The Egyptian Journal of Hospital Medicine (Oct 2012) Vol., 49: 705– 717

Effect of Morin against Gentamicin-Induced Nephrotoxicity in Young Male Rats

Hala A.H. Khattab

Nutrition and Food Science Dept., Faculty of Home Economics, Helwan University

Abstract

Background: Morin, a bioflavonoid with antioxidant properties, is a constituent of many herbs and fruits that are used as herbal medicines. It exhibits many biological activities and possibly even has protective effects against chronic diseases. The present study was conducted to determine the protective effect of morin against gentamicin-induced nephrotoxicity in young male rats.

Material and Methods: Young male rats (n=24) were divided into four groups as follow; (1): control group, (2): gentamicin (GM) group; rats injected intraperitioneally (i.p.) with GM at a dose of 100 mg /kg body weight (b.w.) for five consecutive days to induced nephrotoxicity, (3): morin group; rats administered morin daily at a dose of 30 mg/ kg b. w. via gavage, and (4) GM group pretreated with morin; rats were orally received morin at the same dose and route in group (3) up to two weeks followed by injected i.p. with GM as in group (2). Separated serum samples were used for determination of protein metabolism parameters, kidney functions, malondialdehyde (MDA), and ionic sodium (Na^+) and potassium (K^+).

Results: GM injection induced marked nephrotoxicity as evidenced by significant elevation in serum levels of albumin, creatinine, urea, uric acid, MDA and K^+, with significant reduction in serum levels of total protein, Na^+ and albumin/creatinine ratio (ACR). Pretreatment with morin protected the rats from GM-induced nephrotoxicity as evidenced by significant improvement of these investigated parameters. Histological examination of renal tissues showed marked glomerular thickening, vacuolations of the wall of blood vessels associated with necrobiotic changes in GM injected group, meanwhile there were amelioration in rats group received morin pre-GM injection.

Conclusion: Morin exert potential antioxidant activity and offer nephroprotective effect against GM-induced nephrotoxicity in young rats.

Key words: Morin, gentamicin, nephrotoxicity, antioxidant, young male rats.

Introduction:

Aminoglycosides are natural or semi-synthetic antibiotics with a heterocyclic structure formed by two or more amino sugars linked by glycoside bonds to an aminocyclitol ring, the most widely used drug in this category is gentamicin. The incidence of renal dysfunction following aminoglycoside administration was detected by many workers. Gentamicin, as an aminoglycoside broad spectrum antibiotic, is used against pathogenic gram negative and positive bacteria. Unfortunately, acute renal failure is major complication in 10-20 % of patients receiving the drug (Adbel Naim *et al*., 1999, Avdagić *et al*., 2008, Vanessa *et al*., 2009 and Ibrahim and Saleh, 2012). It has been demonstrated that gentamicin-induced nephrotoxicity is characterized by direct tubular necrosis, which is localized mainly in proximal tubules, basal membrane disruption, mesangial cell contraction, proliferation and apoptosis, thus indicated by decreases in glomerular filtration and alteration in intraglomerular dynamics (Pedraza-Chaverri *et al*., 2003 and Martinez-Salgado *et al*., 2007). The exact mechanism of gentamicin-induced nephrotoxicity still remains unclear. Recent evidence showed that reactive oxygen species (ROS) play a pivotal role in gentamicin-mediated nephrotoxicity, it induced impairment of renal function through liberation of ROS in rats (Heibashy and Abdel Moneim, 1999 and Heibashy *et al*., 2009). Walker *et al*. (1999) have demonstrated that oxidative stress induced by gentamicin, is the central pathway responsible for renal injury. Some studies have reported that

antioxidant administration ameliorates GM-induced nephropathy (Pedraza-Chaverri *et al*., 2003, Avdagić *et al*., 2008 and Ibrahim and Saleh, 2012).

Flavonoids comprise a class of natural products which are found in fruits, nuts, seeds, herbs, and spices. They are consumed regularly as a part of the human diet, and have aroused considerable interest due to their broad pharmacological activity (Galvez *et al*., 2001). Plant flavonoids are emerging as potent therapeutic drugs effective against a wide range of free radical mediated diseases. Morin (2',3,4',5,7-pentahydroxyflavone), a kind of flavonoid belonging to the group of flavonols, it is a natural compound found in fig, guava leaves, onion, apple, and other members of the Moracease family, which are used as dietary agents. It is also widely distributed in tea, cereal grains and a variety of fruits and vegetables. (Kang *et al*., 2004, Lotito and Frei, 2006 and Xie *et al*., 2006). Morin has two aromatic rings linked by an oxygen-containing heterocyclic (Subash and Subramanian, 2008). Abundant in the human diet, morin, with potent antioxidant and metal ion chelating capacities, possesses various biological and biochemical effects including anti-inflammatory, anti-neoplastic, and cardio-protective activities (Middleton *et al*., 2000). Morin exerts antioxidant potential and offers protection against the oxidative stress induced by hydrogen peroxide (Zhang *et al*., 2009).

Morin has been shown to exhibit intestinal anti-inflammatory activity in the acute phase of the trinitrobenzenesulfonic acid model of rat colitis (Galvez *et al*., 2001), chemopreventive effects on chemically induced rat tongue carcinogenesis (Kawabata *et al*., 1999), as well as suppresses the proliferation of a wide variety of tumor cells, including leukemia (Krol *et al*., 2002), and colon cancer (Ranelletti *et al*., 1999). In view of the above findings and the ongoing search for antioxidants that may reduce drug induced oxidative stress, the present work has been carried out to evaluate the antioxidative effect of morin against GM-induced nephrotoxicity in young male rats.

Material and Methods:

Drugs and chemicals:

Morin (morin hydrate: 2',3,4',5,7-pentahydroxyflavone) used in this study was purchased from Sigma Chemical Co.,(St. Louis, Mo, U.S.A.). Gentamicin was kindly provided by Memphis Co. for Pharm. & Chem. Ind. Cairo Egypt, as gentamicin sulphate. Chemical Kits were obtained from Biodiagnostic Co. Egypt. All chemicals used were analytical grade of the highest laboratory purity. Casein was obtained from Misr Scientific Co. Dokki, Giza, Egypt. Trichloroacetic acid, cellulose and L-cystine were purchased from Morgan Co. Cairo, Egypt. Starch and corn oil were obtained from local market. Diethyl ether, vitamins and minerals constituents, and sucrose were obtained from El-Gomhoriya Pharm. and Chem. Ind. Co. Cairo, Egypt.

