Effect of Licorice Extract against Gentamicin-Induced Nephrotoxicity in Male Rats

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Abstract: Background: Nephrotoxicity induced by several synthetic drugs represents a major problem of modern population. Licorice *Glycyrrhiza glabra L.*is one of the most widely used herbal drugs around the world, being present in most pharmacopoeias of eastern and western countries. It exhibits many biological activities and possibly even has protective effects against chronic diseases. **Aim:** The present study was conducted to determine the protective effect of licorice extract against gentamicin-induced nephrotoxicity in male rats. **Material and Methods:** Forty male rats 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): licorice extract group; rats administered licorice extract daily at a dose of 150 mg/ kg b. w. *via* gavage, and (4) GM group pretreated with licorice extract; rats were orally received licorice extract at the same dose and route in group (3) up to seven days followed by injected i.p. with GM as in group (2). Separated serum samples were used for determination of kidney functions and ionic sodium (Na^+) and potassium (K^+). One kidney was used for estimation of lipid peroxides (MDA) and reduced glutathione (GSH), while the other kidney was examined histopathological. **Results:** GM injection induced marked nephrotoxicity as evidenced by significant elevation in serum levels of creatinine, urea, uric acid and K^+, with significant reduction in serum levels of Na^+. Antioxidant status in kidney tissues showed that, in GM there was significant increase in MDA with significant depletion in GSH activity as compared with control group. Pretreatment with licorice extract protected the rats from GM- induced nephrotoxicity as evidenced by significant improvement of these investigated parameters, with restore antioxidant status. Histological examination of renal tissues showed marked glomerular, vacuolations of the wall of blood vessels associated with necrobiotic changes in renal tubules in GM injected group, meanwhile there were amelioration in rats group received licorice extract pre-GM injection. **Conclusion:** Licorice extract exert potential antioxidant activity and offer nephroprotective effect against GM- induced nephrotoxicity in male rats.
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Key words: Licorice extract, gentamicin, nephrotoxicity, antioxidant, rats.

1. Introduction:

Gentamicin is a bactericidal aminoglycoside antibiotic with wide clinical use but disturbing toxicity. Nephrotoxicity and ototoxicity are the most common adverse reactions **(Martínez-Salgado *et al.*, 2007** and **Rybak and Ramkumar, 2007)**. Therapeutic doses of gentamicin and other aminoglycoside antibiotics can produce nephrotoxicity in humans and animals and use of this class of antibiotics is known as one of the most common causes of acute renal failure **(Cuzzocrea *et al.*, 2002)**, possibly due to increased renal uptake of the antibiotic, mainly by the proximal tubules. The effect of gentamicin on biological membranes appears to be important in its toxicity. It has been proposed that the accumulation of aminoglycosides in proximal tubular epithelial cells leads to membrane structural disturbance and cell death by reactive oxygen species (ROS) involvement **(Kadkhodaee *et al.*, 2005)**. Reactive oxygen species produce cellular injury and necrosis *via* several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage **(Cuzzocrea *et al.*, 2002)**. Various attempts at controlling gentamicin toxicity have been suggested some usefulness of the approach in controlling gentamicin toxicity **(Rybak and Ramkumar, 2007)**. Licorice extract is natural remedies from medicinal plants and considered to be effective and safe alternative treatments for inflammation, liver and kidney disorders and oxidative damage.

Licorice, the root of the *Glycyrrhiza glabra* L. (*Fabaceae*) plant species, has been used medicinally for more than 4000 years **(Aoki *et al.*, 2005).** These medicinal plants are used as flavorings, sweeteners and herbal medicine, and also for improving health, detoxification and cures for injury (**Cherng *et al.*, 2006)**. They have been traditionally used for respiratory, gastrointestinal, cardiovascular, genitourinary, eye, and skin disorders, as well as for their antiviral effects **(Zhang and Ye, 2009)**. It has held claim for therapeutic use for fevers, liver ailments, dyspepsia, gastric ulcers, sore throats, asthma, bronchitis, Addison's disease and rheumatoid arthritis and has been used as a laxative, antitussive

and expectorant **(Schulz *et al*., 1998 and Wang *et al*., 2000)**. Derivatives of licorice root have been used in Asia to treat children with biliary atresia, a cholestatic liver disease **(Eric *et al*., 2005)**. Licorice has chemical constituents as an active component such as glycyrrhetinic acid, glycyrrhizin and their aglycones, which are originally isolated from aqueous extracts and help to improve the function of its pharmacological properties **(Gumpricht *et al*., 2005)**. Therefore, the present study was undertaken to evaluate the nephroprotective effects of licorice extract against oxidative stress induced by gentamicin in rat.

