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Protective effects of rutin against sodium fluoride induced nephrotoxicity and blood toxicity in rats

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ABSTRACT

Background: In present study, protective effect of Rutin against sodium fluoride (NaF)-induced nephrotoxicity was evaluated in rats.
Materials and methods: Renal injury was induced by daily administration of sodium fluoride 600 ppm in distilled water. Group I-control, group II-NaF given through drinking water, group III-vitamin E (100 mg/kg), group IV-rutin (50 mg/kg) and group V (70 mg/kg) along with sodium fluoride once a day for 2 weeks. Protective effects of rutin on sodium fluoride-induced nephrotoxicity in rats were determined for biochemical parameters in urine, serum, various antioxidant enzymes.
Results: Administration of nephrotoxic NaF treatment significantly ($P<0.01$) increased the level of specific gravity, urea, uric acid, protein, and creatinine clearance level whereas decreased ($P<0.01$) the pH and creatinine as compared to the control group. Treatment of rutin attenuated the NaF intoxication, dose dependently, and decreased ($P<0.01$) the specific gravity, urea, uric acid, protein and creatinine clearance while increased the pH and creatinine of urine. Activity of antioxidants catalase, superoxide dismutase, reduced glutathione contents were decreased ($P<0.01$) whereas lipid peroxidation product was increased. A significant decrease in body and organ weights occurred compared to control group. Treatment with rutin effectively ameliorated the alterations in the studied parameters of rats.
Conclusions: These results suggest that rutin works as an antioxidant *In vivo* by scavenging reactive oxygen species and this serves to prevent oxidative renal and haematological damage in rat treated with sodium fluoride.

Key words: Sodium fluoride; Rutin; nephrotoxicity; oxidative stress.

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INTRODUCTION

Fluoride is a well-known soil, water, and air contaminant, and it's toxicity to humans has been widely studied. Intake of excess of fluoride through drinking water, food, or inhalation causes a wide range of toxic effect known as fluorosis (Rao et al., 2011). Various forms of F are commonly used in toothpastes, mouth rinses, and even in certain processed beverages and public supplies to help prevent dental caries (Buzalaf et al., 2004). However, excessive intake of F leads to fluorosis, weakened antioxidant defence system, and increased oxidative stress in rat liver (Rupal et al., 2011). Thus it is toxic to living cells because it can generate reactive free radicals and cause alterations in biochemical indices including oxidative stress in a variety of animal species (Chawla et al., 2008). Fluoride ions can easily spread from the lungs and GIT to the blood. Numerous disorders have been connected to the systemic fluoride consumption (Inkielewicz and Krechniak, 2003; Blaszczyk et al., 2009). Previous studies demonstrated that antioxidant compounds such as vitamin E and vitamin C can protect kidney from oxidative stress (Simsek et al., 2005; Ozkaya et al., 2011).

Rutin works as a scavenger of reactive oxidative species (ROS) by donating hydrogen atoms to peroxy radicals, superoxide anions, and singlet oxygen and hydroxyl radicals (Henry et al., 2005); it also functions as a terminator and chelator of metal ions that are capable of oxidizing lipid peroxidation (Noorozi et al., 1998; Middleton et al., 2000). Rutin has been shown to function as an anti-cancer, anti-viral, anti-bacterial and anti-inflammatory agent. It is also used to treat cardiovascular and neurodegenerative disorders because of its appreciable free radical-scavenging and antioxidant

capacities (Wattenberg, 1992). Additionally, studies suggest that rutin alters signal transduction (Williams et al., 2004), causes activation of transcription factors and gene expression (Kampkotter et al., 2007, and may also protect DNA by interacting with carcinogens that have escaped detoxification processes (Hanasaki et al., 1994; 1 Srinivasan et al., 2005; Adewole et al., 2007).

The present study was therefore aimed at investigating the effect of rutin on NaF-induced nephrotoxicity in male Wistar rats by the determination of biochemical parameters and by histological examination.