Induction of nephrotoxicity by gentamicin:

Gentamicin, as gentamicin sulphate, used was in the form of water soluble solution each 1ml containing 40 mg gentamicin sulphate. Renal toxicity was induced by intraperitioneally (i.p.) injection with GM at a dose of 100 mg /kg b.w. for five consecutive days according to (Morales *et al*., 2002).

Pretreatment with morin and mode of administration:

Morin was freshly suspended in dis. water (Sreedharan *et al*., 2009), and administrated daily to rats at a dose of 30 mg / kg b.w., orally using an intragastric tube for a period of two weeks according to (Subash and Subramanian, 2009).

Experimental animals:

Twenty-four young male albino rats, *Sprague Dawley* strain, weighing (80-100 g) were purchased from the animal house of the National Research Center, Dokki, Egypt. Animals were housed in plastic cages, fed on standard casein diet according to Reeves *et al*. (1993) and given tap water *ad libitum*. All rats were handled in accordance with the standard guide for the care and use of laboratory animals.

Experimental design:

After the period of adaptation (one week), animals were divided into four groups (each of 6 rats) as following: **The first (control group):** Rats were administered orally by gavage a single daily dose of dis. water for two weeks, then injected i.p. with saline for five days. **The second (GM group):** Rats were administered orally by gavage a single daily dose of dis. water for two weeks, then injected i.p. with GM at a dose of 100 mg /kg b.w. for five consecutive days. **The third (morin group):** Rats received morin at a dose level of 30 mg / kg b.w. via oral administration up to two weeks, and then injected i.p. with saline for five days. **The fourth (GM pretreated with morin group):** Rats were orally received morin daily at the same dose and route in group (3) up to two weeks, followed by i.p. injection with GM daily consecutively up to five days as in group (2). During the experimental period all animals were weighed to monitor changes and to adjust the dosages of morin and GM accordingly.

Blood collection and serum separation:

One day after the end of GM injectio, rats from each group were fasted overnight. Blood samples were withdrawn by heparinized capillary tube from the retro orbital plexu of each rat under anesthesia with diethyl ether according to the method of Cocchetto and Bjornsson (1983). Blood samples were allowed to clot, and then centrifuged at 3000 rpm for 20 min to separate serum, which kept at -20 ºC till biochemical analysis. The kidneys were collected immediately after scarification of rats in all groups, fixed in 10% formalin, and prepared for histopathological examination.

Determination of protein metabolism parameters:

Separated serum samples were used for determination of total protein (TP) and albumin according to (Henry, 1964 and Doumas *et al*., 1971, respectively). While globulin and albumin to globulin ratio (A/G ratio) were calculated according to (Oser, 1971).

Determination of kidney functions:

Serum samples were used for determination of creatinine, urea and uric acid, according to (Henry, 1974, Patton and Grouch, 1977 and Fosssati *et al*., 1980, respectively). While albumin/creatinine ratio (milligrams of albumin/grams of creatinine) (ACR) was calculated according to (Holly *et al*., 2002).

Determination of malondialdehyde and ionic sodium and potassium:

Serum samples were used for determination of malondialdehyde (MDA) as a measure of lipid peroxidation according to (Yoshioka *et al*., 1979). For ionic analysis serum samples were mixed with 10% trichloroacetic acid after centrifugation, the diluted supernatant (10%) was used for estimation of sodium (Na^+) and potassium (K^+) metals using atomic absorption spectrophotometer (Pye-Unicom) according to (Niels *et al*., 1984).

Histopathological examination:

Sections were taken from kidney tissues from different animals in each group immediately after sacrificed. The tissues were washed with ice-cold saline solution to remove blood, fixed immediately in 10% neutral buffered formalin, dehydrated in different grades of alcohol, embedded in paraffin wax, sectioned at 4-6 µm thick, stained with Haematoxylin and Eosin and cleared in xylene according to (Bancroft *et al*., 1996) and examined microscopically.

Statistical analysis:

Results were expressed as a (mean ± SE). Data were analyzed statistically by analysis of variance, for statistical significance using L.S.D. test, one way ANOVA, post hoc multiple comparisons according to Snedecor and Cochron (1989). An IBM computer with a software system SPSS version 20 was used for these calculations.

Results:

Biochemical results:

The obtained data shown in Table (1) revealed that, serum levels of total protein and globulin exhibited significant decrease (p<0.001), while serum level of albumin and A/G ratio showed significant increase (p<0.001) in GM group when compared with the corresponding values of control group. On the other hand, oral administration of morin had no effect on the tested

protein parameters, their values tended to match with the control values, thus indicating its safe use under the experimental conditions. Pretreatment with morin showed significant improvement in all tested parameters, there were significant difference (p<0.01) when compared with GM group, while recorded non-significant differences when compared with the corresponding values in the control group.

The data shown in Table (2) presented serum levels of creatinine, urea and uric acid, as well as albumin/creatinine ratio (ACR). Serum creatinine, urea and uric acid exhibited significant elevation (p<0.001) in rats injected with GM in compared with the control group, with percentage of increase equal to 192.59, 368.3 and 270.89 %, respectively. Administration of morin pre-GM injection recorded a significant noticeable improvement in these parameters when compared with GM injected group, the values of tested kidney function parameters in GM-pretreated with morin group were significant difference (p<0.001) as compared with GM untreated group. Concerning albumin/creatinine ratio (ACR), GM induced significant reduction (p<0.001) in ACR as compared with control group. Administration of morin to rats showed non-significant change in ACR as compared to control group, the value of ACR tended to match with control value. While, in group received morin pre-GM injection, there was non-significant change in ACR when compared with control group. On the other hand, administration of morin pre-GM injection recorded significant (p<0.001) improvement in ACR value when compared with GM group.

Table (1): Effect of morin on serum levels of protein metabolism parameters in GM-induced nephrotoxicity in young male rats.