2. Material and Methods:

2.1. Drugs and chemicals.

Gentamicin (Gentam-80) as gentamicin sulphate (each 1ml of gentam-80 contains gentamicin sulphate equivalent to 40 mg gentamicin base) was obtained from King Abdulaziz University (KAU) Hospital, Jeddah, KSA. Licorice (*Glycyrrhizza glabra*), derived glycyrrhizic acid a standardized corticosteroid supplement prepared from licorice extract (pure: 98.7%), in capsules, was obtained from GNC, General Nutrition Centers in Saudi Arabia. All chemical and kits with high grade obtained from Sigma-Aldrich (St. Louis, MO) Chemical Co.

2.2. Pretreatment with licorice extracts and mode of administration.

Licorice extracts was suspended in distal water, and administrated daily to rats at a dose of 150 mg / kg b.w. dissolved in distilled water **(Huo, 2011)**, orally using an intragastric tube for a period of seven days.

2.3. Induction of nephrotoxicity by gentamicin.

Gentamicin, as gentamicin sulphate, used was in the form of water soluble solution each. 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)**.

2.4. Experimental animals.

Forty male albino rats, *Sprague Dawley* strain, weighing (120-150 g) were housed in plastic cages, fed on standard diet and given tap water *ad libitum*. All rats were handled in accordance with the standard guide for the care and use of laboratory animals.

2.5. Experimental design.

After the period of adaptation (one week), animals were divided into four groups (each of 10 rats) as following: **First group (control):** Rats were administered orally by gavage a single daily dose of dis. water for seven days, then injected i.p. with saline for five days. **Second group (GM):** 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. **Third group (licorice extract):** Rats received licorice extract at a dose level of 150 mg / kg b.w. *via* oral administration up to seven days, and then injected i.p. with saline for five days. **Fourth group (GM pretreated with licorice extract):** Rats were orally received licorice extract daily at the same dose and route in group (3) up to seven days, 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 seven days and GM accordingly.

2.6. Samples collection and serum separation.

One day after the end of GM injection, 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 specimens were collected immediately after scarification of rats in all groups, one of them were frozen until used for estimation of lipid peroxides (MDA) and reduced glutathione (GSH), while the second were fixed in 10% formalin and prepared for histopathological examination.

2.7. 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).

2.8. Determination of kidney non-enzymatic and enzymatic antioxidants.

Kidney tissues were washed with ice-cold PBS. Tissues were homogenized in approximately 5.0 volumes of ice-cold phosphate buffer (pH 8.0, 0.01 M) using a polytron homogenizer (pt 3100) (five cycles of 10 s at 3000 rpm). Homogenates (20% w/v) were then prepared by sonication in ice-cold phosphate buffer (pH 8.0, 0.01 M). Aliquots were prepared and used for the assessment of malondialdehyde, a reactive aldehyde that is a measure of lipid peroxidation and was expressed as nmol/g tissue **(Uchiyama and Mihara, 1979)** and reduced glutathione (GSH) was expressed as umol/g tissue **(Ellman, 1959**).

2.9. Determination of ionic sodium and potassium.

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).**

2.10. 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.

2.11. 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.

3. Results:

3.1. Effect of licorice extracts on kidney functions.

Table (1) demonstrates the effect of licorice extract on serum levels of creatinine, urea nitrogen and uric acid. Administration of licorice extract to rats showed insignificant change (p>0.05) in all tested kidney function parameters as compared with control group. There was marked significant (p<0.001) increase in serum creatinine, urea nitrogen and uric acid levels were noted in GM injected rats as compared with control group, with percentage of increase equal to 164.15%, 126.73% and 73.30 %, respectively as percent change from control group. However, pretreatment with licorice extract exhibited significantly (p<0.001) reduced kidney functions parameters as compared with GM group.