MATERIALS AND METHODS

Chemicals

Reduced glutathione (GSH), sodium dihydrogen phosphate, sodium fluoride(NaF), potassium dihydrogen phosphate, gamma-glutamyl transferase (GGT), 1,2-dithio-bis nitro benzoic acid (DTNB), 5,5dithiobis(2-nitro benzoic acid)(Ellman's reagent), thiobarbituric acid (TBA), picric acid, sodium hydroxide, trichloroacetic acid (TCA) were purchased from Sigma-Aldrich Chemicals Co. St. Louis, USA. Kits for measurement of urea, uric acid, creatinine, magnesium, potassium, calcium were purchased from Sigma-Aldrich. Other reagents were of analytical grade or of higher purity.

Experimental animals and treatment

Six-week-old male Wistar rats weighing 180-220 g were provided with food and water *ad libitum* and kept at 20-22°°C on a 12-h light-dark cycle. The rats were acclimatized to laboratory condition for 7 days before commencement of experiment. All procedures were conducted as per guidelines of the committee for the purpose of control and supervision of experimental animals. All the pharmacological experimental protocols were approved by the Institutional Animal Ethics Committee (Reg no: MRCP/CPCSEA/IAEC/2013-14/MPCOL/10).

Experimental design

Group I: normal control; Group II: animals were treated with NaF (600ppm) through drinking water for two weeks. Group III: Vitamin-E (100 mg/kg, p.o) along with NaF (600ppm) for two weeks; Group IV: NaF (600ppm) through drinking water and rutin (50 mg/kg, p.o.) for two weeks; Group V: NaF (600ppm) through drinking water and rutin (70 mg/kg, p.o.) for two weeks.

After the completion of dosages rats were kept individually in metabolic cages for 24 h; urine was collected and volume was determined. All the animals were sacrificed; blood was withdrawn prior to the excision of organ. The serum was stored at −80°C after separation until it was assayed as described below. Half of kidney tissues were treated with liquid nitrogen and stored at −80°C for further enzymatic analysis while the other portion was processed for histology.

Analysis of urine

Urine samples were assayed for pH, specific gravity, urea, creatinine, protein and uric acid by using standard diagnostic kits (Span diagnostics Ltd., India). Urinary creatinine clearance was estimated by using the formula:

CrCl ¼ UxV=PXT

Where U: concentration of creatinine in urine
P: concentration of creatinine in plasma
V: 24 h of urinary volume
T: Time in minutes

Analysis of serum

Analysis of serum for blood urea, uric acid, creatinine, total protein (Lowry et al., 1951), calcium, magnesium, potassium was estimated by using standard span diagnostic kits, India.

Assessment of antioxidant profile

Renal tissue was homogenized in 10 volume of 100 mmol KH_2PO_4 buffer containing 1mmol EDTA (pH 7.4) and centrifuged at 12,000 × g for 30 min at 4°C. The supernatant was collected and used for the estimation of catalase (Chance and Maehly, 1955), SOD (Kakkar et al., 1984), reduced glutathione (Jollow et al., 1974) and MDA according to the standard procedures.

Statistical analysis

The values were expressed as the mean ± SEM for the 06 rats in each group. Differences between groups were assessed by one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) software package for Windows (version 13.0). Post hoc testing was performed for intergroup comparisons using the least significant difference (LSD) test. A value corresponding to $P<0.05$ was deemed to be statistically significant.

RESULTS

Effects of rutin on urine profile

Reactive oxygen species (ROS) especially nephrotoxic chemicals effects the urinary profile of kidney. Table 1 shows the changes in renal profile including pH, specific gravity, creatinine, creatinine clearance, urea and uric acid. Administration of nephrotoxic NaF treatment significantly ($P<0.01$) increased the level of specific gravity, urea, uric acid, protein, and creatinine clearance level whereas decreased ($P<0.01$) the pH and creatinine as compared to the control group. Treatment of rutin attenuated the NaF intoxication, dose dependently, and decreased ($P<0.01$) the specific gravity, urea, uric acid, protein and creatinine clearance while increased the pH and creatinine of urine.