Experimental groups	TP (g/dl)	Alb (g/dl)	Globulin (g/dl)	A/G ratio
Control	7.50 ± 0.13	3.69 ± 0.18	3.81 ± 0.15	0.97 ± 0.10
GM	6.38 ± 0.10 ^{a***}	4.75 ± 0.18 ^{a***}	1.63 ± 0.14 ^{a***}	2.91± 0.24 ^{a***}
Morin	7.49 ± 0.08	3.41 ± 0.19	4.08 ± 0.18	0.84 ± 0.08
GM pretreated with Morin	7.08 ± 0.25 ^{b**}	3.99 ± 0.17 ^{b**}	3.09 ± 0.46 ^{b**}	1.29 ± 0.28 ^{b**}

- Values are means ± SE; n=6 in each group.
- ^{a} Significant difference from control group at $p < 0.05^{*}$, highly significant difference at $p < 0.01^{**}$ and very highly significant difference at $p < 0.001^{***}$.
- ^{b} Significant difference between GM group and GM group pretreated with morin at $p < 0.05^{*}$, highly significant difference at $p < 0.01^{**}$ and very highly significant difference at $p < 0.001^{***}$.

The recorded data in Table (3) revealed significant (p< 0.001) elevation in lipid peroxide (MDA) in GM-injected group as compared with control group, the percentage of increase amounted 183.62 % as compared with control group. Rats received morin pre-GM injection resulted in significant improvement (p< 0.001) in MDA content as compared with GM group. Concerning serum content of ionic Na^+ and K^+ presented in Table (3), the results revealed that ionic Na^+ and K^+ in GM-injected group recorded significant difference (p< 0.001) comparing with control group, there were significant decrease in Na^+ concomitant with significant increase in K^+ after GM injection. Morin was found to have no significant effect on serum content of ionic Na^+ and K^+ as comparing with control group. Administration of morin pre-GM injection ameliorated the effects of GM, there were significant difference in ionic content of Na^+ and K^+ (p<0.001) when compared with GM group.

Table (2): Effect of morin on serum levels of kidney functions, and albumin/creatinine ratio (ACR) in GM-induced nephrotoxicity in young male rats.

Experimental groups	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	ACR
Control	0.81 ± 0.02	24.86 ± 1.26	2.13 ± 0.057	4.56 ± 0.24
GM	1.56 ± 0.046 ^{a***}	91.56 ± 3.01 ^{a***}	5.77 ± 0. 15 ^{a***}	3.04 ± 0.14 ^{a***}
Morin	0.74 ± 0.019	23.82 ± 1.01	2.09 ± 0.053	4.61 ± 0.24
GM pretreated with Morin	$^{a*\ b***}$ 0.91 ±0.021	$^{a*\ b***}$ 32.56 ± 1.94	$^{a*\ b***}$ 2.52 ± 0.099	^{b***} 4.38 ± 0.14

- Values are means ± SE, n=6 in each group.
- ^{a} Significant difference from control group at $p < 0.05^{*}$, highly significant difference at $p < 0.01^{**}$ and very highly significant difference at $p < 0.001^{***}$.
- ^{b} Significant difference between GM group and GM group pretreated with morin at $p < 0.05^{*}$, highly significant difference at $p < 0.01^{**}$ and very highly significant difference at $p < 0.001^{***}$.

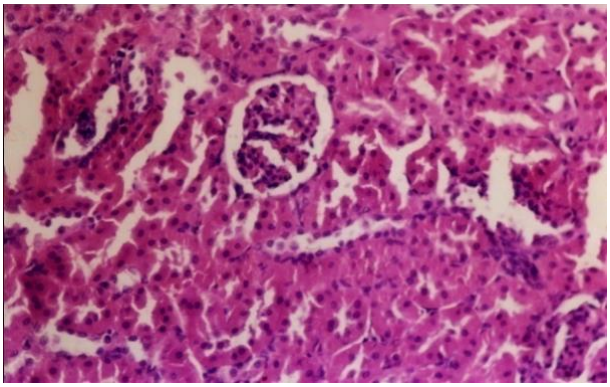
Table (3): Effect of morin on serum levels of malondialdehyde (MDA), sodium (Na^{+}) and potassium (K^{+}) in GM-induced nephrotoxicity in young male rats.

Experimental groups	MDA (µmol/l)	Na^{+} (mmol/l)	K^{+} (mmol/l)
Control	77.37 ± 0.92	145.63 ± 1.28	5.52 ± 0.18
GM	142.07 ± 1.51 ^{a***}	88.54 ± 1.59 ^{a***}	14.22 ± 0.29 ^{a***}
Morin	74.86 ± 1.03	145.42 ± 1.45	5.37 ± 0.12
GM pretreated with Morin	81.21 ± 0.94 ^{a*b***}	141.55 ± 1.73 ^{b***}	6.06 ± 0.07 ^{b***}

- Values are means ± SE; n=6 in each group.
- ^{a} Significant difference from control group at $p < 0.05^{*}$, highly significant difference at $p < 0.01^{**}$ and very highly significant difference at $p < 0.001^{***}$.
- ^{b} Significant difference between GM group and GM group pretreated with morin at $p < 0.05^{*}$, highly significant difference at $p < 0.01^{**}$ and very highly significant difference at $p < 0.001^{***}$.

Histological results:

Microscopically, Kidney sections from control rats revealed the normal structure of renal parenchyma (Fig 1). Kidney of rats injected with GM showed severe changes as evidenced by thickening and vacuoletions in the tunica media of renal blood vessels, preivascular leucocytic cells infiltration together with intratubular eosinophilic and flocculent renal casts (Fig 2), vacuolations of endothelial lining the glomerular tufts associated with hypercellularity of the tufts, necrobiotic changes of renal tubular epithelium and pyknosis of their nuclei (Fig 3), and focal interstitial nephritis associated with mononuclear cells infiltration (Fig 4). Interstial nephritis associated with marked mononuclear cell infiltration were also recorded (Fig 5). Kidney of rats group received morin showed no histopathological alterations (Fig 6). Concerning kidney sections of GM group pretreated with morin showed no histopathological changes (Fig 7), except slight congestion of peritubular capillaries (Fig 8).



