Comparative study of Quercetin or/and Urate Oxidase against Gentamicin -induced Nephrotoxicity and Oxidative Stress in Rat kidneys

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Abstract: This study was conducted to show whether quercetin and urate oxidase would offer ameliorating effects against abnormal alterations in kidney function tests in gentamicin induced nephrotoxic rats. Two experiments were carried out, the first one showed that daily injection of 80 mg gentamicin /kg b. wt interaperitonealy (I.P) for two weeks induced acute renal failure indicated by significant elevation in serum levels of urea, creatinine, uric acid, potassium, inorganic phosphorus and Parathyroid hormone (PTH) and a significant decline in serum sodium, total and ionized calcium associated with a remarkable decrease in the content of glutathione (GSH) and in the activities glutathione peroxidase (Gpx) and catalase (CAT) and in the concentration of thiobarbituric acid reactive substances (TBARS) in the kidney of nephrotoxic rats when compared with their corresponding values in saline injected rats (Normal animals group). In the second experiment, four comparisons were made between gentamicin induced nephrotoxic rats and other nephrotoxic groups received daily i.p. injection of quercetin (50mg/kg b.wt) and urate oxidase (10mg/kg b. wt) for 2 & 4 weeks after the incidence of nephrotoxicity. A remarkable correction was occurred in the levels of serum urea, creatinine, uric acid, potassium, sodium, total and ionized calcium, inorganic phosphorus and PTH in quercetin or urate oxidase treated groups exhibited significant reduction than nephrotoxic untreated rats dependent on the time of treatment (2 & 4 weeks). In the kidney tissues, a considerable amelioration effect was occurred in the content of the levels of GSH and in the activities Gpx and CAT and in the concentration of TBARS after the nephrotoxic rats treated with quercetin or urate oxidase. These corrections were dependent on the time of treatment (2 & 4 weeks). Thus, it may be concluded that quercetin or urate oxidase can be applicable as therapeutic agent with gentamicin therapy. The best beneficial effect was more prominent when nephrotoxic rats treated with both agents (quercetin or urate oxidase) at last interval (4 weeks). The obtained data were discussed according to available obtained researches.

[Marwan Abdel-Lattif Ibrahim and Afaf Abbass **Comparative study of Quercetin or/and Urate Oxidase against Gentamicin -induced Nephrotoxicity and Oxidative Stress in Rat kidneys.** Journal of American Science 2012; 8(1): 600-607]. (ISSN: 1545-1003). http://www.americanscience.org. 84

Key words: Quercetin, urate oxidase, Oxidative Stress, Nephrotoxicity, Rats.

1. Introduction

One of the most common manifestation of nephrotoxic damage is acute renal failure which characterized by decline in glomerular filtration rate with resulting azotaemia . The incidence of renal dysfunction following aminoglycoside administration was detected by many workers (**Garetz and Schacht 1996; Baliga *et al.*, 1997 and Abdel Naim *et al.*,1999).** Gentamicin is aminoglycoside broad spectrum antibiotic used against pathogenic gram negative and positive bacteria **(Taha, 1993).** Its administration into rats induced impairment of renal function through liberation of oxygen free radicals **(Heibashy & Abdel Moneim, 1999 and Heibashy *et al.*,2009).**

Moreover, ROS can induce renal injury both by direct cellular toxicity **(Agarwal *et al.*,1996)** and by promoting production of ox-LDL, which, in turn, further inactivates NO **(Baud & Ardaillou, 1993 and Cardillo *et al.*,1997)** and directly contributes to tubulointerstitial disease **(Agarwal *et al.*,1996)** and glomerulosclerosis **(Ding *et al.*,1997).**

Superoxide anions and other ROS may be generated by several different enzymatic and non-enzymatic mechanisms **(John & Schmieder, 2003).** In the vascular endothelium the main source for superoxide is NADPH-oxidase, but additional enzymes can induce ROS production [e.g., cyclooxygenase, uncoupled endothelial nitric oxide synthase (eNOS), and xanthine oxidase (XO)]. XO can lead to superoxide production during the purine degradation process, which involves metabolism of hypoxanthine and xanthine to uric acid. XO activity has been demonstrated to be elevated in the plasma of hypercholesterolemic subjects and to contribute to endothelial dysfunction in hypercholesterolemia animals **(White *et al.*, 1996)** and humans **(Burnier *et al.*,1996 and Cardillo *et al.*,1997).** In the kidney, XO is also involved in ischemic injury. However, the contribution of XO-derived ROS to endothelial

dysfunction in the kidney in early atherosclerosis has not been determined.

