Arab Journal of Nuclear Science and Applications, 46(1), (205-213) 2013

The Synergistic Effects of Rutin and Urate Oxidase on Nephrotoxicity in Rats

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Received: 20/3/2012 **Accepted: 30/4/2012**

ABSTRACT

This study was conducted to investigate the possible anti-nephrotoxic effect of rutin or/and urate oxidase in gentamicin (GM) induced nephrotoxic rats. Two experiments were carried out, the first one showed that daily injection of 80 mg GM/kg b.wt ineraperitonealy (I.P) for two weeks induced acute renal failure indicated by significant elevation in serum urea, creatinine, uric acid, potassium, inorganic phosphorus, parathyroid hormone and thiobarbituric acid reactive substance and a significant decline in serum sodium, total & ionized calcium with a pronounce decrease in the content of glutathione and the activities of oxidized glutathione, glutathione peroxidase, superoxide dismutase and catalase when compared with their corresponding values in saline injected rats. In the second experiment, comparisons were made between GM induced nephrotoxic rat and other nephrotoxic groups received daily additional oral rutin (100 mg/kg diet), urate oxidase injection (10 mg/100 g b.wt.) and their mixture for 15 & 30 days after the incidence of nephrotoxicity. All the previous parameters were corrected to some extent after the nephrotoxic rats treated with rutin, urate oxidase and their combination.

Keywords: Nephrotoxicity / Rutin / Urate Oxidase / Rats.

INTRODUCTION

One of the most common manifestation of nephrotoxic damage is acute renal failure which is characterized by decline in glomerular filtration rate with resulting azotaemia. The incidence of renal dysfunction following aminoglycoside administration was detected by Abdel-Naim *et al.* [1]. Gentamicin (GM) is an aminoglycoside antibiotic that is very effective in treating life-threatening gram-negative infections and positive bacteria [2]. Unfortunately, 30% of patients treated with GM for more than 7d show some signs of nephrotoxicity [3]. It has been reported that GM-induced nephrotoxicity is characterized by direct tubular necrosis, which is localized mainly in the proximal tubule. The specificity of gentamicin for renal toxicity is apparently related to its preferential accumulation in the renal proximal convoluted tubules (50 to 100 times greater than serum) [4]. The exact mechanism of GM-induced nephrotoxicity is unknown. However, GM has been shown to enhance the generation of reactive oxygen species (ROS) causing deficiency in intrinsic antioxidant enzymes [5]. ROS have been suggested as a cause of death for many cells in different pathological states including various models of renal and cardiac diseases [6]. Accordingly, the use of several compounds with antioxidant activity has been successfully used to prevent or ameliorate GM-induced nephrotoxicity [7].

Flavonoids comprise a large group of secondary metabolites occurring widely throughout the plant kingdom including food plants [8]. It has been become increasingly popular in terms of health protection because they possess a remarkable spectrum of biochemical and pharmacological activities

[9].Flavonoids affect basic cell function such as growth, differentiation and apoptosis. Also, they were showed to be potent antioxidant because of their radical-scavenging activity; ability to complex heavy metal ion and to antagonize a broad spectrum of enzymes such as tyrosine protein kinase [10]. Rutin is a flavonol glycoside comprised of the quercetin and the disaccharide rutinose (rhamnose and glucose). It is found in many plants, fruits and vegetables. The richest source is buckwheat. Also, it is found in citrus fruits, noni, black tea and apple peel. It has shown pharmacological benefits including anti-tumor [11] ; anti-allergic [12] ; anti-mutagenic [13] ; myocardial protecting [14] ; immunomodulator [12] and hepato-protective activities [15] . However, until now, the protective effect of rutin against GM-induced nephrotoxicity has not been investigated.

Urate oxidase is an enzyme that catalyses the conversion of uric acid into allantoin which is 10 times more soluble than uric acid and more readily eliminated by the kidney. Human and certain primates lack this enzyme. It is a potent and fast acting urate lowering drug used for the prevention of acute urate nephropathy [16].

