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Effect of nettle (*Urtica dioica*) extract on gentamicin induced nephrotoxicity in male rabbits

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ABSTRACT

Objective: To investigate the antioxidant effect of an orally administered ethanol extract of nettle (*Urtica dioica*) and its protective role in preventing or ameliorating oxidative stress as a major factor in gentamicin-induced nephrotoxicity in male rabbits.

Methods: Twenty rabbits were divided into 4 equal groups: (G1) control group, (G2) gentamicin treated group (100 mg/kg), (G3) nettle treated group (100 mg/kg), (G4) combination treated group with both gentamicin (100 mg/kg) and nettle (100 mg/kg) for 10 days. The antioxidant properties of nettle were evaluated using different antioxidant tests, such as determination of glutathione and malondialdehyde levels and total phenolic content analysis.

Results: Biochemical and histopathological study revealed that gentamicin caused nephrotoxicity observed clearly in the histopathological section of the kidney in the gentamicin treated group. Serum creatinine and blood urea nitrogen were biochemical indicators for nephrotoxicity which increased significantly in gentamicin treated group; other groups have no significant change in these two parameters. Nettle extract protected the rabbits from alteration in the level of blood urea nitrogen and serum creatinine when given after inducing of gentamicin nephrotoxicity. The nettle treated group showed a great effect as an antioxidant factor by increasing the glutathione level and reducing malondialdehyde level. No significant changes in biochemical parameters and no renal histopathological changes observed in the groups treated with nettle extract, which meant nettle had powerful antioxidant activity.

Conclusions: Therefore, it can be assumed that the nephroprotective effect shown by nettle in gentamicin-induced nephrotoxicity can reserve intracellular levels of biological pathways and supportively enhance excretion of toxic levels of gentamicin.

1. Introduction

Nettle (*Urtica dioica*) belonging to the family Urticaceae is recommended for complaints associated with osteoarthritis and urinary tract infections, rheumatoid arthritis, allergies, Alzheimer's, asthma, bladder problems, bronchitis, bursitis, gingivitis, gout, cough, hair growth, kidney stones, prostate enlargement, and tendinitis [1].

Nettle is one of the most valuable herbs; it contains vitamins A, thiamine (B1), riboflavin (B2), C, D, E, K, and is loaded with minerals such as calcium, cobalt, magnesium, chromium, phosphorus, copper, iron, potassium, silicon, sulfur and zinc [1,2].

Various phytochemicals and their effect on suppression of active oxygen species by natural antioxidants have been intensively studied [3,4].

Drugs such as gentamicin, throughout the endocytic pathway, take up into the epithelial cells of the renal proximal tubules and stay for a long time, leading to nephrotoxicity [5,6]. Gentamicin is a bactericidal antibiotic that binds to 30S ribosome and inhibits bacterial protein synthesis. They are polycations, and their polarity is responsible for their pharmacokinetic properties shared by members of this group. Acidic phospholipids broadly distributed in the plasma membrane in various tissues, which could be the binding site of aminoglycosides in brush border membrane of proximal tubular cells. Free radicals play a major role in the

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pathogenesis of gentamicin nephrotoxicity. Gentamicin can induce suppression of $Na^{(+)}$–$K^{(+)}$ ATPase activity and DNA synthesis in proximal tubules leading to renal injury, which may be due to the generation of reactive oxygen metabolites [4–6].

2. Materials and methods

2.1. Preparation of ethanol extract of nettle

Nettle was collected from Iraqi Kurdistan region/ Rania district/ Doli Plingan town and was defined as *Urtica dioica* in the Department of Plant, Faculty of Agriculture, University of Sulaimani. The plant was washed under tap water, and then dried at room temperature in the shade. The dried plant was crushed by a laboratory blender. Organic solvent extraction of nettle was carried out by using ethanol (95% ethyl alcohol); this was done by using a Soxhlet apparatus. The instrument was used at the College of Science, University of Sulaimani. About 10 g of plant leaf powder were put inside the temple and 50 mL of 95% ethanol was put inside the flask, then the extract was dried. The dry ethanolic extract dissolved in dimethylsulphoxide to prepare concentration of 200 mg/mL used for this study [6–8].

2.2. Blood sampling

Blood samples were collected from the rabbit's marginal ear vein at 10 days post treatment, centrifuged at 3 000 r/min for 10 min. Serums were collected; tests of blood urea nitrogen and serum creatinine were analyzed by an autoanalyzer named as LISA-200 which enabled a wide range of analyses of biochemical assays.

