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Effect of Couch Grass (Agropyrum Repens) on Gentamicin-Induced Nephrotoxicity

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ABSTRACT

Couch grass is used to clean the urinary tract during infections due to its diuretic and antimicrobial effects. The aim of this study was to investigate the possible protective or therapeutic effects of couch grass on gentamycin-induced nephrotoxicity in rats. Four groups of 10 rats were formed in the study. First group, Control: Rats were fed with standard rat feed and water. Second group (couch grass): 3 gr/ L couch grass was added to the drinking water of rats for 7 days. The third group (gentamicin): Gentamicin 80 mg/ kg/ day/ i.p. was given for 7 days. The fourth group (gentamicin + couch grass): Gentamicin 80 mg/ kg/ day/ i.p. and 3 g / L couch grass was added to drinking water for 7 days. One week after the experimental application, blood samples were taken and serum was separated. Albumin, BUN, creatinine, urea, GGT, ALP, Na, Cl and K levels were determined in autoanalyzer. Cystatin C was measured by ELISA. Serum BUN, creatinine, urea, Na, K levels were highest in the gentamicin group. Na, K and cystatin C levels differences between groups were not statistically significant. The increase in BUN, urea and creatinine after gentamicin administration was statistically significant ($p<0.05$). The use of gentamycin and couch grass caused BUN, urea and creatinine levels to decrease. The difference between the groups was found to be statistically significant ($p<0.05$). The administration of gentamycin and couch grass decreased cystatin C levels, statistical significance was found between the groups ($p<0.05$). No statistically significant difference was found between the groups in albumin, Cl, GGT, ALP levels ($p>0.05$). As result, positive changes in serum parameters of gentamycin-induced nephrotoxicity when couch grass were given, showed that couch grass had a kidney protective effect and decreased nephrotoxic damage.

Key words: Cough grass, kidney, gentamicin, nephrotoxicity, rat, cystatin C

Introduction

Kidney failure means partial or complete loss of kidney function. Urinary excretion may also decrease as a result of the kidneys, whose function has been damaged, cannot remove waste materials from the body. As a result, various diseases may occur in the organism. Due to the ability of the kidneys to tolerate the workload, early diagnosis may not be detected in endogenous indicators. It is known that GFR (Glomerular Filtration Rate) is the primary reliable criterion for the determination of kidney damage (1, 2).

The term acute renal failure (ARF) has recently been used to describe the rapid destruction of kidney functions (3). In acute kidney damage, there is no or very little excretion of waste products and urine from the body. ARF is often caused by anemia and toxicity. ARF is divided into clinical stages cross-sectionally. These; prerenal, renal and postrenal. These three should be determined immediately after the ARF is finalized (4, 5).

The irreversible destruction of kidney structures over time is called chronic kidney failure (CRF) (6). CRF can also be defined as the presence of bilateral structural or functional kidney damage for more than 3 months, with or without a decrease in glomerular filtration rate (GFR) (6).

Considering the etiology of CRF; chronic pyelonephritis, chronic glomerulonephritis, acute kidney failure, hypertension, diabetes mellitus, sickle cell anemia, amylodosis, multiple myeloma, polycystic kidney disease. In CRF, nephrons are irreversibly destroyed, and as a result of this, GFR gradually decreases. signs and symptoms of CRF; high level of metabolic reaction residues of proteins in plasma, fluid electrolyte and acid-base balance disorders can be counted. These disorders can affect all systems of the body. The changes that occur in the systems in CRF are directly

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related to the degree of kidney failure and the rate of development of the patient. CRF is not considered to have completely lost function of the affected system/organ. Glomerular filtration level above 35-50 ml/min may not give any symptoms. Initially, the symptoms are related to anemia and nocturia, which is the result of fatigue. When the glomerular filtration level rises to 20-25 ml/min, uremic symptoms occur (7).

Recent studies have shown that plants are a source of many secondary metabolites with a wide range of pharmacological and therapeutic effects (8, 9). It has been shown that plant extracts prevent the oxidative process, protect against diabetes and have antimicrobial effects (10, 11, 12, 13). Couch grass is used as a relaxing diuretic and to relieve pain and spasm in the urinary tract. It is also used in the control of symptoms (cystitis, urethritis, prostatitis) of urinary disease, prostate disease, rheumatism, kidney stones and urinary infections in children with urinary system diseases such as enuresis and urinary incontinence (14). Couch grass has also been used to clear the urinary tract during infections due to its diuretic and antimicrobial effects (15).

In this present study, the protective effect of couch grass on nephrotoxicity induced by gentamicin was investigated.

Materials and Method

In the study, 40 female Wistar albino rats with 250-300 g live weight, provided by the Experimental Research Unit of Van Yuzuncu Yil University Faculty of Medicine, were used. For adaptation, the experimental animals were fed ad libitum with standard rat food in a ventilated room with 12 hours of light and 12 hours of darkness at 24±3 °C for one week.

In this study, rats were randomly divided into 4 groups with 10 animals in each group.

Control group: Standard rat food and water were given for 7 days.

Couch grass group: The animals were given 3 g/L of couch grass with drinking water for 7 days (16).

Gentamicin group: Gentamicin was given as 80 mg/kg/day/i.p for 7 days.

Gentamicin + couch grass: Gentamycin 80 mg/kg/day/i.p was administered for 7 days + 3 gr/L Couch grass drinking water for 7 days (16).

The pre-supplied couch grass was first shredded with a hand blender. It was then weighed on a precision scale and taken into the brewing container. It was waited for 15 minutes for it to brew by adding boiled water on it. The brewed couch grass was filtered through filter paper and made ready for use (16).

