Original Article

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Protective Effect of Hydroalcoholic Extract of Lavandula Officinalis L. on Gentamicin Induced Nephrotoxicity in Rats

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ABSTRACT

BACKGROUND AND OBJECTIVE: Gentamicin is an aminoglycoside antibiotic used in the treatment of Gram-negative bacterial infections. Given that the prevalence of kidney damage has been reported about 10% while taking the drugs. In this study, the protective effect of hydroalcoholic extract of *Lavandula officinalis* on gentamicin-induced nephrotoxicity was studied.

METHODS: In this experimental study, thirty Wistar male rats were divided randomly into five groups of six in each group. The first group received normal saline (5 ml/kg) and the second group received gentamicin 80 mg/kg intraperitoneally for 10 days. Groups 3-5 received respectively 100, 200 and 400 mg/kg of hydroalcoholic extract of *Lavandula officinalis* intraperitoneally 3 hours after gentamicin injection for 10 consecutive days. One day after the last injection, Serum creatinine, BUN, malondialdehyde (MDA) and glutathione (GSH) were measured in left renal tissue. The right kidney was maintained in 10% formalin for Hematoxylin and Eosin (H&E) staining and histological examination.

FINDINGS: The results showed that gentamicin changed significantly serum creatinine (3.4±0.27 mg/dl), BUN (62.79±4.46 mg/dl), Kidney tissue MDA (1232±188.1 nmol/mg protein) and GSH (2.82±0.33 nmol/mg protein) in rats compared to controls (0.57±0.16, 19.55±3.3, 369.40±58.57 and 6.22±0.74 respectively) ($p<0.05$). Extract at a dose of 200 mg/kg 400 significantly inhibited gentamicin-induced enhancement of serum creatinine, BUN and tissue MDA levels ($p<0.05$). Histological results showed that gentamicin could lead to kidney damage and tubular necrosis.

CONCLUSION: The results showed that hydroalcoholic extract of *Lavandula officinalis* reduces biochemical indices and oxidative stress parameters against gentamicin-induced nephrotoxicity icity.

KEY WORDS: *Gentamicin, Nephrotoxicity, Lavandula officinalis, Rat.*

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Introduction

Gentamicin is an aminoglycoside drug which has a major role in the treatment of infectious and microbial diseases. Different reports indicate renal toxicity of the drug in humans and experimental models. The prevalence of renal damage during treatment with this drug is about 10% (1). According to recent studies, selective accumulation of the gentamicin in the kidney cortex and the production of reactive oxygen species (ROS) leading to oxidative stress and damage to the proximal tubule of the kidney, causes of that renal toxicity. Multiple amino groups on the aminoglycoside molecule at physiological pH create a cationic charge.

As a result, aminoglycoside molecule easily connects to Anionic phospholipids in the plasma membrane of proximal tubule cells via electrostatic saturation, and then gathers in intracellular organelles such as mitochondria and nuclei by endocytosis. Gentamicin increased production of superoxide anion, hydrogen peroxide and hydroxyl radicals in the mitochondria of kidney. These free radicals react with unsaturated lipids and caused peroxidation of membrane lipids and phospholipids, changes in membrane fluidity and permeability enhancement and ultimately lead to cell damage. Oxidative stress caused by reactive oxygen species in addition to degradation of cell membranes causes protein denaturation and break the chains of DNA and tissue damage by nucleic acid and protein oxidation that the result is a reduction in glomerular filtration and acute renal toxicity (2-4). Anti-oxidants by neutralizing the effect of reactive oxygen metabolites on cellular components play an important role in preventing oxidative damage and related diseases.