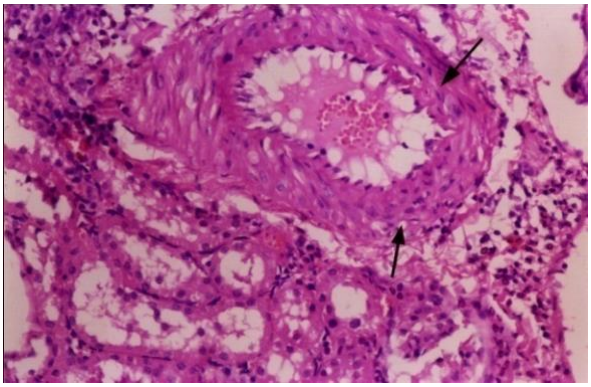
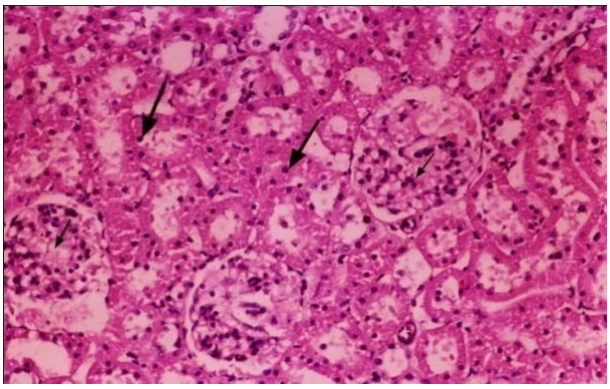


Fig (1): Kidney of control rat showing the normal structure of renal parenchyma. (H &E x 200)

Fig (2): Kidney of rats injected with GM showing marked thickening and vaculation in the wall of blood vessel (arrow), as well as perivascular leucocytic cells infiltration. (H &E x 200)



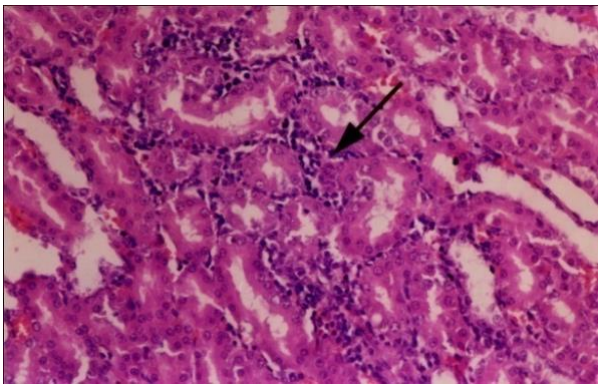
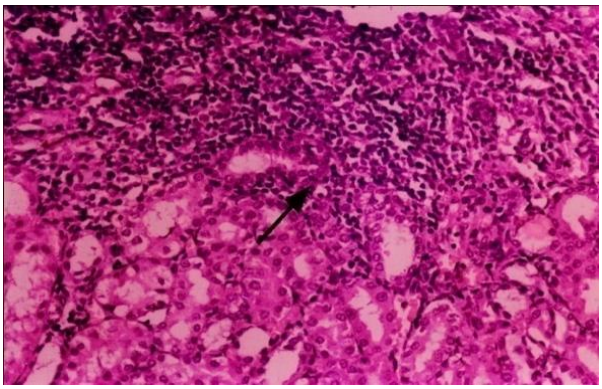


Fig (3 &4): Kidney rats injected with GM showing vacuolations of endothelial lining the glomerular tufts (small arrow), associated with hypercellularity of the tufts, necrobiotic changes of renal tubular epithelium and pykenosis of their nuclei (large arrow) (Fig 3), and Focal interstitial nephritis with mononuclear cells infiltration (arrow). (H &E x 200)



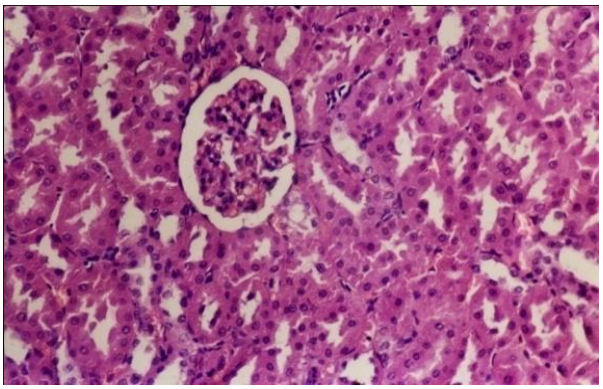
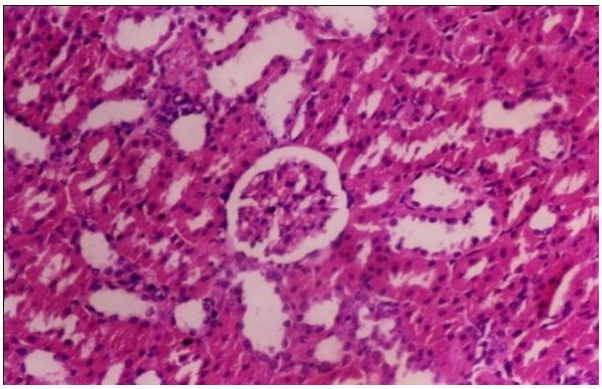


Fig (5): Kidney of rats injected with GM showing interstial nephritis associated with marked mononuclear cell infiltration (arrow). (H &E x 200)

Fig (6): Kidney of morin group showing no histopathological alterations. (H &E x 200)



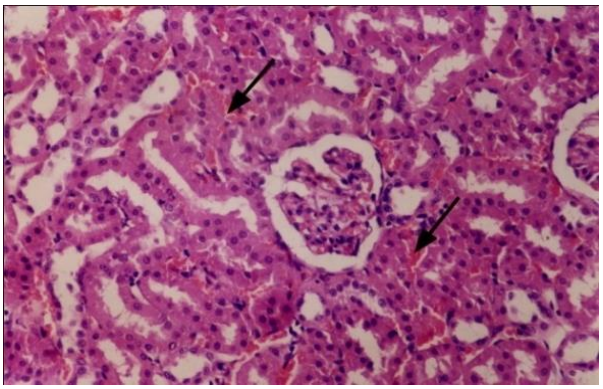


Fig (7 & 8): Kidney of GM rats pretreated with morin showing no histopathological changes (Fig 7), except slight congestion of peritubular capillaries (Fig 8). (H &E x 200)