Tables 2 and 3 show the assessment of serum biomarkers i.e. concentration of magnesium, calcium, potassium; creatinine, total protein; albumin and globulin indicate the functional integrity of the kidneys. NaF administration significantly ($P<0.01$) increased the level

of magnesium, creatinine, urea, uric acid, serum GGT while significantly (P<0.01) decreased the calcium, total protein as compared to control group. Serum level of these parameters were significantly (P<0.01) improved by administration of rutin as compared to NaF treated rats. However, more restoration effects on studied parameters were observed at the higher dose 70 mg/kg of rutin.

Table 1. Effects of Rutin on physical and biochemical parameters of urine.

Treatment Group	pH	Specific gravity	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Creatinine clearance (mg/ml)
Normal control	7.24±011	1.31 ±0.01	51.33±1.26	110.35±0.30	150.83±1.76	0.049 ±0.00
NaF (600ppm) Induced	6.59±0.09**	1.52±0.05**	201.00±0.71**	128.28±0.30**	39.00±0.71**	0.008±0.00**
VitE(100 mg/kg)+ NaF	6.75±0.03	1.01±0.01++	101.00±0.68++	115.33±0.36++	67.86±0.70++	0.025±0.00++
Rutin (50mg/kg) +NaF	6.61±0.09++	1.2 ±0.07++	124.67±0.49++	117.70±0.38++	56.17±0.60++	0.014±0.00++
Rutin (70mg/kg)+NaF	7.07±0.04++	1.2±0.06++	74.17±0.60++	112.50±0.25++	137.17±0.60++	0.039±0.00++

Values are expressed as Mean ±SEM (n=6).
The intergroup variation between various groups was measured by Dunnets multiple comparison test.
**indicates significance from the control group at P<0.01
++ indicates significance from the NaF group at P<0.01

Table 2. Effects of rutin on serum electrolyte profile

Treatment	Total protein (mg/dl)	Calcium (mg/dl)	Magnesium (mg/dl)	Potassium (Mmol/L)
Normal control	38.96 ±0.35++	17.05 ±0.34++	4.68 ±0.45++	9.20±0.32++
NaF	25.97±0.78**	16.24 ±0.41**	10.75± 0.62**	26.51±0.93**
Vit E+ NaF	26.55 ±0.56++	17.70 ±0.45++	6.02±0.33++	12.08±0.53++
Rutin (50mg/kg) + NaF	26.95 ±0.37**++	23.12 ±0.45++	5.12±0.47++	21.08±0.52++
Rutin (70mg/kg) + NaF	29.40 ±0.60++	26.42±0.55++	4.8 ±0.28++	10.60±0.45++

Values are expressed as mean±SEM (n=6). The intergroup variation between various groups was measured by Dunnets multiple comparison test.
**indicates significance from the control group at P<0.01
++ indicates significance from the NaF group at P<0.01

Table 3. Effects of rutin on serum urinary profile.

Treatment	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Serum GGT (mg/dl)
Normal control	49.50±0.76++	12.88±0.40++	5.86 ±0.31++	7.22±0.007++
NaF	174±0.71**	14.26 ±0.41**	7.89± 0.13**	12.34±0.011**
Vit E+ NaF	73.67 ±0.67++	13.53 ±0.41++	4.61±0.33++	8.73±0.011++
Rutin (50mg/kg) + NaF	99.17 ±0.60++	13.97 ±0.41++	6.77±0.36 ++	9.10±0.004++
Rutin (70mg/kg) + NaF	74.51 ±0.76++	13.63±0.31++	6.08 ±0.35++	8.14±0.004++

Values are expressed as mean±SEM (n=6). The intergroup variation between various groups was measured by Dunnets multiple comparison test.
**indicates significance from the control group at P<0.01
++ indicates significance from the NaF group at P<0.01

Effects of rutin on antioxidant profile

The results regarding the protective effects of rutin against the toxic affect of NaF in rat on kidney protein and activities of antioxidant enzymes such as CAT, SOD, GSH and MDA are shown in Table 4. Activities of antioxidant enzymes such as CAT, SOD and GSH were reduced, whereas MDA level increased (P<0.01) by treatment of NaF as compared to control group. This reduction in enzymes activity was reversed significantly (P<0.01), in a concentration dependent way, by the treatment of rutin as compared to the NaF group.