That's why one of the most important strategies to prevent diseases caused by oxidative damage is the utilization of antioxidant substances. So far, several antioxidant compounds, including: Melatonin, Vitamin E, Probucol, N-acetyl cysteine, L-carnitine, Theophylline, Fenoldopam and etc for the prevention of renal toxicity of gentamicin and other aminoglycosides have been used (5-10). The uses of plants in the treatment of many diseases have traditionally been common. Nowadays, herbal remedies such as the use of supplements and plants extracts rich in polyphenols, especially flavonoids and phenolic acids is usual throughout the world. Because it has been shown that phenolic compounds have protective effects against reactive oxygen species and also improved the body's antioxidant system (9-11). Lavender (Lavanda) with scientific name of *Lavandula officinalis* is a perennial plant with shrub to a height of about one meter and that in the lower parts of the square stems are, woody.

Many of its applications in Iranian traditional medicine in the treatment of many diseases have been proven. Researchers have reported multiple health properties of this plant, such as, antianxiety, antidepression, anti-inflammatory, antispasmodic, analgesic, antibacterial, antiparasitic, antiviral, sedative and antioxidant (11-14). Due to having active antioxidant ingredient in Lavender, the protective effect of the extract of this plant in gentamicin-induced renal toxicity checked out in this study, as if to show the preventive effects of this plant, simultaneous use of daily specified amount of Lavender and gentamicin can be recommended, in patients with resistant infections due to antibiotic use in high doses and long-term, to prevent the nephrotoxicity complications caused by gentamicin.

Methods

Chemicals: 1,1,3,3-tetraethoxypropane (TEP), Bovine Serum Albumin (BSA), 2-thiobarbituric acid (TBA), 5, 5- dithiobis (2-nitrobenzoic acid) (DTNB), Coomassie Blue G, Trichloro acetic acid (TCA), reduced glutathione (GSH). All other chemicals were of analytical grade and prepared from Merck Company (Darmstadt, Germany).

Animals: For this experimental study, 30 Wistar male rats, weighing 20 ± 200 g were used. Animal were obtained from animal house of Ahvaz Jundishapur University of Medical Science, Iran. Rats were kept in polypropylene cages and given standard rat chow and drinking water ad libitum. The animals were maintained at a controlled condition of temperature (20±2˚C) with a 12 h light: 12 h dark cycle. The investigation was performed according to the Animal Ethics Committee Guidelines for the use of experimental animals.

Extract preparation: After harvest in the growing season, and identify and verify the scientific name, leaves were dried and then milled. 200 g of powder was obtained for 72 hours in ethanol solvent (water 30: 70 ethanol), then extract was passed through filter paper and the filtrate was concentrated by rotary device and after putting in the oven (30-40° C) dried extract was obtained.

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Study design: In this study, animals were divided randomly into five groups of six in each group. The first group were received daily 2 ml saline intraperitoneally for 10 days. The second group was received gentamicin at dose of 80 mg/kg intraperitoneally for 10 days. The third, fourth and fifth groups received respectively 100, 200 and 400 mg/kg of lavender extract intraperitoneally 3 hours after gentamicin injection for 10 consecutive days. 24 hours after the last injection (eleventh day), After induction of anesthesia by ether, their stomachs were opened by surgical scissors and 2 ml of blood was taken from their heart and for 15 minutes was centrifuged at 3000 rpm. Isolated serum was transferred to biochemistry laboratory and to assess kidney damage, two indicators of blood urea nitrogen (BUN) and serum creatinine were assessed.

Right kidney was removed from each rat and was fixed in 10% formalin solution and was sent to the pathology laboratory to determine the extent of tissue damage. Left kidneys for the evaluation of MDA and GSH were isolated. For this purpose, half a gram of kidney tissue in 0.1 M phosphate buffer pH = 7.4 and the concentration of 10% v / w was homogenized. Bradford method was used, to determine the protein concentration (15). Bradford reagents including Coomassie Blue G, phosphoric acid 85% and ethanol 96% were prepared. Color absorption of a homogenous mixture of 50 µl of diluted tissue and 2.5 ml of Bradford reagents was measured by spectrophotometer at a wavelength of 595 nm, and absorbing was placed in calibration equation protein of known concentrations of 100 to 1000 mg/ml of BSA and concentration was obtained.