Discussion:

Gentamicin is an aminoglycoside antibiotic widely used for the treatment of bacterial infections. Therapeutic doses of gentamicin and other aminoglycoside antibiotics can produce nephrotoxicity in humans and animals, for a more overall insight in pathophysiological mechanisms in the development of toxic tubular damage induced with gentamicin, as well as in new therapeutic approach, numerous studies were conducted in experimental model and indicated that reactive oxygen species (ROS) are potential mediators involved in gentamicin-induced renal injury. ROS can alter the basic cellular constituents and their organelles. Such oxidant-induced alterations can profoundly affect cellular vitality. ROS impair enzymatic and structural protein molecules through such mechanisms as oxidation of sulfhydryl group, carbonyl formation and deamination. They also may destabilize cytoskeletal proteins, which facilitate the attachment of cells to the neighboring extracellular matrix. Thus, oxidants have a capacity to attack and disable multiple critical cellular targets and thereby provoke cell death (Cuzzocrea *et al*., 2002, Kopple *et al*., 2002, Avdagić *et al*., 2007 and Poormoosavi *et al*., 2010)).

Flavonoids have recently attracted great interest as potential therapeutic agents against a variety of diseases, most by involving radical damage. These polyphenolic compounds, ubiquitous in higher plants, are commonly major dietary constituents. Morin (2',3,4',5,7-penta-hydroxyflavone), is a flavone originally isolated from the Moracease family, which used as a dietary agents and also herbal medicines (Lotito and Frei, 2006 and Xie *et al*., 2006). Morin has been shown to exhibit antiproliferative, antitumor, and anti-inflammatory effects (Sunil *et al*., 2007).

This study showed that i.p. injection with GM at a dose of 100 mg /kg b. w. to rats for five consecutive days reduces glomerular function, as reflected by marked significant increase in the serum levels of albumin, creatinine, urea and uric acid concomitant with significant decrease in serum levels of total protein and ACR in young rats injected with GM due to renal damage. Rats received morin did not produce detectable changes in protein metabolism and kidney function parameters. While GM group pretreated with morin exhibited significant improvement in these investigated parameters, thus may be due to effective protective action of morin.

These findings were in coincidence with the observation of Soliman *et al*. (2007) and Patil *et al*. (2010) who used gentamicin (at a dose 80 and 100 mg/kg, respectively) for experimental nephro-toxicity in rats and their results were similar to the present study. Ihab *et al*. (2010) reported that GM induced elevation in serum total protein, BUN, creatinine and TBARS levels. 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 administered 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,

and then developed injury in the proximal tubular epithelial cells of kidney, that caused acute renal failure (Swan, 1997). In addition, the obtained results may be attributed to gentamicin enhance generation of superoxide anions, peroxinitrite anions, and hydrogen peroxide from renal cortical mitochondria, as well as induced enhanced generation of nitric oxide as occurs by activation of inducible nitric oxide synthase (iNOS), which cause deleterious to the kidney tissues (Katusic, 1996 and Avdagić *et al*., 2007).

Uric acid is the end product of purine metabolism, hyperuricemia is associated with impaired renal function, lowering of elevated uric acid level in the blood could be achieved by xanthine oxidase inhibitors and inhibitors of renal urate reaborption (Rott and Agudelo, 2003 and Yu *et al*., 2006). Creatinine and urea is increased under severe renal dysfunction and reduced renal blood flow (Rajendran, 2002). In addition, decreased ratio of albumin/creatinine (ACR) is an important predictor of progression of kidney diseases (Holl *et al*., 2002 and Kestebbaum and Boer, 2010). However, the protective role of morin may be explained by potent inhibitory action on urate uptake in rat renal, inhibitory effect of morin on xanthine oxidase, and the free radical scavenging capacity and antioxidant activity of morin (Yu *et al*., 2006 and Venkatesan *et al*., 2010).

A relationship between oxidative stress and nephrotoxicity has been well demonstrated in many experimental animal models (Somova *et al*., 2003). In gentamicin-injected rats, a significant increase in lipid peroxidation products as malondialdehyde (MDA) suggesting that the involvement of oxidative stress, which has been reported, while administration of morin pre-injection with GM tended to normalize the level of MDA. A role of lipid peroxidation in gentamicin-induced nephrotoxicity has also been described in previous studies. Cuzzocrea *et al*. (2002) reported that, injection of gentamicin at a dose of (100 mg/kg b.wt.) for five days to rats induced a marked renal failure, characterized by significant increase in lipid peroxidation and kidney myeloperoxidase activity. El-Ashmawy *et al*. (2006) reported that gentamicin injection at a dose of (80 mg/kg b.wt.) induced kidney damage as indicated by significant increase in serum levels of urea, creatinine and MDA, and pronounced changes in kidney histology. Meanwhile, pretreatment of rats with hydroxyl radical scavengers has been shown to protect against gentamicin-induced acute renal failure (Kumar *et al*., 2000). The nephroprotective effect of morin may be due to morin reduce lioperoxidation by direct antioxidant effect (Bartosikova *et al*., 2003).

The kidney plays a central role in the regulation the balance of body salt and water, and then disordered regulation of renal functions is responsible for the altered balance of salt and water. In the present study, serum electrolytes were disturbed significantly (p <0.001) in GM injected rats as compared with control rats. 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 increase 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 (Padmini and Kumar, 2012). These results are in harmony with the data obtained by Heibashy and Abdel Moneim (1999) and Heibashy *et al.* (2009). The obtained results may be due to kidney damage caused by the oxidative stress by increasing the formation of free radicals (Prahalathan *et al*., 2012).

Oral administration of morin considerably reduced the raised serum level of K^+ and increased the low level of Na^+, bringing about remarkable recovery in kidneys. Considerable improvement in these levels might be due to the antioxidant property of morin. These findings are correlated well with the kidney histology examination. Kidney oxidative stress was effectively modulated by morin administration, morin significantly improved the status of kidney antioxidants and decreased the levels of ammonia, urea and TBARS, thus may be attributed to antioxidant effects of morin against oxidative stress in the kidney, induced by ammonium chloride (Subash and Subramanian, 2011).