Table (1): Effect of licorice extract pretreatment on creatinine, urea nitrogen and uric acid in GM-Induced nephrotoxicity in male rats

Experimental groups	Creatinine (mg/dl)	Urea nitrogen (mg/dl)	Uric acid (mg/dl)
Control	0.65 ± 0.062	37.33 ± 3.23	1.91 ± 0.12
Licorice	0.692 ± 0.067	36.54 ± 2.41	1.87 ± 0.14
GM	1.717 ± 0.133 [a***]	84.64 ± 2.47 [a***]	3.31 ± 0.18 [a***]
Licorice+ GM	0.755 ± 0.06 [a*b***]	40.87± 4.10 [a*b***]	2.10 ± 0.20 [a*b***]

All values are represented as mean ± SDE (n= 10 rats). [a] versus control group.
[b] versus GM group (* p <0.05, ** p <0.01 and *** p <0.0001).

3.2. Effect of licorice extracts on kidney MDA and GSH levels.

Injection of rats with GM for 5 days consecutive at a dose level of (100mg/kg b.wt) caused a significant (p <0.001) increase with percentage (69.17%) in kidney MDA levels compared with the control values (Fig. 1). On the other hand, GM caused a significant decrease in kidney GSH contents compared to the control values (Fig.2). Pretreatment of GM-injected rats with licorice extract for 7 days before GM and daily thereafter for 5 days significantly (p<0.001) ameliorate the kidney levels of MDA levels and GSH contents as compared with GM rats.

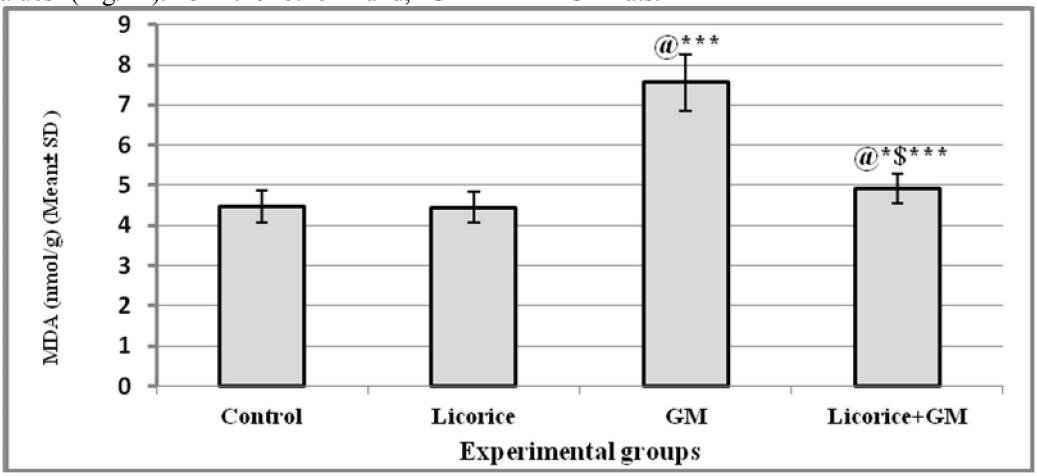

Figure (1): Effect of licorice extract pretreatment on kidney MDA level in GM-Induced nephrotoxicity in male rats.

All values are represented as mean ± SDE (n= 10 rats).
[@] *vs.* control group. [$] *vs.* GM group ([*] p<0.05, [**] p<0.01 and [***] p<0.0001).

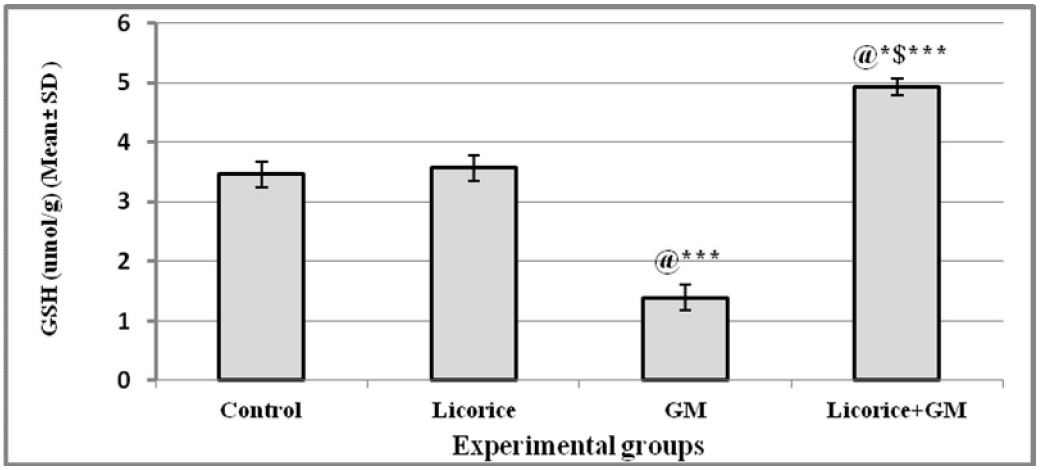

Figure (2): Effect of licorice extract pretreatment on kidney GSH content in GM-Induced nephrotoxicity in male rats.

