



Research article

Nephroprotective roles of local licorice, peppermint extracts and their mixture on gentamicin-induced renal insufficiency in Wistar albino rats

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Abstract

Aminoglycosides (AGs) such as gentamicin (gen) are considered as the optimum therapy for many infections and diseases. Unfortunately, AGs treatment has been linked to acute kidney injury that is also referred to as nephrotoxicity. In the current study, we sought to explore the protective roles of peppermint (pep) and licorice (Lic) extracts as well as their mixture on gen-induced nephrotoxicity. Forty male and female Wistar albino rats were divided into 5 experimental groups: C- was administered with 2 ml of normal saline as a single daily dose intraperitoneally (i.p.) for 14 d. C+ was administered with once-daily i.p. injections of 100 mg/kg BW gen for 8 d. T1 group was administered once-daily i.p. of gen (100mg/kg BW) for 8 d. and Lic ethanolic extract (EE) (100mg/kg BW) every 12 hr for 14 d. T2 was administered once-daily i.p. of gen for 8 d. and pep EE (100mg/kg BW) every 12 hr for 14 d. T3 was administered once-daily i.p. of gen for 8 d. in addition to lic and pep EE mixture (100mg/kg BW) every 12 hr for 14 d. At the end of the study, all rats were anesthetized and trunk blood collected to study renal injury parameters. Those include serum enzymes such as AST, ALT, and ALP; kidney damage markers including urea, creatinine, and total protein; serum electrolytes including Cl, K, and Na levels as well as other renal function markers such as Glutathione peroxidase (GSH) and superoxide dismutase (SOD). All the parameters that indicated the nephrotoxicity occurrence in the C+ group in response to gen were found to be highly improved in most of the studied aspects in T1, T2, and T3. We found that Lic and pep extracts improve renal injury, kidney damage, and renal function markers opposed to gentamicin treatment. In conclusion, our study suggests prescribing those remedies to AGs receiving patients.

Key words: Licorice, Peppermint, Gentamicin, Renal damage, Wistar rats.

Introduction

Gen is one of the AGs family members, and it is derived from *Micromonospora purpurea* gram-positive bacteria. Gen has been used for many decades to treat aerobic gram-negative bacterial infections potentially. As a positively charged chemical, gen combines easily and strongly to the negatively charged components of the proximal tubules making it possible to enter cells and accumulate their rapidly (1). The drug accumulation leads to changes in the fluidity of the renal brush border as a result

of alteration in electrolytes transportation through the membrane (2). It is also implicated in renal cell death due to alteration in cellular protein contents such as lactate dehydrogenase and Na-K-ATPase (3). So, gen accumulation in the renal tubules was found to be a big problem in clinics around the world due to nephrotoxicity that sequences with damage to brush border network (4). Of importance, several histopathological studies fundamentally associate the gen related renal failure to the



necrosis of the kidney tubules (3). In human and after few days of gen treatment, it has been clinically confirmed that around 15% of patients suffer renal dysfunction and the nephrotoxicity is manifested (5). In animals, many evidence of nephrotoxicity occurrence due to small doses of the gen that ranged from 10-20 mg/kg body weight of laboratory rats (6). Accordingly, many efforts were undertaken to ameliorate nephrotoxicity that resulted from aminoglycosides, and more specifically gen. conspicuous recognition of cases at risk and once daily dosing regimen (7) are practically followed in clinics, but these efforts still not resolving the problem even partially. Therefore, other approaches should be studied to prevent or improve AG resulted nephrotoxicity. In this regard, the implementation of natural compounds in the treatment of stubborn diseases is a new trend in modern clinical medicine. This is owing to the fact that natural substances are mostly efficient in clinical use due to their availability and low toxicity. Hence, we aimed at ameliorating AGs nephrotoxic effect by conducting the current study that includes using pep and lic extracts and gen concomitantly. Herbal remedies such as plant extracts have been widely used, about 80% of population, along the history of human being (8). One of the well-known herbs in this regard is pep (*Mentha piperita L.*) that has been used in alternative medicine worldwide such as in bile ducts and gastrointestinal tract disorders, cold, cough/bronchitis, sinusitis, fever, nausea, vomiting, and indigestion, colic, anthelmintic, and antimicrobial (9). This wide use of pep in folk medicine is because it is generally considered to be safe. Lic (*Glycyrrhiza glabra*) is another popular plant that has been used universally since decades in herbal formulas to treat many diseases (10). One of the active ingredients in the plant extract is the Glycyrrhizin (11). Lic used in most factories as a substantial food and medicine sweetener (12). Alongside, Lic has been used to treat conditions such as hepatitis, gastric ulcers, allergy, diuretic and