2.3. Histopathological study

At the end of the experiment, animals were sacrificed by anesthetic. Kidneys from rabbits were fixed immediately in 10% neutral buffered formalin for a period of at least 48 h, then dehydrated in graded alcohol (70%–100%), embedded in paraffin, cutted into 4–6 μm thick sections, stained with hematoxylin–eosin stain. Slides were coded and examined for the pathological changes of nephrotoxicity [9].

2.4. Preparation of tissue homogenate

After the animals were sacrificed, kidneys were quickly excised, placed in a chilled phosphate buffer solution (pH 7.4) at 4 °C, blotted with filter paper and weighed. One gram of kidney was then taken to prepare 10% tissue homogenate using the same buffer solution by utilizing tissue homogenizer at set 3 for 1 min at 4 °C [8,10].

2.5. Measurement of lipid peroxidation

Malondialdehyde (MDA), the end product of lipid peroxidation, was analyzed according to the method of Buege and Aust (1978), which was based on the reaction of MDA with thiobarbituric acid to form MDA-thiobarbituric acid complex, a red chromophore which can be quantitated spectrophotometrically [10,11].

2.6. Determination of glutathione (GSH) level

Total thiol groups contents, which can be used as an indicator for reduced GSH, were determined according to the method of Ellman [8].

2.7. Total phenol content analysis

The total phenolic contents of the leaves of nettle expressed as milligrams of gallic acid equivalent (GAE) per mg dry weight were analyzed by using following-cockatoo method. The total phenols are 128.9 in milligrams of GAE per mg dry weight [10,12,13].

2.8. Statistical analysis

Data were expressed as mean ± SE, where a significant interaction between major factors was identified by ANOVA SPSS version (17.0). Duncan's tests were used to identify significant differences between mean values at $P < 0.05$ [14].

2.9. Experimental design

Twenty rabbits were randomly allocated to four groups of five rabbits for each group ($n = 5$). Group 1 (G1) treated with normal saline (1 mL/kg) for 10 days, Group 2 (G2) treated with gentamicin (100 mg/kg), Group 3 (G3) rabbits treated with ethanol extract of nettle (100 mg/kg), the last group (G4) (combination treatment group) treated with gentamicin (100 mg/kg) and nettle extract (100 mg/kg).

3. Results

The gentamicin treatment group (G2) showed a significant increase in the serum blood urea nitrogen level, in comparison with the control (G1), and the nettle treated group. However, in G4 the treated rabbits showed values met the normal values of the control group (Table 1).

Rabbits in G3 showed a significant increase at the statistical level of $P < 0.05$ in the serum creatinine levels.

Treatment of rabbits with ethanol extract of nettle for consecutive 10 days resulted in a decline in MDA and a significant increase in G2 and G3 (Table 2).

The result of a histopathological study of a control group of the renal section showed normal histological structure; the renal corpuscle consisted of a tuft of capillaries, the glomerulus, surrounded by a double-walled epithelial capsule called a bowman's capsule. The tubules (convoluted tubules and Henle loop) which were lined by cuboidal epithelial cells had a normal luminous appearance.

Table 1
Effect of gentamicin and ethanol extract of nettle on serum creatinine and blood urea nitrogen levels (mg/dL).

Groups	Treatments	Serum creatinine	Blood urea nitrogen
G1	Control	1.44 ± 0.38[a]	12.70 ± 0.43[a]
G2	Gentamicin	3.80 ± 0.14[b]	17.44 ± 0.48[b]
G3	Nettle	1.80 ± 0.22[a]	13.74 ± 0.33[a]
G4	Gentamicin and nettle	1.62 ± 0.25[a]	12.70 ± 0.27[a]

Values expressed as mean ± SE, values in the same column with different letters mean significant differences.

Table 2
Effect of gentamicin and ethanol extract of nettle on renal tissue MDA and GSH levels in the experimental animal model.

Groups	Treatment	MDA nmol/g tissue	GSH µmol/g tissue
G1	Control	210.00 ± 0.81[a]	18.00 ± 0.25[a]
G2	Gentamicin	410.00 ± 0.98[c]	16.52 ± 0.89[a]
G3	Nettle	239.00 ± 0.29[b]	25.43 ± 0.83[b]
G4	Gentamicin and nettle	230.00 ± 0.95[b]	35.23 ± 0.98[c]

Values expressed as mean ± SE, values in the same column with different letters mean significant differences, value with (c) letter is the most significant group at $P < 0.05$.