All values are represented as mean ± SDE (n= 10 rats).
[@] *vs.* control group. [$]*vs.* GM group ([*] $p<0.05$, [**] $p<0.01$ and [***] $p<0.0001$).

3.3. Effect of licorice extracts on ionic sodium and potassium.

Administration of licorice extract did not produce any significantly per se effect on serum ionic Na^+ and K^+ in normal albino rats as compared with control rats (Fig 3 and Fig.4). The results revealed that, GM injection induced significant decrease ($p<0.001$) in Na^+ level concomitant with significant increase ($p<0.001$) in K^+ as compared with control rats. Pretreatment of GM-injected rats with licorice extract for 7 days before GM and daily thereafter for 5 days significantly ameliorated the effects of GM, there were significant difference in ionic content of Na^+ and K^+ ($p<0.001$) when compared with GM group.

3.4. Histopathological results:

Microscopically, kidney sections from control and licorice extract groups revealed the normal structure of renal parenchyma (Fig.5(1-2)). Kidney sections of rat from GM-injected group showing marked hypertrophy, vacuolation of glomerular tufts, necrobiotic changes of renal tubules, protein cast in the lumen of renal tubules, vacuolations of endothelial and lining glomerular tuft (small arrow), peritubular focal hemorrhage and peritubular few leucocytic cell infliteration (Fig.5(3.a-d)). Kidney sections of rat from GM group pretreated with licorice extract showing apparent no histological changes of renal cells, expect some sections showing slight congestion of glomerular tufts (Fig. 5(4.a-b)).

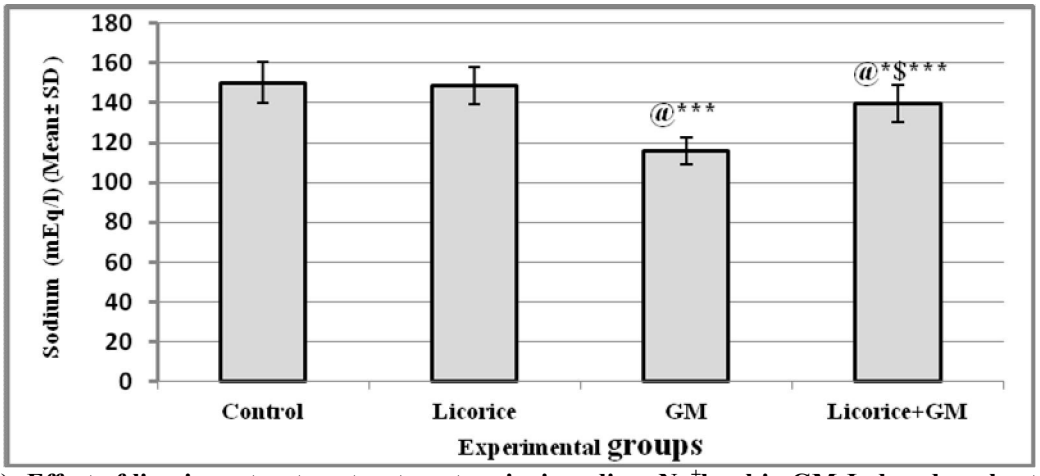

Figure (3): Effect of licorice extract pretreatment on ionic sodium Na^+level in GM-Induced nephrotoxicity in male rats.

All values are represented as mean ± SDE (n= 10 rats). [@]*vs.* control group.
[$]*vs.* GM group ([*] $p<0.05$, [**] $p<0.01$ and [***] $p<0.0001$).