Therefore, the current study was aimed to evaluate the therapeutic effects of rutin or urate oxidase and their mixture in nephrotoxic rats following gentamicin administration in male albino rats.

MATERIAL AND METHODS

Sixty adult male albino rats *(Rattus rattus)* were employed in this study. They were housed in a well-ventilated vivarium of Biological Applications Department, Nuclear Research Center, Atomic Energy Authority, Egypt. They were caged in wire bottom galvanized metal wall boxes under controlled environmental and nutritional conditions (25°C and 55-60% relative humidity) and they fed on a standard diet according to NRC [17] and fresh tap water was available at all times.

The study included two experiments; the first one was carried to investigate the changes in kidney function tests as a result of a gentamicin (GM) administration. So, two groups of rats were selected. The first group (50 rats) was received daily I/P injection of GM (Memphis Co. Pharm and Chem. Ind. Cairo, ARE) at a dose of 80 mg/kg b.wt for two weeks as described by Ohtani *et al.* [18] to induce experimentally acute renal failure. The other group (10 rats) was received daily injection of normal saline (0.9% NaCl) for two weeks and served as control group.

In the second experiment, four comparisons were made between forty rats from the previous test with gentamicin-induced acute renal failure arranged equally into four groups. The first nephrotoxic rats group was left as recovery positive control. The second one was received additional oral dose of rutin (Sigma Chem. Co. ST Louis, Mo, USA) 100 mg/kg diet/day [19] for 15 & 30 days after the end of gentamicin course. The third group was interaperitonealy injected with urate oxidase 10 mg/100 gm b.wt. for the same previous periods as described by Ding *et al.* [20]. The fourth group was received a daily mixture of the same doses of rutin and urate oxidase for the same previous intervals (15 & 30 days).

At the end of each experimental period, blood samples were collected from each group by decapitation killing. Blood samples were left to clot at room temperature then centrifuged at 3000 r.p.m for 15 minutes for serum separation. Sera were then kept at -20° C till determination of biochemical parameters. Serum urea, creatinine and uric acid were determined colorimetrically using commercial kits (Randox, UK.) according to the methods of Fawcett & Scott [21] ; Seeling & Wust [22] and Barham & Trinder [23] respectively. Sodium (Na) and potassium (K) analysis were accomplished by emission flame photometry after suitable dilution as described by Dean [24]. Serum calcium was determined colorimetrically with the help of (Perkin-Elimer) atomic absorption spectrophotometer, using commercial kits (Human, Gesell Schaft for Biochemical and Diagnostic, Wiesbaden, Germany) according to the method of Barnett *et al.* [25] . The concentration of ionized calcium was calculated according to Mclean & Hasting [26] . Inorganic phosphorus was determined colorimetrically using kits supplied by Biomerieux (France) according to the method of Goldenberg & Fernands [27]. Parathyroid

hormone (PTH) was assayed using radioimmunoassay kit using solid phase component system (Diagnostic Products Corporation DPC, Los Angeles, California, USA).

Kidneys were dissected at the end of each experimental period, washed with saline solution (0.9% NaCl) and homogenized in ice-cold 0.25 M sucrose containing 1mM diethylenetriamine penta-acetic acid (1:1 w/v). Each sample was then centrifuged for 20 min at 20,000 g and 4°C. The supernatant was used as the source of experimental product. The content of kidney glutathione (GSH) and oxidized glutathione (GSSG) were estimated according to Baker *et al.* [28] and Aseni *et al.* [29] respectively. The enzymatic activity of renal glutathione peroxidase (GP_x) [30] , superoxide dismutase (SOD) [31] and catalase [32] were assayed by ELISA technique using commercial kits (IBL, Gesellschaft, Hamburg, Germany). Moreover, thiobarbituric acid reactive substance (TBARS) level in kidney tissues was determined colorimetrically according to Hogberg *et al.* [33] using commercial ELISA kit (Cayman Chem. Co., USA).