Quercetin a member of flavonoids, found in fruits, vegetables, leaves and grains **(Chen *et al.,* 2005)**. It is also one of antioxidants with property of protecting our body in fighting against forming of free radicals cause of mutation of cells` DNA **(Erlund *et al.,* 2006)**. Quercetin is readily absorbed from the intestinal tract and blood levels rise as the dosage of the quercetin supplement increases. Quercetin is commonly present as a glycoside and is converted to glucuronide / sulfate conjugates during intestinal absorption and conjugated metabolites are found in circulating blood **(Renugadevi & Milton, 2009 and Koli *et al.,* 2010).**

In numerous experimental studies on both animals and humans, quercetin has been found to protect kidney tissues against age-related insult. NF-kB activity in the kidneys increases with age and leads to increased oxidative stress. Caloric restriction, which is known to extend life span, has been found to reduce NF-kB activity in the kidneys of rats **(Kim *et al.,* 2002)**.

Researchers tested the effect of quercetin on the activation of NF-kB in cultured rat kidney cells. The cells were proximal tubular cells (PTC's), which play a pivotal role in progressive kidney diseases by regulating the accumulation of macrophages **(Wang *et al.,*1999; Satyanarayana *et al.,*2001 and Cornish *et al.,* 2002).** The authors found that quercetin potently inhibited NF-kB activation in PTC.10 Since NF-kB regulates inflammatory signaling and adhesion molecules in PTC, these findings may explain earlier findings that preventive administration of quercetin inhibited tubular injury and the upregulation of inflammatory cytokines in the renal cortex.

Ischemia and reperfusion, discussed earlier in relation to cardiovascular disease, also damages the kidneys. Quercetin protects the kidneys during ischemia and reperfusion by preserving higher levels of the enzyme xanthine dehydrogenase relative to the injurious enzyme xanthine oxidase **(Sanhueza *et al.,* 1992).**

In humans and other primates, urate oxidase (uricase), a hepatic enzyme, is inactive as a result of a non-sense mutation, originating a stop codon. So, only animals which possess uricase are able to transform uric acid in a more soluble (5-10 times more than uric acid) and more eliminable molecule: allantoin. A side product of this reaction is hydrogen peroxide, toxic for kidney that is converted in H_2O and O_2 by catalase. A hypothesis considers this mutation as a result of phylogenetic evolution, because uric acid has antioxidant properties that protect against neurological degenerative diseases and increases longevity **(Scott & Hooper, 2001 and Vogt, 2005).** Yet, the loss of this enzyme arises the consequences derived from uric acid poor solubility. Mice with gene inactivation of urate oxidase have hyperuricemia and renal tubulopathy **(Kelly *et al.,* 2001).**

Today, therapeutic trials to decrease serum uric acid and treating renal failure are focused on using uricostatic and urolytic drugs. Therefore, the study was aimed to evaluate the protective effects of quercetin or urate oxidase and their mixture following gentamicin administration in rats. These different nephroprotective agents based on their antioxidant and uricostatic properties due to their pharmacokinetics and pharmacodynamics with correlate their effects on some biochemical parameters related to kidney function testes dependent on time of treatment.

2. Material and Methods

Sixty adult male albino rats (*Rattus rattus*) were employed in this study. They were housed in a well ventilated vivarum of Zoology Department, Women's Collage, Ain Shams University. They were caged in wire bottom galvanized metal wall boxes under controlled environmental and nutritional conditions (25°C and 55-60% relative humidity) they fed on a standard diet according to National Research Council ***(NRC, 1977)*** and fresh tap water was *ad libitum.*

The study included two experiments; the first 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. for Pharm and Chem. Ind. Cairo, ARE) at a dose of 80 mg/kg b.wt for two weeks as described by **Ohtani *et al.,* (1995)** 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 four groups of rats with gentamicin-induced acute renal failure (10 rats for each).The first nephrotoxic rats group was left as recovery positive control. The second one has treated interaperitonealy daily with 50mg/kg b.wt of quercetin (Sigma Chem. Co. ST Louis, Mo, USA) for 2 and 4 weeks after the end of gentamicin course **(Renugadevi & Milton, 2009**). The third group was interaperitonealy injected urate oxidase 10 mg/kg b.wt for the same previous intervals **(Ding *et al.,* 1996).** The last group (fourth group) was interaperitonealy received both quercetin and urate oxidase for 2 and 4 weeks.

