, III_a and III_b when compared to respective control (group II). The deterioration of the renal functions induced by cisplatin and the effect of rutin is given in Table-3. Cisplatin reduced creatinine clearance (Cl_{cr}), urine to serum creatinine ratio (U_{cr}/S_{cr}) and increased excretion of urinary protein in group II and IV animals, when compared to normal control animals. In curative regimen, animals

which received 100 mg/kg rutin significantly improved U_{cr}/S_{cr}, Cl_{cr} and reduced urinary protein excretion when compared to group II animals. In prophylactic regimen (group V_a and V_b), animals which received rutin ten days prior to the administration of cisplatin also significantly reduced the serum markers level, excretion of protein in urine, improved U_{cr}/S_{cr}, Cl_{cr} when compared to group IV animals and the protection is dose dependent. Rats treated with cisplatin showed increased malondialdehyde (MDA) content, whereas the rats pre-treated with rutin showed moderate decrease in MDA level and animals which belong to curative regimen showed significant reduction of MDA content (Table-4). The effect of rutin and cisplatin on MDA, glutathione (GSH) content and PAH uptake in renal cortical slices was listed in Table -5. Cisplatin increased the MDA level, reduced the GSH content and inhibited the PAH uptake. Rutin significantly reversed the effects that are caused by cisplatin.

4. Discussion

Cisplatin is a potent anticancer agent. However, its clinical use is limited by its renal toxicity [1-3]. The precise mechanism of nephrotoxicity has not been elucidated, but studies suggest that oxygen free radicals play important role [4-8]. Induction of nephrotoxicity by cisplatin is assumed to be a rapid process involving reaction with protein in renal tubules. As renal damage occurs within an hour after administration of cisplatin [28], it is important that the protective agent be present in sufficient concentrations in the renal tubules before injury occurs. This might explain the rationale behind prophylactic treatment. Cisplatin significantly elevated the levels of BUN and SC in rats. Cisplatin, at 6 mg/kg i.p. dose also caused in deterioration of renal functional parameters. Out of two regimens (curative and prophylactic), rutin was found to be active in curative regimen which was characterized by changes in serum markers level, urinary functional parameters, MDA content in kidney. The present study also showed that cisplatin causes LPO, decreased GSH content and inhibited PAH uptake. Rutin was active in inhibiting the lipid peroxidation, significantly increased the GSH levels and reversed the PAH uptake in renal cortical slices.

Over the past several years, the number of persons suffering from renal troubles is increasing because of inadvertent exposure of chemicals such as pesticides, heavy metals and usage of drugs due to therapeutic necessities. In the prevention of renal toxicity nutraceuticals, such as flavonoids have potential to play a key role. Flavonoids are a group of polyphenolic compounds which are present widely in plant kingdom both in the free State and as glycosides and possess wide biological activities. They have been demonstrated to exert beneficial effects on some diseases involving uncontrolled lipid peroxidation. The capability to interact with protein phosphorylation and the antioxidant, iron chelating and free radicals scavenging activity may account for the wide pharmacological profile of flavonoids [29, 30]. A number of flavonoids are known to possess good antiinflammatory, antitumor, antifungal, antibacterial and antihepatotoxic activities [31]. Rutin, a dietary flavonoid, which is used in the present study was reported to exhibit antiinflammatory, antineoplastic, antiulcer and CNS activities. Rutin and quercetin have now an established place in therapeutics. They have been shown in a number of studies to be potent antioxidants, capable of scavenging free radicals. Quercetin was reported to be effective against cisplatin-induced renal toxicity. However, administration of quercetin either p.o.

or i.p. was associated with toxicities such as mutagenicity [32]. In view of the advantages of rutin, being a non-toxic and non-oxidisable, it is interesting that rutin exhibited good protection in cisplatin-induced nephrotoxicity. Pharmacokinetic studies of rutin revealed that only about 17% of an ingested dose is absorbed [33]. Hence, in the present study, rutin was tested at moderately high doses of 50 mg and 100mg/kg.

In conclusion, our findings showed that rutin exhibited good nephroprotective activity against cisplatin-induced nephrotoxicity *in vivo* and *in vitro*. Hence, rutin supplementation during cisplatin therapy reduces the risk of cisplatin-induced nephrotoxicity. The nephroprotector activity of rutin may be because of its free radical scavenging property. The conclusions of the present study are interesting and give impetus for further studies.

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