All data were subjected to statistical analysis according to Snedecor & Cochran [34] using a computer program (Costate) on way completely randomized. Students "t" test was used in the first experiment and analysis of variance "ANOVA" test followed by Duncan multiple range test [35] were used for analysis of data in the second experiment.

RESULTS AND DISCUSSION

In the current study, rat was used as an animal model for induction of acute renal failure by gentamicin injection at a dose 80 mg / kg b.w. for two weeks equivalent to that used clinically in man as described by Ohtani *et al.* [18] . Acute renal failure is characterized by disorders in some biochemical parameters in GM treated rats as shown in the first experiment presented in table (1). GM produced significant ($p<0.05$) increase in the concentrations of serum urea, creatinine and uric acid. These results confirmed that GM produced nephrotoxicity as previously reported by Ali *et al.* [36] . These changes reflected the severity of renal insufficiency which occurred in association with the sudden fall in glomerular filtration rate because of the majority of administrated GM enters specifically the proximal tubular epithelial cells, binds to anionic phospholipids in the target cells inducing abnormalities in the function and metabolism of multiple intracellular membranes and organelles then developed injury in the proximal tubular epithelial cells of kidney that caused acute renal failure [37] .

Serum electrolytes were disturbed significantly ($p<0.05$) in GM treated rats as compared with control animals. Lower value of serum sodium indicates inability of kidney to conserve sodium and chloride. Haemodilution may be involved in the fall of sodium value *via* excess of water intake and or increased production of endogenous water. In turn, the reversed increases of potassium observed may be due to reduced excretion of potassium aggravated by leakage of intracellular potassium into blood stream as a result of GM induced lesions in renal tubular epithelium. These results are in harmony with the data obtained by Heibashy & Abdel Moneim [38] . Serum phosphate and PTH were significantly ($p<0.05$) increased, conversely, serum total and ionized calcium were significantly ($p<0.05$) decreased in GM injected rats. Similar results were obtained by Heibashy *et al.* [39] . The authors attributed these disturbances to the elevated parathormone level which produced after GM administration. Furthermore, increased glucocorticoids levels enhance deposition of calcium as calcium phosphate and carbonate in injured skeletal muscle [40] . Also, the toxicity of GM may cause an increase in the urinary excretion of calcium and inhibited calcium intake into mitochondria and stimulate ionized calcium from mitochondria [41] .

Glutathione (GSH) has a very important role in protecting against oxygen free radical damage by providing reducing equivalents for several enzymes; GSH is also a scavenger of hydroxyl radicals and singlet oxygen [42]. In the present study, the content of GSH and GSSG in rat kidney tissues were significantly ($p<0.05$) reduced after GM injection as compared to control group (Table 1). This result is confirmed by other studies, which have pointed to reduction of GSH levels after GM

administration [43,44] . An explanation to GSH depletion after GM treatment might be due to NADPH depletion or increased consumption of GSH in non-enzymatic removal of oxygen-radicals [45] . In addition, oxidation of GSH to GSSG by the oxidant stress, with efflux of GSSG being the major factor responsible for maintenance of the redox ratio. GSSG is of great biological importance, since it allows fine-tuning for the cellular redox environment under normal conditions and upon the onset of stress, and provides the basis for GSH stress signaling [46]. Sinha *et al.* [47] have reported that galactosamine, an established experimental toxin, decreases the reduced glutathione (GSH) and enhances the renal tissue content of the oxidized form (GSSG). The conversion of GSSG to GSH is mediated through the enzyme glutathione reductase. Therefore, GM may act like *S*-(1,2-dichlorovinyl)-L-cysteine a known nephrotoxicant and interferes with the recycling of GSSG into GSH by inhibition of the enzyme glutathione reductase [48] .

Table (1): Nephrotoxic effects of gentamicin on some biochemical parameters in rats after two weeks administration.