Table 4. Effect of rutin on renal antioxidant profile.

Treatment	MDA (nm/g tissue)	SOD (U/mg protein)	Catalase (U/min)	GSH (µmol/g tissue)
Normal control	74.00±0.58^{++}	19.05±1.54^{++}	18.2±2.2^{++}	1.72±0.157^{++}
NaF	141.67±0.42**	7.58±2.08**	7.5±0.85**	0.73±0.15**
Vit E+ NaF	95.00±0.58^{++}	19.75±1.4^{++}	17.37±1.17^{++}	1.68±0.17^{++}
Rutin (50mg/kg) + NaF	110.50±0.43^{++}	12.83±1.87^{*++}	11.5±2.2^{*++}	1.28±0.19^{*++}
Rutin (70mg/kg) + NaF	92.00±0.58^{++}	17.62±2.14^{++}	16.5±2.6^{++}	1.58±0.16^{++}

Values are expressed as mean±SEM (n=6). The intergroup variation between various groups was measured by Dunnets multiple comparison test.
**indicates significance from the control group at P<0.01
++ indicates significance from the NaF group at P<0.01

Effects of rutin on increase in body weight, absolute kidney weight and relative kidney weight

Protective effects of different dosages of rutin against NaF administration to rat, increase in body weight, absolute kidney weight and relative kidney weight are shown in Table 5. Administration of NaF to rats significantly increased (P<0.01) the absolute kidney weight, relative kidney weight while significantly decreased (P<0.01) the percent increase in body weight for two experimental weeks as compared to the control group. Administration of rutin consistently restored (P<0.01) the percent increase in body weight, kidney weight and relative kidney weight as compared to the NaF group. More protective effects of rutin for the percent increase in body weight, kidney weight and relative kidney weight against the NaF intoxication to rats were observed at the higher concentration of rutin (70 mg/kg).

Table 5. Effect of Rutin on organ weight of NaF treated rats.

Treatment	Adrenal glands (mg)	Heart(mg)	Kidneys(mg)	Brain(mg)	Liver(mg)
Normal control	44.00±0.57	393.16±1.55	1391.00±2.06	1682.67±3.76	6026.67±4.41
NaF	74.49±0.47**	571.99±0.71**	1641.93±1.02**	1747.33±1.50**	5455.17±2.43**
Vit E+ NaF	32.99±0.49^{++}	353.99±1.11^{++}	893.49±1.40^{++}	1111.64±0.81^{++}	2353.33±1.67^{++}
Rutin (50mg/kg) + NaF	53.66±0.49^{++}	491.66± 0.57^{++}	1454.99±1.28^{++}	1587.38±1.03^{++}	4827.50±3.82^{++}
Rutin (70mg/kg) + NaF	46.16±0.42^{++}	476.99±0.71^{++}	1233.83±2.45^{++}	1313.96±0.94^{++}	4723.1±1.46^{++}

Values are expressed as mean±SEM (n=6). The intergroup variation between various groups was measured by Dunnets multiple comparison test.
**indicates significance from the control group at P<0.01
++ indicates significance from the NaF group at P<0.01

DISCUSSION

Rutin modulates several biological functions and exhibits anti-cancer, anti-viral, anti-bacterial and anti-inflammatory activities due to its appreciable free radical-scavenging and anti-oxidant capacities (Williams et al., 1992). At the end of the study period, NaF treatment was found to have decreased the percent increase in body weight but increased the absolute kidney weight and relative kidney weight as compared with the control group. Similar alterations for these parameters with NaF have been determined previously (Kurokawa et al., 1990). In the present study, the observations made with respect to percent increase in body weight, absolute kidney weight and relative kidney weight in male wistar rats appeared to suggest that rutin can alleviate NaF-induced toxicity in these animals.