suggested malignant ascites, immune function, anti-parasitic, antioxidant, antiviral, antitumor, and chronic viral hepatitis (13). Importantly, like pep, Lic considered generally safe and has no adverse pathological effects on liver and kidney tissues in mice (11). It is noteworthy that Lic components have found to have a therapeutic effect on obstructive nephropathy that was induced experimentally in rats (14). Lic found to be effective in mitigating the sepsis-induced acute kidney injury (AKI) by alleviating malady disorders and boosting pathological parameters that measurably improve the survival rate in rats with AKI (15). In the previous study, the researchers established that Lic active ingredients improve survival rate by inhibiting the programmed cell death accompanying the AKI condition in rats (15). In the current study, our aim was to uncover the nephron-protective effects of pep and Lic extracts, in addition to their mixture against renal damage induced by gen.

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 378

Plants preparation:

The fresh leaves of *Lic and pep* were collected in October 2016 from the region outskirts of Al-Diwaniyah city in the middle Euphrates of Iraq. The plants samples were dried in open air and shady conditions until completely dried and then grinded by an electric blender separately to obtain powder. All experiments were performed with one batch of Lic leaf and pep extracts, separately.

Preparation of ethanolic plant extracts

The preparation of alcoholic extract of both studied plants were performed by weighting 200 g of powdered leaves added to 1000 ml of 96% ethanol to obtain 20% concentration. The products were put into dark bottle, poured into shaker apparatus for



3 d. and then filtered through 0.2 μ m filter papers (Millipore TM Membrane Filter, USA). The extract was stored at 4°C until use.

Animals:

Forty healthy albino rats from both sexes, weighing 150-200 g were obtained from the animal house of Veterinary Medicine College/ University of Al-Qadisiyah. The animals were kept under a 12 hr. light/ dark cycle controlled condition at room temperature (22 \pm 2 °C) and had free access to food and water. Both animal sexes were injected intraperitoneally according to In et al., (2014) that found that ip injection is without chronic or sever harm to the male rats (16).

Anti-nephrotoxic activity:

A Total of 40 male and female Wistar albino rats were divided randomly into 5 experimental groups; 8 animals each as follow:

1. C-, was injected with N.S. at a single daily dose of 2 ml, ip for 14 d.
2. C+, was administered at a single daily dose of 100 mg/kg BW gen ip for 8 d.
3. T1, was injected at a single daily dose of gen (100mg/kg BW) for 8 d and lic EE (100mg/kg BW) every 12 hr for 14 d.

4. T2, was injected at a single daily dose of gen (100mg/kg BW) for 8 d and pep EE (100mg/kg BW) every 12 hr for 14 d.

5. T3, was administered at a single daily dose of gen (100mg/kg BW) for 8 d in addition to Lic and pep EE mixture (100mg/kg BW) every 12 hr. for 14 d.

Blood collection & Biochemical assays:

At the end of the study, all rats from all groups were anesthetized and trunk blood collected to obtain serum in order to study renal injury parameters including serum enzymes such as AST, ALT, and ALP; kidney damage markers including urea, creatinine, and total protein; serum electrolytes including Cl, K, and Na levels. As well as, other renal function markers such as GSH and SOD as well.