Parameters	Normal control rats (n=10)	Nephrotoxic rats (n=10)
Urea (mg/dL)	19.31 ± 0.16	77.83 ± 1.67 *
Creatinine (mg/dL)	0.51 ± 0.02	2.12 ± 0.07 *
Uric acid (mg/dL)	0.37 ± 0.01	1.02 ± 0.09 *
Na (meq/L)	134.2 ± 0.58	119.87 ± 1.73 *
K (meq/L)	4.15 ± 0.08	5.51 ± 0.12 *
Inorganic Ph (mg/dL)	8.02 ± 0.13	9.11 ± 0.21 *
Total calcium (mg/dL)	9.13 ± 0.16	7.88 ± 0.16 *
Ionized calcium (mg/dL)	2.31 ± 0.08	1.68 ± 0.13 *
PTH (pg/ml)	14.42 ± 0.17	23.83 ± 0.33 *
GSH (mg/g protein)	179.23 ± 1.82	121.68 ± 1.41 *
GSSG (mg/g protein)	2.11 ± 0.09	1.43 ± 0.07 *
GPx (µmole/min/mg protein)	154 .08 ± 1.51	113.0 ± 1.08 *
SOD (NU/30 min/g protein)	5.36 ± 0.62	3.79 ± 0.38 *
CAT (µM/mg/g protein)	516.33 ± 2.37	339.41 ± 1.84 *
TBARS (nmol/g tissue)	112.05 ± 1.71	185.17 ± 2.88 *

- Values are expressed as mean ± SE. - n = number of rats.
- * Means within the same row are differ significantly at ($P<0.05$).

The activities of endogenous antioxidant enzymes (GPx, SOD and CAT) were greatly reduced in GM-treated rats compared with control group (Table 1). GPx serves to detoxify peroxides by reacting them with GSH [49] . The scavenging of superoxide radicals is achieved through an upstream enzyme, SOD, which catalyses the dismutation of superoxide to H_2O_2 [50]. This reduction in GPx, SOD and CAT activities after GM injection has been previously recorded [40] suggesting that oxidative stress is one of the causes of GM-induced renal damage.

The obtained data from table (1) showed significant ($p<0.05$) increase in TBARS which is a lipid peroxidation product. This result is in harmony with Ali *et al.* [36] who reported that GM

nephrotoxicity showed increased lipid peroxidation in the renal cortex and explains the nephrotoxicity of GM due to tissue damage by free radicals resulted from GM administration. Also, Cuzzocrea *et al.* [3] reported that GM is able to generate free radicals as hydrogen peroxide, hydroxyl radical and superoxide anions in rat renal mitochondria. Moreover, GM causes rapid changes in membrane lipid composition. These changes of membrane lipid composition may be induced by free radical-initiated lipid peroxidation [51]. This view is supported by increased MDA level which is a one of the product of lipid peroxidation in GM treated rats kidney [52].

Table (2) : Ameliorating effects of rutin, urate oxidase or their co-administration on some biochemical parameters of gentamicin induced nephrotoxic rats.