Blood sampling:-

At the end of each experimental period (2 and 4 weeks) and after overnight fasting, animals were scarified the blood samples were collected from each rat on each time interval. Blood samples were centrifuged for 10 minutes at 3000 rpm within an hour of the blood collection and the sera were obtained. Sera were separated and divided into considerable aliquots to avoid the effects of repeated thawing and freezing. All specimens of sera were stored at -20°C until use.

Determination of serum urea, creatinine and uric acid:-

The serum parameters were analyzed spectrophotometrically by using double beam UV-Visible spectrophotometer (UV-Visible spectrophotometer, VIS-JR, model 1601). Estimation of serum urea and creatinine were carried out using respective diagnostic kits purchased from Randox Ltd., Co. (UK) according to the methods of **Fawcett & Scott (1960)**, **Seeling & Wust (1969)** and **Barham & Trinder (1972)** respectively.

Determination of serum electrolytes:-

Sodium (Na) and Potassium (K) analysis were accomplished by emission flame photometry after suitable dilution as described by **Dean (1960).** Serum calcium was determined colorimetrically using commercial kits (Human, Germany) according to the method of **Barnett *et al.* (1973).** The concentration of serum ionized calcium was calculated according to **Mclean & Hasting (1935).** Serum inorganic phosphorus was determined colorimetrically using kits supplied by Randox Ltd., Co. (UK) and according to the method of **Goldenberg & Fernands (1966).**

Estimation of serum parathyroid hormone (PTH):-

Parathyroid hormone (PTH) was assayed by radioimmunoassay (RIA) kit using solid phase component system (Phoenix Pharmaceuticals, Inc., USA) as described by **Patrono & Peskar (1987).**

Determination of antioxidant enzymes and lipid peroxidation:-

After animals sacrifice, the kidneys were quickly dissected, weighed (calculated as g/100 g body weight) and dipped in liquid nitrogen for 1 min then preserved at -20 ºC (Ultra-low freezer) untill analysis carried out. The kidneys were washed in ice-cold saline solution (0.9 % NaCl). One hundred milligrams of kidney tissue was 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 aspirated and used for the estimation of reactive oxygen metabolites in terms of lipid peroxidation as thiobarbituric acid reactive substances (TBARS) concentration according to **Hogberg *et al.* (1974),** glutathione (GSH) content according to **Baker *et al.* (1990)**, glutathione peroxidase (Gp_x) activity according to **Rotruck *et al.* (1973)**, catalase (CAT) activity according to **(Aebi, 1974),** and total protein estimation ***(Lowry et al., 1951).*** The commercial ELISA kits of TBARS, GSH, Gp_x and CAT were purchased from Cayman Chem. Co., USA.

Statistical Analysis:-

Data were presented as mean ± standard error (SE) and were statistically analyzed using Students "t" test in the first experiment. The data in the second experiment were statistically analyzed using analysis of variance (ANOVA) followed by Duncan's multiple range test according to **Snedecor & Cochran (1982)** by the aid of SPSS program, Version 10, USA.

3. 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 80mg/kg b.w. for 14 days equivalent to that used clinically in man as described by **Ohtani *et al.* (1995).** Acute renal failure is characterized by disorders in some biochemical parameters in gentamicin treated rats as shown in the first experiment presented in table (1). Gentamicin produced highly significant ($p<0.001$) increases in the concentration of serum urea, creatinine and uric acid. These results confirmed that gentamicin produced nephrotoxicity as previously reported by **Ali *et al.*, 2003, Goto, 2004 and Heibashy *et al.*, 2009.** 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 multipleintracellular membranes and organelles then developed injury in the proximal tubular epithelial cells of kidney that caused acute renal failure **(Swan, 1997).**

