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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.