Parameters		Nephrotoxic recovery group n=10	Nephrotoxic treated with rutin n=10	Nephrotoxic treated with urate oxidase n=10	Nephrotoxic treated with co-administration n=10
Urea (mg/dL)	15 days	72.62 ± 1.68 ^{A}	69.12 ± 1.59 ^{B}	65.92 ± 1.62 ^{C}	62.98 ± 1.55 ^{E}
	30 days	69.87 ± 1.56 ^{B}	64.47 ± 1.64 ^{C}	61.01 ± 1.51 ^{D}	51.32 ± 1.41 ^{F}
Creatinine (mg/dL)	15 days	1.95 ± 0.08 ^{A}	1.78 ± 0.08 ^{C}	1.56 ± 0.09 ^{E}	1.55 ± 0.07 ^{E}
	30 days	1.67 ± 0.08 ^{B}	1.40 ± 0.07 ^{D}	1.29 ± 0.07 ^{F}	1.01 ± 0.08 ^{G}
Uric acid (mg/dL)	15 days	0.99 ± 0.09 ^{A}	0.92 ± 0.08 ^{C}	0.82 ± 0.07 ^{E}	0.81 ± 0.06 ^{E}
	30 days	0.76 ± 0.09 ^{B}	0.73 ± 0.09 ^{D}	0.65 ± 0.07 ^{F}	0.58 ± 0.05 ^{G}
Sodium (meq/L)	15 days	123.22 ± 1.95 ^{A}	123.14 ± 1.94 ^{A}	124.12 ± 1.84 ^{A}	126.98 ± 1.89 ^{B}
	30 days	127.11 ± 1.91 ^{B}	126.89 ± 1.81 ^{B}	133.40 ± 1.77 ^{C}	134.38 ± 1.92 ^{C}
Potasium (meq/L)	15 days	5.41 ± 0.11 ^{A}	5.38 ± 0.11 ^{A}	5.41 ± 0.10 ^{A}	5.32 ± 0.09 ^{A}
	30 days	4.86 ± 0.08 ^{B}	4.59 ± 0.12 ^{B}	4.67 ± 0.08 ^{B}	4.22 ± 0.08 ^{C}
Inorganic Phosphorus (mg/dL)	15 days	9.61 ± 0.18 ^{A}	9.36 ± 0.16 ^{A}	9.30 ± 0.16 ^{A}	9.29 ± 0.17 ^{A}
	30 days	8.97 ± 0.16 ^{B}	8.99 ± 0.13 ^{B}	8.61 ± 0.14 ^{B}	8.49 ± 0.15 ^{B}
Total Calcium (mg/dL)	15 days	7.81 ± 0.19 ^{A}	8.02 ± 0.16 ^{B}	8.03 ± 0.18 ^{B}	8.21 ± 1.14 ^{B}
	30 days	8.09 ± 0.17 ^{B}	8.48 ± 0.18 ^{C}	8.53 ± 0.17 ^{C}	9.23 ± 1.16 ^{D}
Ionized Calcium (mg/dL)	15 days	1.69 ± 0.11 ^{A}	1.77 ± 0.11 ^{B}	1.85 ± 0.14 ^{B}	1.87 ± 0.11 ^{B}
	30 days	1.82 ± 0.08 ^{B}	1.98 ± 0.08 ^{C}	2.12 ± 0.11 ^{D}	2.34 ± 0.8 ^{E}
PTH (pg/ml)	15 days	24.58 ± 0.36 ^{A}	21.99 ± 0.34 ^{B}	21.01 ± 0.31 ^{B}	19.89 ± 0.29 ^{D}
	30 days	20.93 ± 0.25 ^{B}	18.32 ± 0.29 ^{C}	17.88 ± 0.29 ^{C}	15.08 ± 0.25 ^{E}

- Values are expressed as mean ± SE. - n = number of rats.
- Means bearing different superscript (A, B, C, D, E, F & G) within the same parameter are differ significantly at ($P<0.05$).

From the data of the second experiment which presented in table (2), it was obvious that rutin administration proved to have some ameliorating effects against undesirable changes in kidney function following GM injection for two weeks. With the progress of time after the GM was discontinued, serum urea, creatinine, uric acid and PTH were corrected significantly ($p<0.05$) in rutin group as compared with recovery nephrotoxic group (positive control), although the levels of all those

variables were still significantly ($p<0.05$) higher than saline injected rats (negative control). On the other hand, serum sodium, potassium and inorganic phosphorus levels did not show any significant changes between rutin treated group and the recovery nephrotoxic one. Total and ionized calcium showed a significant ($p<0.05$) increase all throughout the period of treatment with rutin than recovery nephrotoxic group. These results could be in accord with several other researches, which reported that compounds with antioxidant properties like *S*-allylmercaptocysteine [53] and dia llyl sulfide [54] inhibited the increased urinary excretion of total protein induced by GM in rats.