The interstitial tissue was devoid of degeneration and hemorrhage (Figure 1A and 1B). The section of the kidney in the gentamicin group induced pronounced changes in the structure of renal corpuscle including swelling appearances, increasing of urinary spaces, destruction of the urinary pole that continue with damaged tubules (Figure 2A). Degeneration of the epithelium of collecting tubules and Henle loop characterized by presence of varying degrees of tubular epithelial vacuolar degeneration, diminishing of brush border, attenuation, loss of epithelial cellular detail with abundant epithelium of proximal and distal convoluted tubules contained massive cytoplasmic aggregation of hyaline droplet; some of cytoplasmic droplets were coalesced to forming hyaline cast that was eosinophilic proteinaceous material (most likely proximal convoluted tubules) and slight interstitial hemorrhage (Figure 2B).

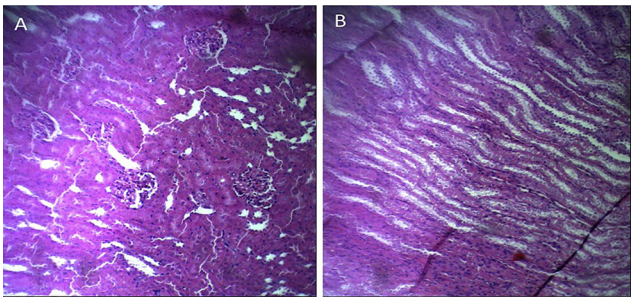

Figure 1. Kidney section and Henle loops.
A: Shows normal glomerular and collecting tubular structure; B: Show normal histological feature in the control group (hematoxylin and eosin stain, ×100).

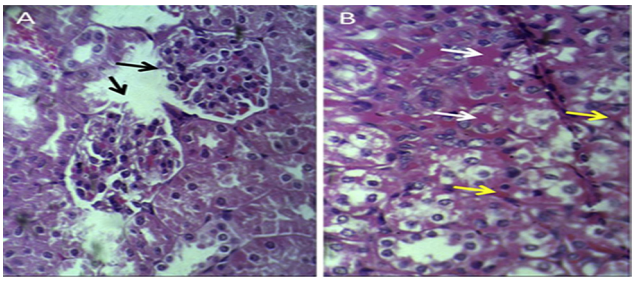

Figure 2. Slightly swelling of glomeruli and hydropic degeneration.
A: Leads to increasing of urinary spaces with the destruction of urinary pole that continue with damaged tubules (black arrow); B: The cytoplasm of renal tubules and Henle loop have multiple opaque vacuoles with centrally located nuclei with the presence of hyaline cast in the lumen of proximal convoluted tubules (white arrow) and interstitial hemorrhage as indicated by yellow arrow (hematoxylin and eosin stain, ×400).

Sections of renal tissue from rabbits treated with ethanol extract of nettle showed adequate numbers of glomeruli and renal tubules. The renal glomeruli had normal capillary loops with no evidence of shrinkage or swelling, and the tubules were lined by cuboidal epithelial cells with brush borders and had normal lumina with no evidence of protein cast. The interstitial tissue was devoid of inflammatory cells (Figure 3).

Sections of renal tissue in a rabbit group treated with both ethanol extracts of nettle and gentamicin, showed almost normal renal tissue architecture with a few focal hydropic swelling of tubular epithelial cells, which indicated the partial to complete role of nettle as a protective effect against the nephrotoxicity side effect of gentamicin (Figure 4).

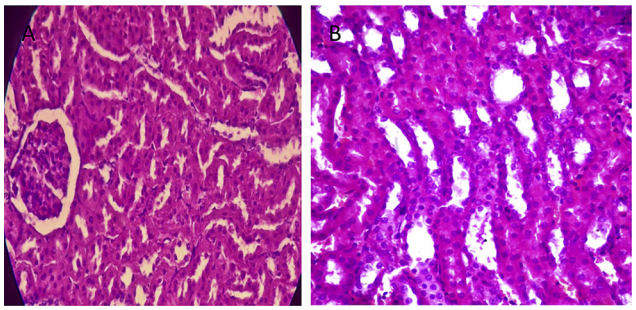

Figure 3. Section of renal tissue from group of rabbits treated with ethanol extract of nettle, the group shows adequate numbers of glomeruli and renal tubules, the interstitial tissue is devoid of inflammatory cells (hematoxylin and eosin stain, ×400).