Biochemical data were concordant with pathological findings. Kidney sections from rats injected with GM showed severe changes, marked as thickening, preivascular leucocytic cells infiltration, vacuolations of endothelial lining the glomerular tufts, necrobiotic changes of renal tubular epithelium and pyknosis of their nuclei, and focal interstitial nephritis associated with mononuclear cells infiltration. These results confirmed with (Harlalka *et al*., 2007). Rats received morin alone showed normal appearance of the kidney without any pathological changes. This indicates that morin does not possess any adverse effect under the experimental conditions. The histopathological findings of the present study confirmed the obtained biochemical results, where oral administration of morin pre-GM injection considerably normalized the tested biochemical and bringing about remarkably recovery in kidney as evidenced microscopically. These may be due to the antioxidant property of morin. Administration of morin remarkably minimized the structural changes in kidney, thus may be explained by increase intake of antioxidants avoided or minimized kidney injury by reducing oxidative stress (Prahalathan *et al*., 2012).

In conclusion, gentamicin as an aminoglycoside antibiotic produces nephrotoxicity, due in part to an imbalance of pro and anti-oxidants (oxidative stress). The correction of oxidative stress biomarkers by morin was consistent with amelioration of the biochemical and histopathological changes induced by GM-injection. Thus, protective effect of morin against GM-induced renal damage may be explained by its antioxidant and free radicals scavenger properties of morin.

Acknowledgment:

I am grateful to **Dr. Kawkab A. Ahmed**, Professor of Pathology, Faculty of Veterinary Medicine, Cairo University, for her help to carry out the histopathological study of this work.

References:

Abdel-Naim AB, Abdel-Wahab MH and Attia FF (1999): Protective effects of vitamin E and probucol against Gentamicin nephrotoxicity in rats. Pharmacol Res., 40 (2): 183-187.

Avdagić N, Ćosović E, Nakaš-Ićindić E, Mornjaković Z, Začiragić A and Hadžović-Džuvo A (2008): Spirulina platensis protects against renal injury in rats with gentamicin-induced acute tubular necrosis**.** Bosn. J. Basic Med. Sci., 8 (4): 331-336.

Avdagić N, Nakaš-Ićindić E, Rašić S, Hadžović-Džuvo A, Začiragić A and Valjevac A (2007): The effects of inducible nitric oxide synthase inhibitor L-N6 -(1-iminoethyl) lysine in gentamicin-induced acute tubular necrosis in rats. Bosn. J. Basic Med. Sci., 7(4):345-351

Bancroft D, Stevens A and Turmer R (1996): Theory and practice of histological technique, 4th ed., Churchill Living Stone, Edinburgh, London, Melbourne. p: 47-67.

Bartosikova L, Necas J, Suchy V, Kubinova R, Vesela D, Benes L, Bartosik T, Illek J, Salplachta J, Klusakova J, Bartosova L, Strnadova V, Frana P and Franova J (2003): Monitoring of antioxidative effect of morin in alloxan-induced diabetes mellitus in the laboratory rat. Acta Vet. Brno., 72:191-200.

Cocchetto DM and Bjornsson TD (1983): Methods for vascular access and collection of body fluids from the laboratory rat. J. Pharm. Sci., 72 (5): 465- 492.

Cuzzocrea S, Mazzon E , Dugo L, Serraino I, Di-Paola R, Britti D, De- Sarro A, Pierpaoli S, Caputi A, Masini E and Salvemini D (2002): A role for superoxide in gentamicin-mediated nephropathy in rats. Eur. J. Pharmacol., 450(1):67-76.

Doumas BT, Watson WR and Biggs HG (1971): Measurement of serum albumin with bromocresol green. Clin. Chem. Acta, 31: 87.

El-Ashmawy IM, El-Nahas AF and Salama OM (2006): Grape seed extract prevents gentamicin-induced nephrotoxicity and genotoxicity in bone marrow cells of mice. Basic Clin. Pharmacol. Toxicol., 99 (3): 230-236.

Fosssati P, Prencipe L and Berti G (1980): Uric acid measurements with enzymatic colorimetric method. Clin. Chem., 26:227-273.

Galvez J, Coelho G , Crespo ME, **Cruz T, Rodríguez-Cabezas ME, Concha A, Gonzalez M and Zarzuelo A (2001):** Intestinal anti-inflammatory activity of morin on chronic experimental colitis in the rat. Aliment Pharmacol. Ther., 15 (12) : 2027-2039.

Harlalka GV, Patil CR and Patil MR (2007): Protective effect of *Kalanchoe pinnata pers*. (Crassulacease) on gentamicin- induced nephrotoxicity in rats. Indian J. Phatamacol., 39(4): 201-205.

Heibashy MIA and Abdel Moneim AE (1999): Kidney and liver function tests after late dimethyl sulfoxide (DMSO) administration in rats with gentamicin induced acute renal failure. J. Egypt German Soc. Zool., 30(A): 35-48.

Heibashy MIA, El-Nahla AM, Ibrahim AI and Saleh YA (2009): Comparative study between dimethyl sulfoxide (DMSO), allopurinol and urate oxidase administration in nephrotoxic rats induced with gentamicin. 43rdAnnual Veterinary Medical Symposium, College of Veterinary Medicine Nursing and Allied Health, Tuskegee University, Alabama, USA.

Henry RJ (1964): Determination of total protein by colorimetric method. Clinical Chem., Harper and Row Publishers, New York, p:181.

Henry RJ (1974): Creatinine measurements with colorimetric method. Clin. Chem., principles and techniques. 2nd ed., Harper and Row Publishers, New York, p: 525.

Holly JM, Chi-Yuan H, Shimon S and Gary C (2002): Use of the albumin/creatinine ratio to detect microalbuminuria: implications of sex and race. J. Am. Soc. Nephrol., 13 (4): 1034-1039.

Ibrahim MA and Saleh AA (2012): Comparative study of quercetin or/and urate oxidase against gentamicin-induced nephrotoxicity and oxidative stress in rat kidneys. J of Am. Sci., 8(1):600-607.