Rutin exerts its antioxidant effects by scavenging free superoxide and hydroxyl radicals on one hand and by inhibiting xanthine oxidase activity and lipid peroxidation on the other [55]. In the current study, treatment with rutin markedly enhanced the content of GSH & GSSG and the activities of GPx, SOD and CAT enzymes but, reduced the elevated levels of TBARS (Table 3). These results indicating the ability of rutin to transfer electrons and free radicals in addition to activation of antioxidants enzymes and decreases oxidative stress through its antioxidant properties [55]. Also, it has been reported that rutin has effectively reversed the biochemical, behavioral, and neurochemical changes in rat treated with haloperidol [56] and improved the antioxidants enzymes system in human hepatoma cell line (Hep G2) by inhibition MDA levels and increasing CAT activity and therefore preventing or delay oxidative damage and its adverse effects [57]. Moreover, the antioxidant enzyme status was increased after rutin feeding in normal liver and in diabetic liver and kidney [58].

Table (3) : Ameliorating effects of rutin, urate oxidase or their co-administration on some antioxidant enzymes and lipid peroxidation of kidney nephrotoxic rats.

Parameters		Nephrotoxic recovery group n=10	Nephrotoxic treated with rutin n=10	Nephrotoxic treated with urate oxidase n=10	Nephrotoxic treated with co-administration n=10
GSH (mg/g protein)	15 days	128.25 ± 1.49^{A}	135.04 ± 1.56^{B}	142.97 ± 1.60^{C}	154.86 ± 1.66^{D}
	30 days	134.46 ± 1.51^{B}	143.98 ± 1.52^{C}	155.06 ± 1.62^{D}	161.13 ± 1.58^{E}
GSSG (mg/g protein)	15 days	1.49 ± 0.12^{A}	1.61 ± 0.11^{C}	1.63 ± 0.11^{C}	1.78 ± 0.10^{E}
	30 days	1.72 ± 0.11^{B}	1.84 ± 0.12^{D}	1.88 ± 0.12^{D}	2.03 ± 0.09^{F}
GP_X (µmol/min/ mg protein)	15 days	113.31 ± 1.11^{A}	120.18 ± 1.19^{B}	132.19 ± 1.27^{C}	137.99 ± 1.26^{D}
	30 days	119.76 ± 1.17^{B}	125.45 ± 1.21^{C}	141.31 ± 1.24^{D}	152.07 ± 1.34^{E}
SOD (NU/30 min/ g protein)	15 days	3.82 ± 0.94^{A}	4.09 ± 0.86^{B}	4.27 ± 0.79^{C}	4.59 ± 0.78^{E}
	30 days	4.05 ± 0.88^{B}	4.46 ± 0.87^{C}	4.81 ± 0.81^{D}	5.11 ± 0.84^{F}
CAT (µM/mg/g protein)	15 days	357.12 ± 1.90^{A}	374.17 ± 1.91^{B}	385.94 ± 1.97^{D}	401.01 ± 2.05^{C}
	30 days	371.19 ± 1.87^{B}	399.67 ± 1.95^{C}	421.12 ± 1.96^{E}	470.85 ± 2.19^{F}
TBARS (nmol/g tissue)	15 days	180.74 ± 2.62^{A}	163.09 ± 2.36^{C}	165.12 ± 2.28^{C}	156.13 ± 2.35^{D}
	30 days	175.89 ± 1.78^{B}	150.49 ± 1.85^{D}	142.74 ± 1.46^{E}	124.81 ± 1.39^{F}

- Values are expressed as mean ± SE. - n = number of rats.
- Means bearing different superscript (A, B, C, D, E & F) within the same parameter are differ significantly at ($P<0.05$).