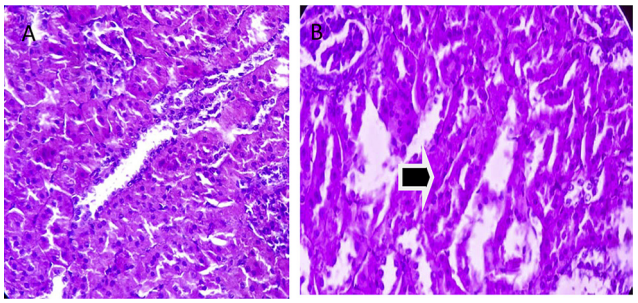

Figure 4. The renal sections of rabbits treated with combination treatment of herb extract and gentamicin, nearly like those of the control group showed only minimal and focal hydropic swelling of tubular epithelial cells (black arrow) (hematoxylin and eosin stain, ×400).

4. Discussion

The creatinine is a waste product excreted by the kidney mainly through glomerular filtration. Any increase in the value of this product indicates decreased excretion or impaired renal function [15,16]. Creatinine clearance enables a estimation of the glomerular filtration rate. Gentamicin treated group showed a significant increase in the serum creatinine level while the control group or other treatment groups showed no significant changes.

The blood urea nitrogen test is indicative of impaired renal function which is one of the dependable tests of a renal disorder. Gentamicin treated group showed a significant increase during the treatment period while those groups treated with nettle had no significant changes [17,18].

Serum creatinine and blood urea nitrogen were in accordance with nephrotoxic effect caused by gentamicin. The results of

both tests can assume the nephroprotective effect shown by nettle in gentamicin-induced nephrotoxicity [15,16].

The histopathological study is one of the conformable tests for the nephrotoxic effect of gentamicin which encourages the formation of reactive oxygen species (ROS) and lipid peroxidation of the membrane lipid and protein denaturation. Slides from kidney section reflected the protective role of nettle in preventing the appearance of renal abnormal changes [17,19].

Gentamicin-induced nephrotoxicity is related to its high concentration attained in the cells of proximal tubules compared to the serum concentration, and its tendency to bind strongly to the components of the brush border membrane of the proximal tubule leads to the most common clinical presentation of acute renal failure, which is characterized by the presence of enzymuria and elimination of fragments from brush border membrane [20,21].

The protective activity of nettle may be due to phenols. Phenolic compounds have antioxidant properties due to their ability of scavenging free radicals and active oxygen species such as single oxygen, free radicals and hydroxyl radicals [22,23].

Oxidative stress has a critical role in the pathophysiology of several kidney diseases, and many complications of these diseases are mediated by oxidative stress, oxidative stress-related mediators and inflammation [13,24,25].

The kidney is an organ highly vulnerable to damage caused by ROS, likely due to the abundance of long-chain polyunsaturated fatty acids on the composition of renal lipids. Aminoglycosides are nephrotoxic agents; their nephrotoxicity is mainly attributed to induction of ROS and depletion of antioxidant enzyme activities in kidney [5,26–28].

Glutathione peroxidase and MDA are two antioxidant parameters act as indicator for free radical levels in the body. They are indicators for oxidative stress status in the body and their concentrations in tissues are more reliable indicator than the concentration in blood. Their significant decrease in kidney of gentamicin treated rabbits may be indicative of exhaustion of these enzymes as a consequence of increased oxidative stress [21,23,28,29].

Research investigations have suggested that a diet rich in polyphenolic compounds and flavonoids is associated with longer life expectancy [19,25].

From the results of this study, the phenol content is 128.9 GAE which is a high quantity of phenol playing a major role in the antioxidant property of ethanol extract of nettle.

Gentamicin induces oxidative stress through induction of ROS such as superoxide, hydroxyl radical anion and hydrogen peroxide, which attacks different cell components as DNA, RNA, proteins, lipids and enzymes leading to many degenerative processes in the renal cells manifested as glomerular disease, renal ischemia, perfusion injury and eventually acute renal failure [29–31].

Vitamin E has nephroprotective effect in gentamicin-induced nephrotoxicity, which could be mediated through its potent antioxidant effect and can preserve intracellular levels of biological pathways by enhancing excretion of toxic levels of gentamicin [16,31].

In a study done by Toldy *et al*. [4], the proposed neuroprotective property of nettle extract can be attributed to the antioxidant activity of *Urtica dioica*.

Peroxidative degradation of membrane lipids of endoplasmic reticulum, rich in polyunsaturated fatty acids leads to the formation of lipid peroxides, which in turn give products like MDA that cause damage to the membrane and alter cellular function.

The results obtained from the present study indicated that ethanol extract of this plant exhibited a wide range of antioxidant activity. The overall antioxidant activity of this plant might be attributed to its phenolic content which plays act as a protective agent in the treatment of nephrotoxicity that caused by gentamicin.

Conflict of interest statement

We declare that we have no conflict of interest.

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