Serum electrolytes were disturbed significantly ($p<0.001$) in GM treated rats as compared with control animals. Lower value of serum sodium indicates inability of kidney to conserve sodium and chloride. Haemodilution too 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 appeared to be due to reduced excretion of K aggravated by leakage of intracellular potassium into blood stream as a result of gentamicin induced lesions in renal tubular epithelium. These results are in harmony with the data obtained by **Heibashy & Abdel Moneim (1999)** and **Heibashy *et al.* (2009).** Serum phosphate and PTH were significantly ($p<0.001$) increased, conversely, serum total and ionized calcium were significantly ($p<0.001$) decreased in gentamicin injected rats. Similar results were obtained by **Hruska *et al.* (1975)** and **Breen *et al.* (1996)**. The authors attributed these disturbances to the elevated parathormone level which produced after gentamicin administration. Furthermore, increased glucocorticoids levels enhance deposition of calcium as calcium phosphate and carbonate in injured skeletal muscle **(Heibashy & Abdel Moneim, 1999 and Abdel Magied &. and Heibashy, 2000).** Also, the toxicity of gentamicin may cause an increase in the urinary excretion of calcium and inhibited calcium intake into mitochondria and stimulate ionized calcium from mitochondria **(Abdel Mageid & Heibashy, 2000 and Heibashy *et al.*, 2009).**

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

Parameters	Normal control group n=10 rats	Nephrotoxic group n=10 rats
Urea (mg/dl)	18.79 ± 0.14	78.14 ± 1.63*
Creatinine (mg/dl)	0.51 ± 0.02	2.15 ± 0.08*
Uric acid (mg/dl)	0.39 ± 0.01	1.05 ± 0.09*
Na (meq/L)	133.36 ± 0.58	118.67 ± 1.71*
K (meq/L)	4.17 ± 0.09	5.51 ± 0.13*
Inorganic Ph (mg/dl)	8.11 ± 0.13	9.23± 0.22*
Total calcium (mg/dl)	9.18 ± 0.17	7.82 ± 0.15*
Ionized calcium (mg/dl)	2.33 ± 0.07	1.62 ± 0.07*
PTH (pg/ml)	14.54 ± 0.19	22.97 ± 0.39*
GSH (mg/g)	181.02±1.79	123.43±1.26*
GPx (µmole/min/mg protein)	154.49±1.47	110.29±1.03*
CAT (µM/mg/g)	516.33±2.92	328.77±1.65*
TBARS (nmol/g tissue)	110.09± 1.57	186.21± 2.92*

- Values are expressed as means ± S.E. - N = number of rats in the group.
- * Significant at $p< 0.001$ between the groups in the same rows.

In the kidney tissues of nephrotoxic rats, a significant ($p<0.001$) decrease in glutathione (GSH) content and a remarkable depression in the activities of glutathione peroxidase (Gp_x) and catalase (CAT) associated with a considerable elevation in the concentration of thiobarbituric acid reactive substances (TBARS) which is a lipid peroxidation product (Table 1). This result is in harmony with **Heibashy & Abdel Moneim (1999); Ali *et al.* (2003)** and **Heibashy *et al.* (2009).** The authours explained these results to gentamicin nephrotoxicity which led to a remarkable elevation in the concentration of lipid peroxidation in the renal cortex and explains the nephrotoxicity of gentamicin due to tissue damage by free radicals resulted from gentamicin administration. As, **Cuzzocrea *et al.* (2002)** reported that gentamicin is able to generate free radicals as hydrogen peroxide, hydroxyl radical and superoxide anions in rat renal mitochondria.

From the data of second experiment presented in table (2) it was obvious that, quercetin administration proved to have some ameliorating effects against undesirable changes in kidney function following gentamicin injection for 14 days. With the progress of time after the gentamicin was discontinued, serum urea, creatinine, uric acid and PTH were corrected significantly ($p<0.05$) in quercetin group as compared with recovery nephrotoxic group (positive control) dependent on the time of treatment (2 & 4 weeks). As time advanced, serum potassium level was decreased significantly ($p<0.05$) only after 2 and 4 weeks after gentamicin discontinuity. While, a significant ($p<0.05$) decline in inorganic phosphorus was detected after quercetin administration dependent on the time of treatment (2 & 4 weeks). Total and ionized calcium showed a significant ($p<0.05$) increase all over the period of treatment with quercetin than recovery nephrotoxic group. Serum sodium level did not show any significant changes between quercetin treated group and the recovery nephrotoxic one at the first intervail (2 weeks). As time advanced, serum sodium level was increased significantly ($p<0.05$) at the last intervail (4 weeks).

The benefits of quercetin treatment include inhibition of biotransformation, free radical scavenging, anti-inflammatory effects and enhancement of blood flow **(Erlund *et al.*, 2006; Renugadevi & Milton, 2009 and Koli *et al.*, 2010).** They reported that quercetin and its derivatives are able to penetrate mucous membranes and organelle membranes. Unlike most penetrating solvents, penetrance or absorption of quercetin is not associated with irreversible membrane damage. Furthermore, quercetin traps free radical hydroxide (OH) also, quercetin reduction metabolite whereas quercetin traps free radical oxygen.