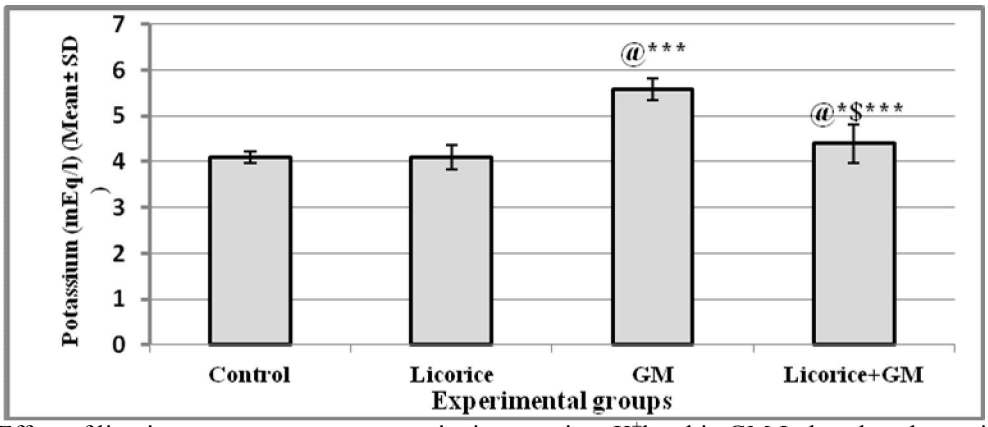

Figure (4): Effect of licorice extract pretreatment on ionic potassium K^+level in GM-Induced nephrotoxicity in male rats.

All values are represented as mean ± SDE (n= 10 rats).
$^{@}$*vs.* control group. $^{\$}$*vs.* GM group (* p<0.05, ** *p*<0.01 and *** *p*<0.0001).