MDA level assay: To determine MDA, Satho method was used (16). 1.5 ml of TCA (10% w/v) was added to 0.5 ml of tissue homogenate. And then round was centrifuged at 4000g for 10 minutes. 2 ml of TBA solution (0.67%, w/v) was added to 1.5 ml of supernatant, and then incubated for 30 minutes in a boiling water bath. And then 2 ml of 1-butanol was added to the solution and was centrifuged at 4000g for 15 minutes. Absorption of pink supernatant was read by a spectrophotometer at 532 nm. MDA concentration was determined by using TEP as standard, and results were expressed as nmol/mg protein. To draw the calibration curve the concentrations of 0.2- 20 µM TEP in 10% sulfuric acid was used.

GSH level assay: GSH content was identified and measured by GSH reaction with Ellman's reagent (DTNB) and the formation of TNB (yellow) (17). In summary 1 ml of Tris-EDTA buffer with pH = 8.6 to a 50 µl homogenized tissue were added and mixed. Then 20 µl of 0.01M of Elman reagents in methanol was added to the above mixture and after 15 minutes at room temperature, Created yellow at a wavelength of 412 nm was measured with a spectrophotometer. GSH standard was used at different concentrations for drawing calibration curve. Due to the amount of protein per ml of homogenized tissue, the GSH content was obtained according to nmol/mg protein.

Statistical analysis: The data were analyzed by SPSS 18 software and statistical tests of one-way ANOVA test followed by Tukey's post hoc analysis and P-value less than 0.05 was considered significant.

Results

Effects of lavender extract on BUN and serum creatinine: Results showed that the serum creatinine and BUN in the gentamicin receiving group were 3.4±0.27 and 62.79 ± 4.46 and in the group receiving saline were 0.57 ± 0.16 and 19.55 ± 3.3 respectively. Gentamicin alone caused a significant increase in the BUN and serum creatinine in comparison to the group receiving saline ($p<0.05$) (Fig 1 and 2). According to the results, the lowest concentration of creatinine and BUN related to the group receiving saline and the highest concentrations of gentamicin related to the group receiving. A significant difference between BUN levels was observed in groups receiving gentamicin and extract at doses of 100, 200 and 400 mg/kg with the group receiving gentamicin alone ($p<0.05$). So that there was no significant difference between BUN levels in the groups receiving the extract at doses of 400mg/kg and the group receiving saline which reflects the positive impact of extract in reducing kidney damage resulting gentamicin inject.

Effects of lavender extract on MDA and GSH in renal tissue: The results showed that the malondialdehyde (MDA) and glutathione of kidney tissues in the group receiving gentamicin were 1232±188.1 and 2.82±0.33 nmol/mg protein and were in the group receiving saline 58.57±369.40 and 74.0±22.6 nmol/mg protein. Gentamicin significantly increased amount of MDA in renal tissue in comparison to the group receiving saline ($p<0.05$).

The extract at dosage of 100, 200 and 400mg/kg decreased significantly gentamicin-induced increase in MDA ($p<0.05$). As there was no significant difference

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of MDA between groups receiving the extract at the dose of 400 mg/kg and the group receiving saline (table 1). Also gentamicin reduced significantly the amount of GSH in renal tissue compared with the group receiving saline ($p<0.05$). Extract at dose of 200 and 400 mg/kg significantly increased the reduction of GSH caused by gentamicin ($p<0.05$).

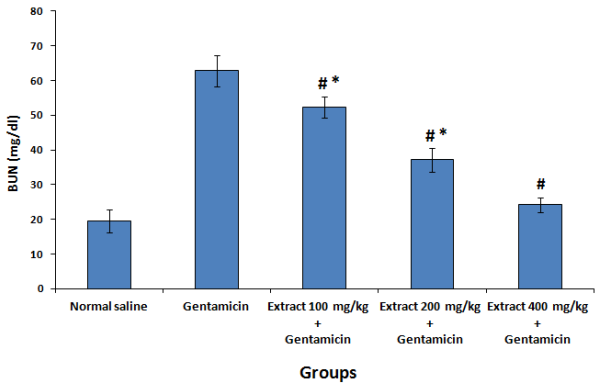

Fig 1. Effect of lavender extract on BUN levels in Gentamicin- induced nephrotoxicity. Values are means±SD (N=6).