Statistical analysis:

Values of the biochemical parameters measured were recorded as the mean \pm SEM (standard error for the mean). The values of the variables were analyzed for statistically significant differences using the one way ANOVA then the least significant differences (LSD), with the help of SPSS for windows version 21 (SPSS Inc., Chicago Ill); values less than 0.05 were regarded as statistically significant.

Results

Our results show that there is a significant (P<0.05) elevation in serum AST, ALT, and ALP levels in the gen treated group (C+) compared to the negative control (C-) and the treatment groups (Table 1). On the other

hand, serum AST, ALT, and ALP levels were significantly (P<0.05) reduced in the T1, T2, and T3 groups compared to the C+ group.

Table (1): the effect of lic, pep & their mixture on serum enzymes levels in gen-induced renal damage in rats

Group	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
T1	105.3 \pm 4.23 ^{BC}	55.68 \pm 3.9 ^B	187.43 \pm 9.9 ^B
T2	101.22 \pm 8.12 ^C	52.15 \pm 3.49 ^B	178.15 \pm 5.49 ^C
T3	112.12 \pm 5.24 ^B	54.72 \pm 5.56 ^B	189.98 \pm 7.56 ^B
C+	167.34 \pm 9.45 ^A	71.12 \pm 4.42 ^A	214.18 \pm 8.42 ^A
C-	86.54 \pm 11.13 ^D	51.38 \pm 2.15 ^B	168.18 \pm 7.15 ^D

• Numbers represent means \pm standard error

• Different superscript letters represent significant difference(P<0.05)

The results show that there is a significant increase (P<0.05) in the urea and creatinine levels in the serum in the C+ compared to the

C- and the treatment groups (Table 2). Our results show higher levels of those parameters in response to gen treatment. In



contrast, animals that received lic (T1) and pep (T2) extracts or their mixture (T3) show measurable reduction in urea and creatinine levels in the serum. Again, this indicates that the plant extracts used in the current study work to reverse gen effect on the kidneys. Table 2 also shows that the total protein level

in the gen treated group was significantly lower than that of the C- and the treatment groups. While, the groups that received the lic and pep as well as their mixture exhibited low levels of total protein in the blood compared to the C+, but there was no difference compared to the C- group.

Table (2): the effect of lic, pep & their mixture on some renal markers levels in gen-induced renal damage in rats

Group	Urea (mg/dl)	Creatinine (mg/dl)	Total protein (g/dl)
T1	31.82±1.03 ^C	0.78±0.42 ^B	8.93±1.02 ^A
T2	38.25±0.52 ^B	0.74±0.52 ^B	8.79±1.02 ^A
T3	29.68±1.24 ^C	0.81±0.34 ^B	8.75±1.02 ^A
C+	55.14±3.28 ^A	2.18±0.98 ^A	6.91±0.84 ^B
C-	26.32±2.12 ^C	0.61±0.07 ^C	8.85±0.58 ^A

- Numbers represent means ± standard error
- Different superscript letters represent significant difference(P<0.05)

We found that levels of Cl, K, and Na were significantly (P<0.05) lowered in the C+ group compared to the C- and the other treatment groups (Table 3). On the other

hand, T1, T2 and T3 groups manifested notable higher electrolyte levels compared to the C+ group.