Quercetin has recently been shown to protect against the kidney damage caused by a well-known nephrotoxic drug. Cyclosporine is a potent immune suppressant, the first-line therapy for solid organ transplant patients and autoimmune disease patients **(Satyanarayana *et al.*, 2001).** It causes kidney damage in the form of fibrosis, arterial damage, and cyst formation, among other changes. Such extensive damage is thought to be due to a combination of

factors, including increased free radical production, increases in renal nerve activity that cause constriction of renal arteries, blockade of the release of calcium from the mitochondria and a resultant rise in intracellular calcium. (If calcium concentrations rise too high, blood vessels become constricted.)

In a study of cyclosporine's effects on rat kidneys, a 20% to 30% reduction in glomerular filtration rate (the rate at which the kidneys filter wastes from the blood) and up to 40% reduction in renal blood flow were found. Rats given 2 mg/kg of quercetin suffered far less damage to their kidneys when given cyclosporine. Their urinary output increased and markers of free radical damage dropped **(Yao *et al.,* 2011).**

Quercetin's antioxidant effects and its enhancement of mitochondrial function-including improved intracellular/extracellular calcium balance - likely explain these protective effects. Protecting the kidneys is paramount, for once they are damaged, and it becomes difficult, if not impossible to restore healthy function however; Quercetin reduces cisplatin toxicity in cultured tubular epithelial cells **(Sanchez-Gonzalez *et al.,* 2011),** protects the kidney against damage indicted by reactive oxygen species **(Singh *et al.,* 2004$_{a\&b}$)**, was shown to be protective in the face of oxidative damage to the kidneys of rats; reduces the kidney damage from ischemia-reperfusion injury (**Shoskes *et al.,* 2005)** and reduces platelet aggregation and adhesion by reducing hydrogen peroxide production **(Anjaneyulu & Chopra, 2004; and Liu *et al.,* 2010).**

Due to the antioxidant powerful of quercetin, a significant ($p<0.05$) correction was occurred in glutathione (GSH) content, glutathione peroxidase (Gp_x) and catalase (CAT) activities associated with a remarkable decrease in the concentration of thiobarbituric acid reactive substances (TBARS) in the nephrotoxic rats dependent on the time of treatment (Table 2).

Regarding treatment of urate oxidase in nephrotoxic rats, a significant ($p<0.05$) decrease was observed in serum urea, creatinine, uric acid, potassium, PTH and inorganic phosphorus in urate oxidase treated group when compared to quercetin treated groups as well as nephrotoxic recovery group especially after at the last intervail (4 weeks). Sodium level was significantly ($p<0.05$) increased in urate oxidase treated group when compared to quercetin treated groups dependent on time of treatment. Moreover, serum total and ionized calcium exhibited a significant ($p<0.05$) elevation in urate oxidase treated group when compared to quercetin treated groups all over the period of experiment (Table 2). These results are in agreement with the data obtained from **Oda *et al.* (2002)** and **Oldfield & Perry (2006)** who reported that urate oxidase a peroxisomal liver enzyme that catalyses the enzymatic oxidation of uric acid into the more water soluble allantoin which 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 **Goldman *et al.* (2001) and Carlos *et al.* (2007)** 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 it is hypouricemic effects that prevent acute renal damage induced by acute urate nephropathy **(Lisa & Mariano, 2007).** Many investigations reported that the administration of urate oxidase had a good option, sometimes better than use of allopurinol in patients with severe acute hyperuricemia **(Wolf *et al.,* 1999).**

Although, the liver plays a major role in drug metabolism the intestine is also an important organ for the biotransformation of drugs **(Ilett *et al.,* 1990 and Krishna & Klotz 1994).** 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 **(Matzke & Frye 1997).** Also, several studies reported that animals with renal failure also exhibit decreased hepatic drug metabolism mediated by cytochrome P_{450} **(Uchida *et al.,* 1995 and Leblond *et al.,* 2001 & 2002).** The correction which occurred in all estimated parameters of nephrotoxic rats treated with urate oxidase than nephrotoxic rats treated with quercetin 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_3 A_1 and CYP_3A_2. The obtained data were confirmed in human by **Klin *et al.* (1995) and Matzke & Frye (1997)** and in rats by **Ding *et al.* (1996) and Leblond *et al.* (2001 & 2002).**

Table (2): Ameliorating effects of quercetin or/and urate oxidase on serum biochemical parameters of nephrotoxic rats.