*significant difference in comparison with the saline group ($p<0.05$)

#significant difference in comparison with the Gentamicin group ($p<0.05$).

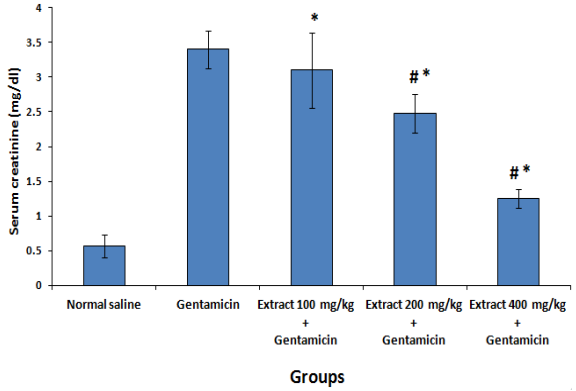

Fig 2. Effect of lavender extract on serum creatinine in Gentamicin- induced nephrotoxicity. Values are means±SD (N=6).

*significant difference in comparison with the saline group ($p<0.05$).

#significant difference in comparison with the Gentamicin group ($p<0.05$).

Effects of Lavender extract on the kidney histopathological: In the group receiving saline, kidney tissue had Normal structure and certain pathological lesions were not observed (Fig 3A). Swelling was observed in cells lining the renal proximal tubule cell in the group receiving gentamicin. In some tubule cell necrosis was visible (Fig 3B). In the group receiving gentamicin and lavender extract at a dose of 100mg/kg, Necrosis and cell swelling was observed in proximal cells (Fig 3C). In the group receiving gentamicin and lavender extract at a dose of 200mg/kg, A significant protective effect And the less damage to the kidneys were seen (Fig 3D). In renal tissue of animals receiving gentamicin and the lavender extract at a dose of 400mg/kg400, Inflation was observed (Fig 3E).

Table 1. Effects of lavender extract on both GSH and MDA in renal tissue of rats exposed to gentamicin

Groups	GSH (nmol/mg protein)	MDA (nmol/mg protein)
Control (saline)	6.22 ± 0.74^{b}	369.40 ± 58.57^{b}
Gentamicin	2.82 ± 0.33^{a}	1232 ± 188.1^{a}
Extract 100 mg/kg +Gentamicin	3.30 ± 0.34^{a}	$865.8\pm141.3^{a,b}$
Extract 200 mg/kg +Gentamicin	$4.31\pm0.53^{a,b}$	$58.74\pm695.2^{a,b}$
Extract 400 mg/kg +Gentamicin	$5.15\pm0.60^{a,b}$	551.4 ± 78.73^{b}

[a] significant difference with saline group ($p<0.05$)

[b] significant difference with gentamicin group ($p<0.05$)

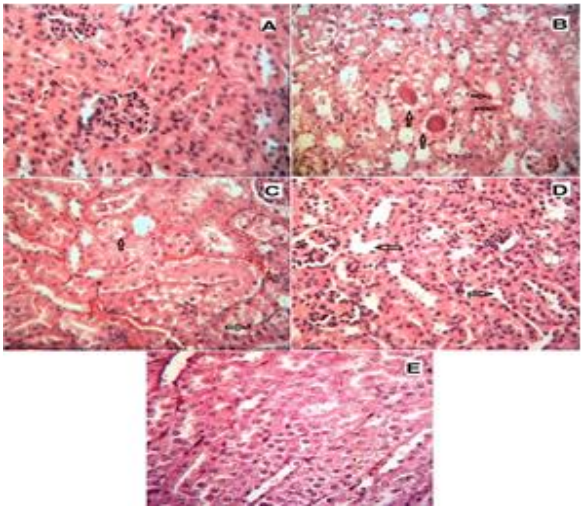

Fig 3. Histopathological observations (kidney sections stained with Hematoxylin & Eosin, magnification x 400) showing effects of lavender extract on gentamicin-induced nephrotoxicity changes in rat kidney. (A) Normal, (B) gentamicin treated group, (C), (D) and (E) are treated with 100, 200 and 400 mg/kg of lavender extract, respectively.