Ihab TA, Gamal AE and Ashraf T (2010): Green tea ameliorates renal oxidative damage induced by gentamicin in rats. Pak. J. Pharm. Sci., 23(1):21-28.

Kang DG, Moon MK, Sohn EJ, Lee DH and Lee HS (2004): Effects of morin on blood pressure and metabolic changes in fructose-induced hypertensive rats. Biol. Pharm. Bull., 27 (11):1779–1783.

Katusic ZS (1996): Superoxide anion and endothelial regulation of arterial tone. Free. Radic. Biol. Med., 20(3):443-448.

Kawabata K, Tanaka T, Honjo S, Kakumoto M, Hara A, Makita H, Tatematsu N, Ushida J, Tsuda H and Mori H (1999): Chemopreventive effect of dietary flavonoid morin on chemically induced rat tongue carcinogenesis. Int. J. Cancer, 83 (3):381-386.

Kestebbaum B and Boer IH (2010): Urine albumin-to-creatinine ratio: What's in a` number?. J. Am. Soc. of Nephrology, 21(8):1243-1244.

Kopple JD, Ding H, Letoha A, **Ivanyi B, Qing DP, Dux L, Wang HY and Sonkodi S (2002):** L-carnitine ameliorates gentamicin-induced renal injury in rats. Nephrol. Dial. Transplant., 17 (12):2122-2131.

Krol W, Dworniczak S, Pietsz G, Czuba ZP, Kunicka M, Kopacz M and Nowak D (2002): Synthesis and tumoricidal activity evaluation of new morin and quercetin sulfonic derivatives. Acta Pol. Pharm., 59 (1):77-79.

Kumar KV, Naidu MUR, Shifow AA and Ratnakar KS (2000): Probucol protects against gentamicin-induced nephrotoxicity in rats. Indian J. of Pharmacol., 32(2):108–113.

Lotito SB and Frei B (2006): Consumption of flavonoid rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon. Free Radic. Biol. Med., 41(12): 1727-1746.

Martinez-Salgado C, Henindez-Lopez FJ and Novoa-Lopez JM (2007): Glomerular nephrotoxicity of aminoglycosides. Toxicol. Appl. Pharmacol., 223(1):86-98.

Middleton E, Kandaswami C and Theoharides TC (2000): The effects of plant flavonoids on mammalian cells: implications for

inflammation, heart disease, and cancer. Pharmacol. Rev., 52 (4):673- 751.

Morales AI, Buitrago JM, Santiago JM, Fernandez-Tagarro M, Novoa JM and Perez-Barriocanal F (2002): Protective effect of trans-resveratrol on gentamicin-induced nephrotoxicity. Antioxid. Redox. Signal., 4(6):893-898.

Niels FA, Peter DW, Jurgen T and Ole SA (1984): Determination of sodium and potassium with ion-selective electrodes. Clin. Chem., 30(3): 433-436

Oser B (1971): Hawk's physiological chemistry. 14th ed., Tata Mc Graw-Hill Publishing Co, Ltd. New Delhi.

Padmini MP and Kumar JV (2012): A histopathological study on gentamicin induced nephrotoxicity in experimental albino rats. J. of Dental and Med. Sci., 1(1):14-17.

Patil CR, Jadhav RB, Singh PK, Mundada S and Patil PR (2010): Protective effect of oleanolic acid on gentamicin induced nephrotoxicity in rats. Phytother Res., 24(1):33-37.

Patton C and Grouch SR (1977): Enzymatic determination of urea. Anal. Chem., 49:464-468.

Pedraza-Chaverri J, Gonzalez-Orozco AE and Maldonado PD (2003): Diallyl disulfide ameliorates gentamicin-induced oxidative stress and nephropathy in rats. Eur. J. Pharmacol., 473(1): 71-78.

Poormoosavi SM, Behmanesh MA and Najafzadeh H (2010): Effect of cimetidine on gentamicin-losartan induced -nephrotoxicity in rats. African J. of Pharmacy and Pharmacol., 4(6): 341-345.

Prahalathan P, Kumar S and Raja B (2012): Effect of morin, a flavonoid against DOCA- salt hypertensive rats: a dose dependent study. Asian Pacific J. of Tropical Biomedicine, 2 (5): 443-448.

Rajendran K (2002): Test procedure on clinical lab technology, J. P. Brothers Pvt ltd, 3rd ed.

Ranelletti FO, Ricci R ,Larocca LM , Maggiano N, Capelli A, Scambia G, Benedetti-Panici P, Mancuso S, Rumi C and Piantelli M (1992): Growth inhibitory effect of quercetin and presence of type-II estrogen-binding sites in human colon-cancer cell lines and primary colorectal tumors. Int. J. Cancer, 50 (3):486-492.

Reeves PG, Nielsen FH and Fahey GC (1993): AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr., 123(11):1939-1951.

Rott KT and Agudelo CA (2003): Gout. J. of the Am. Med. Assoc., 289 (21): 2857-2860.

Snedecor GW and Cochron WG (1989): Statistical methods. 8th ed., Lowa State Univ. Press, Ames, Lowa, USA.

Soliman KM, Abdul-Hamid M and Othman AI (2007): Effect of carnosine on gentamicin-induced nephrotoxicity. Med. Sci. Monit., 13(3): 73-83.

Somova LO, Nadar A, Rammanan P and Shode FO (2003): Cardiovascular, antihyperlipidemic and antioxidant effects of oleanolic and ursolic acids in experimental hypertension. Phytomedicine, 10(3):115–121

Sreedharan V, Venkatachalam KK and Namasivayam N (2009): Effect of morin on tissue lipid peroxidation and antioxidant status in 1,2-dimethylhydrazine induced experimental colon carcinogenesis. Invest. New Drugs, 27(1): 21-30.

Subash S and Subramanian P (2008): Effect of morin on the levels of circulatory liver markers and redox status in experimental chronic hyperammonaemic rats. Singapore Med. J., 49(8): 650-655.

Subash S and Subramanian P (2009): Morin a flavonoid exerts antioxidant potential in chronic hyperammonemic rats: a biochemical and histopathological study. Mol. Cell. Biochem., 327 (1-2): 153-161.