Table (3): the effect of lic, pep & their mixture on some electrolytes levels in gen-induced renal damage in rats

Group	Cl (MEq/L)	K (MEq/L)	Na (MEq/L)
T1	90.22 ± 6.88 ^B	6.28 ± 0.9 ^B	111.71 ± 6.9 ^B
T2	92.54 ± 7.13 ^B	7.1 ± 0.49 ^A	117.3 ± 5.49 ^A
T3	84.62 ± 4.45 ^C	6.12 ± 2.8 ^B	110.75 ± 3.56 ^B
C+	71.44 ± 2.12 ^D	3.78 ± 1.42 ^C	97.11 ± 6.45 ^C
C-	97.66 ± 5.74 ^A	7.13 ± 0.96 ^A	118 ± 5.08 ^A

- Numbers represent means ± standard error
- Different superscript letters represent significant difference(P<0.05)

In Table (4), our results show that the level of GSH was higher in response to gen treatment. In this regard, C+ group shows significant (P<0.05) rise in the levels of GSH compared to the C- and T1, T2, and T3

groups. SOD level on the other side, was lower in response to gen treatment. In this regard, C+ group shows significant (P<0.05) reduction in the level of SOD compared to C-, T1, and T2 groups.

Table (4): the effect of lic, pep & their mixture on oxidative parameters levels in gen-induced renal damage in rats

Group	GSH (nmol/mg)	SOD (nmol/mg)
T1	59.82 ± 3.03 ^C	30.38 ± 4.9 ^B
T2	57.1 ± 2.6 ^C	37.32 ± 6.49 ^A
T3	60.48 ± 3.24 ^B	32.75 ± 2.56 ^B
C+	67.22 ± 8.12 ^A	29.12 ± 1.87 ^B
C-	51.23 ± 4.38 ^D	40.78 ± 2.96 ^A

- Numbers represent means ± standard error
- Different superscript letters represent significant difference(P<0.05)



Discussion

AGs-induced nephrotoxicity has been studied by several researchers though the recent centuries, but still not resolved. In this study, we show that injection with 100mg/kg of gen daily for 8 d had clear evidence of nephrotoxicity. For instance, our results showed that there is a marked elevation in the levels of serum AST, ALT, and ALP which indicate hepatic as well as renal toxicity. These high levels of AST, ALT, and ALP in the sera might be caused by releasing of these enzymes from the liver cells to the blood due to hepatocellulopathy. Gen has been shown to cause hepatocellular damage that destroy the plasma membrane and release the hepatic enzymes such as AST, ALT, and ALP into the circulation (17). Pep treatment reversed the toxic effect of gen in our study. It was reported that pep extracts lower the levels of AST, ALT, and ALP because of the active ingredients of the plant such as α -tocopherol, caffeic acid and menthol that have a protective effect against liver toxicity (18). Similarly, lic was found to be effective in curing liver damage in mice with alcohol hepatotoxicity (19). The authors attributed this effect to the role of lic in boosting liver health by enhancing antioxidant defense and anti-inflammatory response. It was also established that pretreatment with lic extract reversed the CCL4 toxic effects in liver of rats (20). AST, ALT, and ALP levels in the blood are also considered indicators of renal dysfunction and kidney damage since they were found to be significantly elevated in the end-stage renal disease in human patients (17). According to all the previous mentioned work, we suppose that pep and lic extract protect both liver and kidney from the toxic effects of gen. In agreement with the others, our results showed that the serum levels of urea and creatinine were elevated significantly in gen treated rats (20). It has been well known that aminoglycosides-induced nephrotoxicity is manifested by a reduction in the glomerular filtration rate