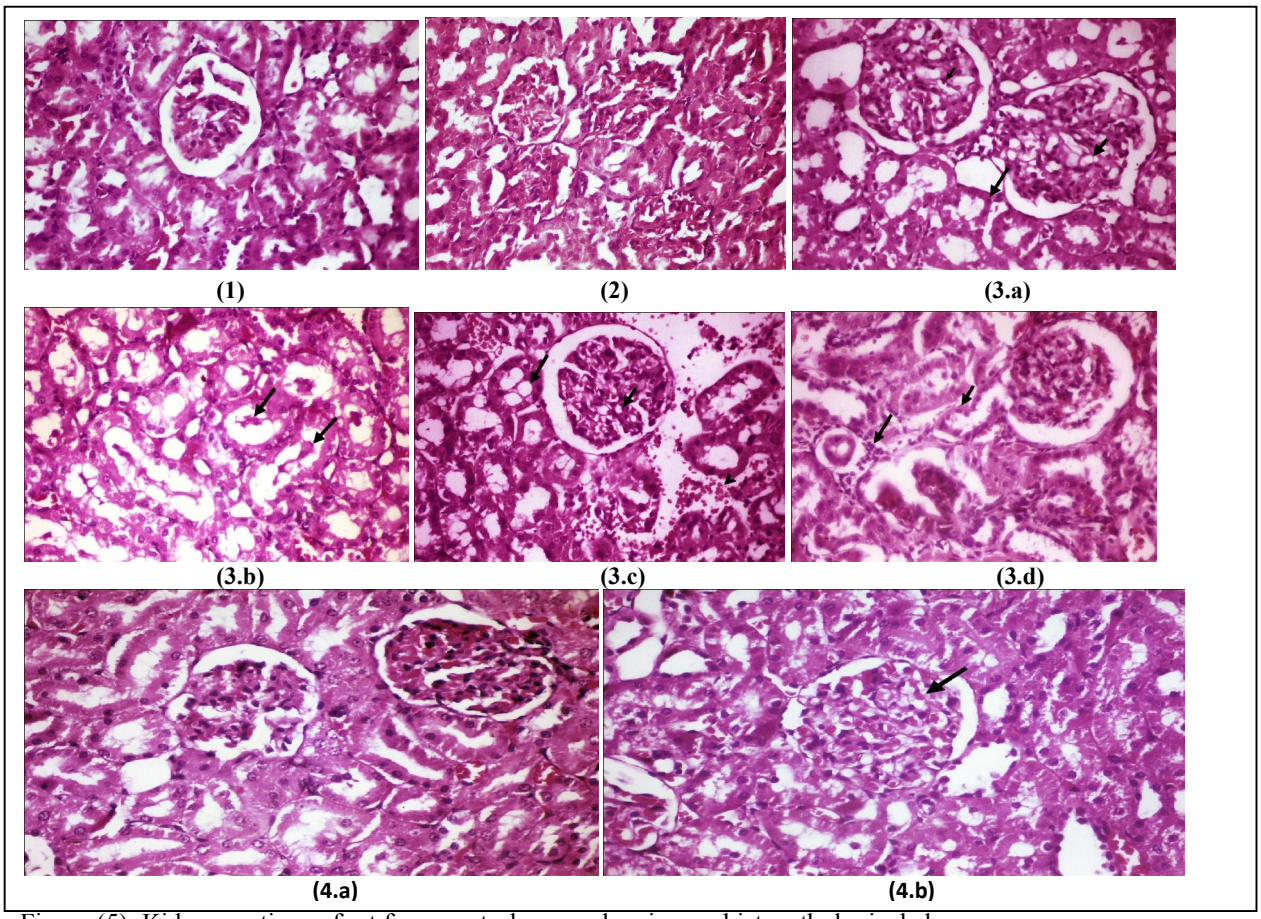

Figure (5): Kidney sections of rat from control group showing no histopathological changes
(1). Sections of rat from licorice group showing normal histological structure of renal parenchyma (2). Kidney sections of rat from GM-injected group showing marked hypertrophy and vacuolation of glomerular tufts (small arrow), as well as necrobiotic changes of renal tubules (large arrow) (3.a), protein cast in the lumen of renal tubules (3.b), vacuolations of endothelial lining glomerular tufts (small arrow), vacuolations of epithelial lining renal tubules (large arrow), as well as peritubular focal hemorrhage (arrow head) (3.c), necrobiotic changes of renal tunular epithelium (small arrow) and peritubular few leucocytic cell infliteration (large arrow) (3.d). Kidney sections of rat from GM group pretreated with licorice extract showing apparent no histological changes of renal cells (4.a), expect some sections showing slight congestion of glomerular tufts (4.b). (H&E X 400)

4. Discussion:

Gentamicin an aminoglycoside antibiotic is used against Gram negative bacteria. However, the use of gentamicin is associated with nephrotoxicity that limit its frequent use. The gentamicin induced nephrotoxicity involves renal oxidative stress, which is accompanied with reduction in renal antioxidant defense mechanisms. In addition, induction of acute tubular necrosis, glomerular damage and renal inflammation are the major events implicated in gentamicin nephrotoxicity **(Abdel Raheem *et al*., 2009)**. Oxidative stress plays a key role in gentamicin induced nephrotoxicity. It is evidenced that the renal accumulation of gentamicin is implicated in the induction of nephrotoxicity **(Watanabe *et al*., 2004)**.

Natural remedies from medicinal plants are considered to be effective and safe alternative treatments for many diseases. Licorice is one of the most widely used herbal drugs around the world, being present in most pharmacopoeias of eastern and western countries **(Biondi *et al*., 2005)**. It is used as an esteemed crude drug in both the orient and occident treatment of various diseases such as cancer, peptic ulcers, stomach cancer, heartburn, inflammation, kidney and liver disorder, influenza, sore throats, asthma, and infections caused by bacteria or viruses. Chinese traditional medicine originated from the dried roots of several *Glycyrrhiza* species **(Hayashi *et al*., 2000).** Licorice plant has also been used probably as a flavoring in food, beverages, candies sweetening agent **(Wang *et al*., 2000)**. Glycyrrhizic acid, the most studied active constituent of Licorice, is a sweet tasting material (**Acharya *et al*.,** 1993). In many countries, GA is used as a major therapeutic agent to treat chronic viral hepatitis and allergic dermatitis **(Tanahashi *et al*., 2002)**. It is also known to have anti-inflammation **(Fujisawa *et al*., 2000)**, antiulcer, antihepatotoxic and antivirus activities **(Ito *et al*., 1997, Cinat *et al*., 2003 and Fu *et al*., 2005).** In view of this, the present study was undertaken to investigate the effect of simvastatin, a lipophilic statin, in gentamicin induced nephrotoxicity in albino rats