Subash S and Subramanian P (2011): Impact of morin (a bioflavonoid) on ammonium chloride-mediated oxidative damage in rat kidney. Inter J. of Nutr. Pharm. Neuro. Diseases,1(2): 174-178.

Sunil KM, Rishi SA, Gautam S, Bharat BA and Govindarajan TR (2007): Morin (3,5,7,2',4'-pentahydroxyflavone) abolishes nuclear factor-ĸB activation induced by various carcinogens and inflammatory stimuli, leading to suppression of nuclear factor-ĸB-regulated gene expression and up-regulation of apoptosis. Clin. Cancer Res., 13(7): 2290-2297.

Swan SK (1997): Aminoglycoside nephrotoxicity: review. Seminars in nephrology 17(1):27-33.

Vanessa BS, Rodrigo- Fagundes LO, Hévio FL, Aurigena AAF, Gerlane CBG, Maria LF, Karla CSQ and Raimundo FA (2009): Gentamicin induces renal morphopathology in wistar rats. Int. J. Morphol., 27(1): 59-63.

Venkatesan RS, Sadiq AM, Kumar JS, Lakshmi GR and Vidhya R (2010): Effect of morin on mercury chloride induced nephrotoxicity. An Inter. Quarterly J. of Environ. Sci., 4 (3):193-196.

Walker PD, Barri Y and Shah SV (1999): Oxidant mechanisms in gentamicin nephrotoxicity. Ren. Fail., 21(3-4): 433- 442.

Xie MX, Long M, Liu Y, Qin C and Wang YD (2006): Characterization of the interaction between human serum albumin and morin. Biochim. Biophys. Acta, 1760 (8): 1184-1191.

Yoshioka T, Kawada K, Shimada T and Mori M (1979): Lipid peroxidation in maternal and cord blood and protective mechanism against activated oxygen toxicity in the blood. Am. J. Obstet. Gynecol., 135 (3):372-376**.**

Yu Z, Fong WP and Cheng CH (2006): The dual actions of morin (3,5,7,2',4'-pentahydroxyflavone) as a hypouricemic agent: Uricosuric effect and xanthine oxidase inhibitory activity. J. of Pharmacol. Exp. Ther., 316 (1): 169-175.

Zhang R, Kang KA, Piao MJ, Maeng YH, Lee KH, Chang WY, You HJ, Kim JS, Kang SS and Hyun JW (2009): Cellular protection of morin against the oxidative stress induced by hydrogen peroxide. Chem. Biol. Interact., 177 (1): 21 27.

الملخص العربي

تأثير المورين ضد السمية الكلوية التي يُحدثها الجنتاميسين في ذكور الفئران الصغيرة

هالة عبد الرحمن حسن خطاب

قسم التغذية وعلوم الأطعمة- كلية الإقتصاد المنزلي- جامعة حلوان

المورين من البيوفلافونيدات التي لها خصائص مضادة للأكسدة، وهو يعتبرمن المكونات للعديد من الأعشاب والفواكه التي تستخدم كأعشاب طبية، ويظهر المورين العديد من الخصائص البيولوجية ولة تأثير وقائي ضد العديد من الأمراض المزمنة. **وقد أجريت هذه الدراسة لتحديد** الـتأثير الوقائي للمورين ضد التسمم الكلوي المحُدث بواسطة الجنتاميسين في ذكور الفئران الصغيرة.

تم تقسيم ذكور الفئران الصغيرة (عددهم =٢٤ فأر) إلي أربع مجموعات: المجموعة الأولي الضابطة، المجموعة الثانية الجنتاميسين (تم حقن الفئران داخل الغشاء البريتوني بمادة الجنتاميسين بجرعة مقدراها ١٠٠مجم/ كجم من وزن الجسم لمدة ٥ أيام متتالية لإحداث سمية في الكلي)، المجموعة الثالثة المعطاة المورين (تم أعطاء الفئران المورين بجرعة مقدراها ٣٠ مجم/ كجم من وزن الجسم عن طريق الفم)، والمجموعة الرابعة مجموعة الجنتاميسين التي تم معالجتها مسبقا بالمورين(تم أعطاء الفئران المورين بنفس الكيفية والجرعة مثل المجموعة الثالثة لمدة أسبوعين ثم تم حقنهم بالجنتاميسين مثل المجموعة الثانية). تم تقدير محتوي مصل الدم من مقاييس التمثيل الغذائي للبروتين، وظائف الكلي، المالوندای ألدهيد، وأيوني الصوديوم والبوتاسيوم.

أوضحت النتائج أن الحقن بمادة الجنتاميسين قد أحدث سمية كلوية بدرجة عالية كما يتضح ذلك من خلال حُدوث إرتفاع ذو دلالة إحصائية في مُحتوي مصل الدم من الالبيومين، الكرياتينين، اليوريا، حمض اليوريك، المالونداي ألدهيد وأيون البوتاسيوم، مع حدوث إنخفاض ذو دلالة إحصائية في محتوي مصل الدم من البروتين الكلي، أيون الصوديوم ونسبة الالبيومين الي الكرياتينين. وقد أدي إعطاء المورين إلي وقاية الفئران من السمية الكلوية المحُدثة بواسطة الجنتاميسين، وقد ظهر ذلك من خلال حدوث تحسنا ملحوظا ذو دلالة إحصائية في كل القياسات المختبرة. وقد أظهر الفحص الهستوباتولوجي في الخلايا الكلوية، زيادة سمك جدران الأوعية الدموية وتنكس فَجويٌ بالكبيبات مع حدوث تغييرات نسيجية وموت موضعي في الخلايا الكلوية في المجموعة المحقونة بالجنتاميسين ، بينما أدت المعالجة المسبقة بمادة المورين الي حُدوث تحسناً ملحوظاً في الخلايا الكلوية.

أتضح من هذه الدراسة أن المورين له تأثيرفعال بدرجة عالية كمضاد للأكسدة وكمادة واقية من التسمم الكلوي المحُدث بواسطة الجنتاميسين.

الكلمات المفتاحية: المورين، الجنتاميسين، السمية الكلوية، مضادات الأكسدة، ذكور الفئران الصغيرة.