(GFR) of the kidneys and injury or even necrosis in the renal proximal tubules (21). The high levels of those markers in our study reveal that the study was well suited, and the renal injury in gen treated rats is confirmed since there was no capacity to eliminate the urea and the creatinine from the blood to the urine (22). Collectively, this ensures renal damage due to gen in our animals. In contrast, rats that were treated with lic and/or pep extract showed clear reduction in urea and creatinine in the serum (Table 2). Pep extract showed protective effect on kidneys that belonged to animals treated with gen by reversing the gen effect that was indicated by improving the urea and creatinine clearance (23). Similarly, lic was also found to ameliorate the nephrotoxic effect of gen in rats by improving the renal prognosis that is characterized by lowering urea and creatinine levels in the blood and increasing their levels in the urine (24). This effect might be related to the role of the plant extract in reducing the levels of inflammatory cytokines, such as TNF- α , IL-1 β , and IL-6, and inhibition of nitric oxide and prostaglandin E2, induced nitric oxide synthase (iNOS) and cyclooxygenase- 2 in kidney (16). The latter study also suggested that lic can induce recovery in sepsis-IKA as a result of blocking the NF- κ B signaling pathway. On the other hand, serum total protein level was significantly reduced in the C+ group compared to the C- (Table 2). This might be related to the gen adverse effect on the liver. Gen is implicated in hepatocellular damage according to many studies (25). Liver is the organ where protein synthesis takes place, and when there is reduction in protein synthesis it is mostly related to liver illness, or to the excessive loss of protein in urine due to kidney disease (26). Our results showed that there is conspicuous effect for both extracts on the liver/and or kidney health since the level of total protein the serum returned to normal which indicate normal protein synthesis/ and or clearance.



Our results indicated that gen treatment decreases the level of the electrolytes in the serum (Table 3). This is in agreement with another study in human where the levels of some electrolytes were high in urine due to excessive waste because of the treatment (27). The authors declared that the mechanism of wasting due to gen treatment is not fully elucidated, but they suggested that this electrolytes wasting is related to the effect of gen on the distal convoluted tubule of the kidney (27). The insult to this part of the kidney prevents electrolytes reabsorption and subsequently low levels of electrolytes in the blood. In another study, the researchers found that there was gradual accumulation of Na and Cl concentrations in the proximal tubules (28) that might be why those electrolytes are low in serum. Gen caused significant excretion of Na and Mg in urine samples that were collected from neonates after treatment (29) that also support our results. In addition, Na, K, and Cl low levels in the serum of animals administered with gen are markers of the incapability of the renal tissue to conserve those electrolytes (30). Several other studies found that gen treatment results in reduction of electrolytes transportation such as Na, K, Cl, and calcium that also diminishes the $\text{Na}^+ - \text{K}^+$ ATPase activity (31). It is also well known that gen, at clinical doses, can lead to lower the levels of the K and Mg in the blood, the cases that clinically known as hypocalcemia and hypomagnesemia and demonstrated in normal human beings (32) and lab rabbits (33). Conversely, our results showed that pep extract administration reversed the effect of gen and increased the electrolytes levels in the serum which agrees with what reported before (23). The reason was reported to be due to the effect of the pep ingredients in fixing the gen side effects on the kidney (23). More studies were done considering the

effect of lic supply on the blood electrolytes. For example, it was suggested that lic can induce Na ion- permease, Na^+/K^+ ATPase, and citrate synthase gene expression indirectly that collectively increase Na retention by renal tubules (34). Finally, the current study showed that there is significant increase in the GSH level in C^+ group. This can be a defense mechanism that the body used to eliminate the gen toxic effect on the body tissues including liver and kidney since GSH is an important antioxidant. This action belongs to the GSH activity in repairing the tissue damage that results from free radicals, peroxides, and heavy metals (35). SOD level was lower significantly in the gen treated group which is attributed to the effect of gen in decreasing the levels of antioxidant enzymes including SOD and catalase for example (36). Those antioxidants were higher in the pep treated rats that might be related to the antioxidant effect of this herb (37). This effect is due to the high content of phenolic compounds like flavonoids in the pep (38). This is in agreement with other study that detected increased levels of GSH and increased of SOD in rats during pep administration (39). Similar results were recorded during lic usage that agrees with ours indicating that lic active ingredients supply improve health by inducing antioxidant defense mechanism such as SOD (40). According to our findings in the current study, pep and/ or lic extracts play effective role as nephroprotective remedies to decrease or completely cure the gen toxic effects on the kidney. Moreover, future recommendation to enhance the safety of gen may incorporate applying a simultaneous therapy system that might include; other herbal remedies and/ or synthetic antioxidants to ameliorate the gen-induced nephrotoxicity.



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