Parameters	Interval	Nephrotoxic Group (recovery) n=10 rats	Nephrotoxic Treated with Quercetin n=10 rats	Nephrotoxic Treated with Urate oxidase n=10 rats	Nephrotoxic Treated with Co-administration n=10 rats
Urea (mg/dl)	2wks n = 5	$73.41 \pm 1.67^{A}_{a}$	$70.09 \pm 1.59^{B}_{a}$	$66.53 \pm 1.61^{C}_{a}$	$63.61 \pm 1.56^{D}_{a}$
	4wks n = 5	$70.13 \pm 1.58^{A}_{b}$	$65.37 \pm 1.64^{B}_{b}$	$60.11 \pm 1.52^{C}_{b}$	$50.94 \pm 1.42^{D}_{b}$
Creatinine (mg/dl)	2wks n = 5	$1.97 \pm 0.09^{A}_{a}$	$1.79 \pm 0.08^{B}_{a}$	$1.58 \pm 0.09^{C}_{a}$	$1.56 \pm 0.07^{D}_{a}$
	4wks n = 5	$1.65 \pm 0.08^{A}_{b}$	$1.41 \pm 0.07^{B}_{b}$	$1.29 \pm 0.07^{C}_{b}$	$1.01 \pm 0.08^{D}_{b}$
Uric acid (mg/dl)	2wks n = 5	$0.98 \pm 0.08^{A}_{a}$	$0.91 \pm 0.07^{B}_{a}$	$0.83 \pm 0.07^{C}_{a}$	$0.82 \pm 0.06^{C}_{a}$
	4wks n = 5	$0.78 \pm 0.08^{A}_{b}$	$0.72 \pm 0.09^{B}_{b}$	$0.63 \pm 0.07^{C}_{b}$	$0.57 \pm 0.05^{D}_{b}$
Sodium (meq/L)	2wks n = 5	$122.32 \pm 1.97^{A}_{a}$	$123.44 \pm 1.94^{A}_{a}$	$123.92 \pm 1.86^{A}_{a}$	$127.74 \pm 1.89^{B}_{a}$
	4wks n = 5	$126.51 \pm 1.92^{A}_{b}$	$127.51 \pm 1.83^{B}_{b}$	$132.32 \pm 1.77^{C}_{b}$	$133.78 \pm 1.92^{C}_{b}$
Potassium (meq/L)	2wks n = 5	$5.51 \pm 0.11^{A}_{a}$	$5.49 \pm 0.12^{A}_{a}$	$5.27 \pm 0.10^{B}_{a}$	$5.02 \pm 0.09^{C}_{a}$
	4wks n = 5	$4.94 \pm 0.08^{A}_{b}$	$4.78 \pm 0.11^{B}_{b}$	$4.69 \pm 0.08^{C}_{b}$	$4.23 \pm 0.08^{D}_{b}$
Inorganic Phosphorus (mg/dl)	2wks n = 5	$9.49 \pm 0.19^{A}_{a}$	$9.38 \pm 0.17^{A}_{a}$	$9.01 \pm 0.16^{B}_{a}$	$8.74 \pm 0.17^{C}_{a}$
	4wks n = 5	$8.91 \pm 0.16^{A}_{b}$	$8.67 \pm 0.14^{B}_{b}$	$8.31 \pm 0.13^{C}_{b}$	$8.11 \pm 0.12^{D}_{b}$
Total Calcium (mg/dl)	2wks n = 5	$7.83 \pm 0.19^{A}_{a}$	$8.04 \pm 0.18^{B}_{a}$	$8.08 \pm 0.18^{B}_{a}$	$8.45 \pm 1.14^{C}_{a}$
	4wks n = 5	$8.11 \pm 0.17^{A}_{b}$	$8.59 \pm 0.18^{B}_{b}$	$8.63 \pm 0.17^{B}_{b}$	$9.21 \pm 1.16^{C}_{b}$
Ionized Calcium (mg/dl)	2wks n = 5	$1.70 \pm 0.11^{A}_{a}$	$1.79 \pm 0.12^{B}_{a}$	$1.89 \pm 0.14^{C}_{a}$	$1.91 \pm 0.11^{C}_{a}$
	4wks n = 5	$1.83 \pm 0.09^{A}_{b}$	$1.97 \pm 0.08^{B}_{b}$	$2.13 \pm 0.11^{C}_{b}$	$2.42 \pm 0.8^{D}_{b}$
PTH (pg/ml)	2wks n = 5	$25.37 \pm 0.36^{A}_{a}$	$22.79 \pm 0.34^{B}_{a}$	$21.04 \pm 0.31^{C}_{a}$	$19.93 \pm 0.29^{D}_{a}$
	4wks n = 5	$21.31 \pm 0.26^{A}_{b}$	$18.64 \pm 0.29^{B}_{b}$	$16.95 \pm 0.29^{C}_{b}$	$14.58 \pm 0.25^{D}_{b}$