In the current study, the elevated levels of serum creatinine, urea and urea nitrogen have been suggested to be an index of renal damage and dysfunction (**Finco and Duncan, 1976)**. The ability of the kidney to filter creatinine (a non-protein waste product of creatinine phosphate metabolism) is reduced during renal dysfunction as a result of diminished glomerular filtration rate. Thus, the increase in serum creatinine level is an indication of renal dysfunction **(Perrrone *et al*., 1992)**. Moreover, the elevated levels of blood urea and urea nitrogen occur during renal dysfunction. In the present study, the gentamicin administration in rats increased the level of serum creatinine. In addition, the blood urea and urea nitrogen levels were increased in gentamicin administered albino rats as compared to normal albino rats. Furthermore, the elevated level of urea protein was noted in gentamicin-administered albino rats as compared to normal albino rats. These results suggest the development of renal damage and renal dysfunction in rats administered gentamicin, which was consistent with earlier studies of others **(Pedrazha chaverri *et al*., 2000, Maldonado *et al*., 2003 and Parlakpinar *et al*., 2005).**

The concurrent administration of licorice extract prevented the elevated level of serum creatinine in rats administered GM. In addition, licorice extract markedly reduced GM-induced increase in blood urea and urea nitrogen in rats. These results suggest that licorice extract has an ability to prevent GM nephrotoxicity in rats, which was agree with **Sharma and Rathore (2011)** who found that prophylactic administration of aqueous suspension of powdered *Glycyrrhiza glabra* roots at three different doses for 7 days to mice could provide appreciable protection against acetaminophen challenge on 8^{th} day in sublethal experiments. These may be explained by the main components of licorice root as triterpene, saponins, glycyrrhizin/glyccyrrhizic acid and glycyrrhetic acid. Moreover, **Gumpricht *et al*., (2005)** and **Yoshida *et al*. (2007)** revealed that, glycyrrhizin is a major active constituent isolated from licorice that scavenges reactive oxygen species (ROS) and has an anti-inflammatory action.

Oxidative stress plays a major role in gentamicin-induced nephrotoxicity **(Walker *et al*., 1999)**. The increase in lipid peroxidation and consequent decrease in reduced form of glutathione are the indicators of oxidative stress **(Kakkar *et al*., 1997)**. In the present study, the renal MDA has been noted to be increased in GM-administered rats. In addition the reduction in renal GSH in gentamicin-administered rats was noted. These results suggest the induction of renal oxidative stress in rats' administration GM. However, administrated licorices extract markedly prevented renal oxidative stress induced by GM by reducing renal MDA and restoring activity of renal GSH. Accordingly, it may be suggested that the anti-oxidant effect of licorice extract may have played a key role in preventing the gentamicin-nephrotoxicity.

Gentamicin - nephrotoxicity associated with decreases in serum levels of sodium and increases levels of potassium suggested that the site of GM action is the distal convoluted tubules causing increased urinary excretion of sodium **(Elliott *et al*., 2000)**. The mechanism of nephrotoxicity caused by GM was attributed to stimulation of generation of

reactive oxygen species (ROS) causing tissue oxidative stress **(Cuzzocrea *et al*., 2002** and **Tavafi *et al*., 2012**). *Glycyrrhiza glabra* could prevent GM induced nephrotoxicity **(Desai *et al*., 2004)**. Antioxidant capacity of licorice is used to treat kidney or urinary system based on oxygen radical absorbance capacity method **(Wajcikowski *et al*., 2007)**.

Examination of kidney sections of GM injected rats revealed a marked necrosis of renal tubules. These results were in agreement with the previously reported by **Martínez-Salgado *et al*. (2007) and Tavafi *et al*. (2012)** who concluded that GM induces nephrotoxicity manifested by biochemical and histological changes in rats. In the current study, the histopathological changes induced by GM in kidney of rats and the ameliorative effects by licorice extract were parallel with the reported biochemical alterations. *Glycyrrhiza glabra* could attenuate peroxynitrite induced renal oxidative damage through inhibition of protein nitration **(Yokozawa *et al*., 2005)**. Moreover, glycrrhizin could prevent lead acetate induced hepatic oxidative stress and hyperproliferative activity in wistar rats. Pretreatment of rats orally with glycrrhizin decreased hepatic microsomal lipid peroxidation and increase in the level of GSH content and lowered DNA synthesis **(Rahman and Sultana, 2006).**

Conclusion:

In conclusion, licorice extract produce nephroprotective effects in rats as their oral administration concomitantly with gentamicin (GM) induced significant reduction in biochemical and morphological renal alterations caused by GM. Nephroprotective effect could be due to the antioxidant activity. Therefore, the study recommends that intake of licorice may be beneficial for patients who suffer from kidney diseases and those on GM therapy.

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