Urinalysis provides important clues about acid-base balance and kidney function. High levels of urea, uric acid and protein in urine reflect the kidney dysfunction and renal injuries induced by NaF treatment (Ogeturk et al., 2005). Increased specific gravity is a basic symptom of dehydration, renal artery steatosis, necrosis, and decreased blood flow to the kidneys. In addition, increased RBC and WBC counts in the urine of NaF-treated rats suggested severe injuries to renal tissues. Higher levels of protein and the number of RBCs and WBCs might have also contributed to the values of specific gravity obtained in the present study. This increase in specific gravity consequently reflects the high degree of damage to kidney tissue. The hematuria and proteinuria observed in the present study could be related to necrosis and kidney dysfunction (Bhattacharya et al., 2005). Ogawa et al. also found that the glomerular capillary wall is permeable to low molecular-weight proteins. Therefore, an appreciably high level of proteinuria indicates the leakage of low molecular weight proteins. The oxidative stress induced by NaF might

promote the formation of various vasoactive mediators that can affect renal function directly by initiating renal vasoconstriction or decreasing the glomerular capillary ultrafiltration coefficient. This action will reduce the glomerular filtration rate, leading to proteinuria. Rutin administration to rats treated with NaF ameliorated the toxicity of NaF in kidneys to restore the level of the studied parameters in a concentration dependent manner. These results suggest that rutin can be used as renoprotective agent against NaF induced toxicity.

The present study revealed that NaF administration caused marked increases in the serum levels of creatinine, uric acid, serum GGT, potassium, and magnesium as reported previously. The results suggested that rutin prevented NaF -induced toxicity, and that the levels of creatinine, uric acid, total protein, and calcium could be altered to those seen in the control group. There is a large body of evidence implicating oxidative stress and ROS in the mechanism of NaF-induced toxicity in animal models. In the present study, the mean activity of the antioxidants CAT, SOD and GSH were found to be significantly lowered in the NaF-treated group compared with that of the control group. Lowered activities of these antioxidant enzymes with NaF in in-vivo experimental models have been reported (Khan et al., 2003). However, the treatment of rutin with NaF modified the biochemical changes caused by NaF in rat. In the present study, the mean activities of antioxidant enzymes were significantly higher compared with those of the NaF -treated group and thus had a potential protective effect. GSH is a vital extracellular and intracellular protective antioxidant against oxidative stress. It reduces hydrogen peroxides and hydroperoxides by its redox and detoxification reactions, and protects protein thiol groups from oxidation. In the present study, the mean level of GSH upon NaF treatment was depleted in the kidneys compared with that seen in the control group. Following decreases in the level of GSH, oxidative stress increases and, thereafter, cell damage occurs (Khan et al., 2003). In the present study, the animals receiving NaF showed glomerular injuries, tubular necrosis, tubular dilatation, tubular cell swelling and tubular brush border loss. Endogenous levels of GSH were found to be increased with an accompanying increase in the mean activities of CAT, and SOD with rutin to that of the NaF treated group. Free radicals and reactive oxygen species mediate the propagation of peroxidation of polyunsaturated fatty acids, this cascade can be prevented through enzymatic and non-enzymatic antioxidants.

CONCLUSION

The protective role of rutin at different levels was evaluated in this manuscript. It may contribute its protective effects by erasing the damaging action of sodium fluoride at various metabolic cycles, and the repair of DNA damage. The protective potential may involve scavenging potential and antioxidant capacity to ameliorate the NaF induced toxicity. This study substantiated the scientific evidence in favors of its pharmacological use in renal injuries.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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