Discussion

The results of this study showed that simultaneous administration of Lavender extract, with gentamicin causes prevention of kidney damage induced by taking this drug in rats. This effect is concerned to the antioxidant and inhibition of lipid peroxidation

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properties of Lavender extract. As observed in this study, the parameters of oxidative stress and renal excretory function had significant differences with each other in control and gentamicin groups. Gentamicin actively reabsorbed in the proximal tubule and the concentration of gentamicin in tubular cell causes damage of this piece and impairs renal circulation that as a result decreased glomerular filtration rate and increased plasma concentrations of creatinine and BUN (18).

Also in this study , comparison of the results in gentamicin group with control group showed that in gentamicin group creatinine , which represents the amount of glomerular filtration rate , was reduced that caused the sharp enhance plasma concentrations of creatinine and BUN in this group. Receiving different doses of lavender extract could reduce the amount of creatinine and BUN in a dose dependent manner. In a study of Nasri et al. in evaluating the effect of garlic extract on the treatment and prevention of gentamicin-induced nephrotoxicity, creatinine and BUN decreased. They believe that reducing the amount of creatinine and BUN as a result of administration of garlic extract is related to its phenolic and antioxidant compounds. Also in this study the effect of doses of 200 and 400 mg/kg of lavender extract on the prevention of gentamicin-induced nephrotoxicity was significant in comparison to gentamicin receiving group. Moreover, the use of lavender extract could reduce MDA levels in kidney tissue and increase the level of glutathione. This represents a reduction of oxidative stress and increase of regenerative potential of kidney tissue. This finding is in accordance with results of other studies (5-7, 9)

In another study Ashtiani et al. examined the effects of *Salvia Officinalis* extract on gentamicin-induced nephrotoxicity. The results showed that glomerular filtration rate which was reduced by gentamicin, partially recovered by sage extract that caused plasma creatinine and urea nitrogen concentration reduction. Also oxidative stress that was enhanced by gentamicin, decreased considerably by the sage extract. There for because of the similarity of active ingredients in lavender and sage extract it is expected that lavender extract has similar effects in reducing the concentration of plasma creatinine and urea-nitrogen thus strengthening in renal function and renal protection (20). So observed renal protection effects of Lavender extract can be attributed to the high content of antioxidant of this plant. The BUN and creatinine are directly related to the amount of kidney damage and impaired renal function that in fact they are criteria and indicators for renal and proximal tubule function. In this study and other studies, these indices significantly reduced in animal groups that received herbal extract with gentamicin that was a document on the improvement of renal function and the protective effect on renal toxicity of these plants. Najafzadeh et al. examined the protective effect of the silymarin (silybum marianum) extract on gentamicin-induced toxicity in dogs that final histopathological studies of kidney tissue revealed that silymarin extract was able to prevent the gentamicin-induced glomerulonephritis in renal tissue that they attributed this antioxidant effect of silymarin as a result of the active ingredients in the plant (21). Other studies have shown that use of antioxidant factors can prevent increase in creatinine and BUN concentration following the use of gentamicin (6). HajHashemi and colleagues examined and demonstrated the anti-inflammatory effect of this extract and polyphenols, as well as essential oil (22). By chromatography experiments revealed that this plant has 26 different matters. Lavender essential oil mainly contains linalool, Linalyl acetate, Ocimene, Camphor and Caryophyllene. Among the other components of the plant can be cited tannins, coumarin, flavonoids and phytosterols (23).

Since Lavender plant has a powerful antioxidant and anti-inflammatory effects therefore, possibly through antioxidant effects and by eliminating free radicals has been able to reduce the toxic effects of gentamicin in renal tubular cells.

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