- Values are expressed as means ± S.E. - N = number of rats in the group.
- A, B, C, D = means bearing different superscripts within the same row are differ significantly ($P<0.05$).
- a, b = means bearing different subscripts within the same column are differ significantly ($P<0.05$).

By reviewing table (3), the best amelioration effect was occurred in all studied parameters of nephrotoxic rats treated which treated by both agents (quercetin and urate oxidase). These data may be attributed to synergistic effects of them and due to improvement in their physical and biochemical properties.

From the data of the present study, it could be concluded that daily intraperitonial injection of rats with 80 mg gentamicin /kg b.w for 14 days caused a serious harmful effects on renal function tests. Thus, it could be suggested that gentamicin 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, gentamicin therapy should be preceded by antioxidant administration and renal function tests must be done to detect any early functional alterations. Further studies with quercetin or/and urate oxidase will be required to give answers about the recommended doses which enhances its protective action and how long after gentamicin treatment.

Table (3): Ameliorating effects of quercetin or/and urate oxidase on kidney biomarker parameters of nephrotoxic rats.

Parameters	Interval	Nephrotoxic Group (recovery) n=10 rats	Nephrotoxic Treated with Quercetin n=10 rats	Nephrotoxic Treated with Urate oxidase n=10 rats	Nephrotoxic Treated with Co-administration n=10 rats
GSH (mg/g)	2wks n = 5	$129.45\pm1.50^{A}_{a}$	$136.11\pm1.56^{B}_{a}$	$144.33\pm1.61^{C}_{a}$	$156.46\pm1.67^{D}_{a}$
	4wks n = 5	$135.38\pm1.51^{A}_{b}$	$145.28\pm1.53^{B}_{b}$	$159.16\pm1.63^{C}_{b}$	$163.92\pm1.59^{D}_{b}$
GPx (µmole/min/mg protein)	2wks n = 5	$113.22\pm1.11^{A}_{a}$	$119.79\pm1.19^{B}_{a}$	$130.89\pm1.28^{C}_{a}$	$135.49\pm1.27^{D}_{a}$
	4wks n = 5	$120.56\pm1.17^{A}_{b}$	$127.95\pm1.22^{B}_{b}$	$139.32\pm1.25^{C}_{b}$	$149.16\pm1.33^{D}_{b}$
CAT (µM/mg/g)	2wks n = 5	$346.32\pm1.91^{A}_{a}$	$368.07\pm1.92^{B}_{a}$	$381.94\pm1.99^{C}_{a}$	$396.24\pm2.06^{D}_{a}$
	4wks n = 5	$365.11\pm1.89^{A}_{b}$	$393.53\pm1.96^{B}_{b}$	$417.12\pm1.97^{C}_{b}$	$464.95\pm2.18^{D}_{b}$
TBARS (nmol/g tissue)	2wks n = 5	$181.62 \pm 2.63^{A}_{a}$	$165.44 \pm 2.38^{B}_{a}$	$164.02 \pm 2.28^{B}_{a}$	$153.24 \pm 2.36^{C}_{a}$
	4wks n = 5	$177.81 \pm 1.79^{A}_{b}$	$152.77 \pm 1.87^{B}_{b}$	$141.84 \pm 1.46^{C}_{b}$	$122.31 \pm 1.39^{D}_{b}$

- Values are expressed as means ± S.E. - N = number of rats in the group.
- A, B, C, D = means bearing different superscripts within the same row are differ significantly ($P<0.05$).
- a, b = means bearing different subscripts within the same column are differ significantly ($P<0.05$).

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12/28/2011