Regarding treatment of urate oxidase in nephrotoxic rats, a significant ($p<0.05$) decrease was observed in serum urea, creatinine, uric acid, inorganic phosphorus, PTH and TBARS in urate oxidase treated group when compared to rutin treated groups as well as nephrotoxic recovery group especially after 30 days. Sodium level was significantly ($p<0.05$) increased in urate oxidase treated group when compared to other treated groups after 30 days. On the other hand, potassium levels was significantly ($p<0.05$) decreased with the progress of time in urate oxidase treated group. Serum total & ionized calcium and all antioxidant enzymes exhibited a significant ($p<0.05$) elevation in urate oxidase treated group when compared to other treated groups throughout the period of experiment (Table 2 &3). These results are in agreement with the obtained data by Oldfield & Perry [59] and Heibashy *et al.* [39]. They reported that urate oxidase, a peroxisomal liver enzyme catalyzes the enzymatic oxidation of uric acid into the more water soluble allantoin, is 10 times more soluble than uric acid and more readily eliminated by the kidney and it may reduce creatinine and blood urea nitrogen levels by improving renal function. Also, Carlos *et al.* [60] mentioned that the treatment with rasburicase (recombinant urate oxidase) which is an urolytic agent reversed the inflammatory changes and lessened tubular injury with an improvement in renal function by proinflammatory pathway mechanism. It has been also developed for the prevention and treatment of chemotherapy-induced hyperuricemia and acute renal failure induced by tumour lysis. The obtained results from urate oxidase might be due to its hypouricemic effects that prevent acute renal damage induced by acute urate nephropathy [61]. Many investigations reported that the administration of urate oxidase had a good option in patients with severe acute hyperuricemia [62].

Although, the liver plays a major role in drug metabolism, the intestine is also an important organ for the biotransformation of drugs [63] . The effects of renal failure on intestine metabolism are unknown. However, several pharmacokinetic studies have revealed that the bioavailability of several drugs reduced in renal failure suggesting a decrease in intestine first pass metabolism [64]. Also, several studies reported that animals with renal failure also exhibit decreased hepatic drug metabolism mediated by cytochrome P_{450} [65] . In this work, the best correction occurred in all estimated parameters of nephrotoxic rats treated with urate oxidase than rutin may be due to the minimal harmful effects of urate oxidase on liver and intestine cytochrome P_{450} isoforms especially CYP_2C_6; CYP_2C_{11} ; CYP_3A_1 and CYP_3A_2. The obtained data were confirmed in human by Matzke & Frye [64] and in rats by Ding *et al.* [20] and Leblond *et al.* [65] .

Co-administration of rutin and urate oxidase to the nephrotoxic rats significantly decreased the GM-induced nephrotoxicity demonstrated by prevention of the increase in urinary enzyme activities and preservation of GSH and antioxidant enzymes levels in kidneys. This could be due to the ability of these antioxidants substance to transfer electrons free radicals chelate metals catalays [66,67] and activate antioxidant enzymes [68,69] . Moreover, the maximum ameliorating effects of two antioxidants pronounced according to the synergistic effects which improve the physical and pharmakinetics properties of them. These improvements depend on the time of administration.

From the cited data of the present study, it could be concluded that daily intraperitonial injection of rats with 80 mg GM /kg b.w. for two weeks caused a serious harmful effects on renal function tests. Thus, it could be suggested that GM must be given in the lowest effective therapeutic doses and for a period not close to each other in patients with normal kidney function. Also, GM therapy should be preceded by antioxidant administration and renal function tests must be done to detect any early functional alterations. Administration of 100 mg rutin / kg diet / day for 30 days inhibits to a great extent the deteriorative changes induced by GM. Urate oxidase in a dose of 10 mg/100 g b.wt. for 30 days proved to have a better protective action against GM nephrotoxicity than rutin. Therefore, urate oxidase agent might open a new therapeutic possibility in patient with sever uncontrolled gout and impaired renal function. Further studies with urate oxidase will be required to give answers about the recommended doses which enhances its protective action and how long after GM treatment. Moreover, the use of rutin in combination with urate oxidase caused a great ameliorating effect in nephrotoxic rats. It is evident now that full recovery from GM nephrotoxicity did not occur ofter 30

days from the onset of giving all treatments, since all the levels of all measured parameters failed to restore to normal values.

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