After the experimental application, 90 mg/ kg ketamine was administered to all rats i.p. to allow them to enter anesthesia. The animals were placed on the table in the dorso-ventral position, the rat's neck was grasped with the right hand, and the forelimbs were firmly held and stretched with the thumb and index fingers. The thorax of the animal was shaved and cleaned with alcohol. After opening with a vertical incision in the midline, the heart was directly cannulated and blood samples were taken into tubes without anticoagulant, centrifuged at 3000 rpm for 10 minutes, and serum was separated. It was stored at -18 °C until analysis. Serum albumin, BUN, creatinine, GGT, ALP, Na, Cl, K levels in the samples were measured with the help of autoanalyzer, and the amount of cistatin C was measured with the ELISA kit method.

Serum albumin (mg/dL), BUN (mg/dL), creatinine (mg/dL), GGT (U/L), Cl (mmol/L), belonging to control, couch grass, gentamicin, gentamicin + couch grass group rats, The averages of Na (mmol/ L), K (mmol/ L), urea (mg/ dL), ALP (U/ L) levels are given in Table 1.

Plasma albumin levels were determined as 4.02±0.17, 4.05±0.35, 3.99±0.13, 4.07±0.17 mg/ dL in rats in the control, couch grass, gentamicin, and gentamicin+couch grass groups, respectively. It was determined that the mean albumin levels of the rats in the control and couch grass groups were close to each other, lower in the gentamicin group, and higher in the gentamicin + couch grass group. There was no statistical significance in the difference between the groups ($p>0.05$).

Plasma BUN levels were found to be 21.7±2.49, 19.7±1.49, 30.04±1.89, 27.1±1.52 mg/ dl in rats in the control, couch grass, gentamicin and gentamicin + couch grass groups, respectively. The highest rate was found in rats in the gentamicin group (30.04±1.89 mg/ dl). It was found to be the lowest in the couch grass group (19.7±1.49 mg/ dl). Statistically significance ($p<0.05$) was found between the gentamicin group and the other groups. There was no statistical significance in the difference between the control group and the couch grass group ($p>0.05$).

Plasma creatinine levels were 0.32±0.04 mg/dl in the control group, and 0.33±0.02 mg/dl in the couch grass group. No statistical significance was

Table 1. Plasma albumin, BUN, creatinine, GGT, Cl, Na, K (mmol/L), urea and ALP (U/L) levels of the rats in the groups

	Control	Couch grass	Gentamicin	Gentamicin + Couch grass	P Value
	X±Sx	X±Sx	X±Sx	X±Sx	
Albumin (mg/ dL)	4.02±0.17[a]	4.05±0.35[a]	3.99±0.13[a]	4.07±0.17[a]	p>0.05
BUN (mg/ dL)	21.7±2.49[a]	19.7±1.49[a]	30.04±1.89[b]	27.1±1.52[c]	p<0.05
Kreatinin (mg/ dL)	0.32±0.04[a]	0.33±0.02[a]	0.62±0.06[b]	0.56±0.05[c]	p<0.05
GGT (U/ L)	1.87±0.83[a]	1.7±0.67[a]	1.9±1.28[a]	2.2±1.13[a]	p>0.05
Cl (mmol/ L)	104.02±2.13[a]	103.91±4.02[a]	102.27±1.95[a]	103.74±3.76[a]	p>0.05
Na (mmol/ L)	143.12±1.45[a]	143.2±1.31[ab]	145.5±1.71[b]	144.5±1.35[ab]	p<0.05
K (mmol/ L)	5.5±0.42[a]	5.34±0.37[a]	5.75±1.03[a]	5.19±0.58[a]	p>0.05
Üre (mg/ dL)	46.36±1.7[a]	42.08±1.5[b]	65.67±3.8[c]	60.62±3.18[d]	p<0.05
ALP (U/ L)	191.25±33.72a	181.4±28.23[a]	183.9±27.27[a]	158.9±18.24[a]	p>0.05
Sistatin C (mg/ dL)	1.23±0.12[a]	1.15±0.27[a]	1.36±0.2[a]	1.29±0.07[a]	p>0.05

a, b: The difference between the group means with different letters on the same line is statistically significant

found in the difference between these two groups. A significant increase was detected in the gentamicin group (0.62±0.06 mg/ dl) compared to the control and couch grass groups (p<0.05). A significant decrease was observed in the gentamicin + couch grass group compared to the gentamicin group (p<0.05).

Plasma GGT levels were found to be 1.87±0.83, 1.7±0.67, 1.9±1.28, and 2.2±1.13 U/L in rats in the control, couch grass, gentamicin, and gentamicin+couch grass groups, respectively. It was determined that the mean GGT levels of the rats in the control, couch grass and gentamicin groups were close to each other, while the gentamicin + couch grass group was relatively high. There was no statistical significance in the difference between the groups (p>0.05).

Plasma Cl levels were found to be close to each other as 104.02±2.13, 103.91±4.02, 102.27±1.95, 103.74±3.76 mmol/ L in the control, couch grass, and gentamicin+ couch grass groups, respectively. A lower value was found in the gentamicin group compared to the other groups. There was no statistical significance between the groups (p>0.05).

The highest plasma sodium level was found in the gentamicin group (145.5±1.35 mmol/ L), and the lowest in the control group (143.12±1.45 mmol/ L), and statistical significance was found between the two groups (p<0.05). There was no significant difference between the other groups (p>0.05).

Plasma potassium levels were found to be 5.5±0.42, 5.34±0.37, 5.75±1.03, 5.19±0.58 mmol/ L in rats in the control, couch grass, gentamicin and gentamicin + couch grass groups, respectively. There was no statistical significance in the difference between the groups (p>0.05).

Plasma urea levels were found to be 46.36±1.7, 42.08±1.5, 65.67±3.8, 60.62±3.18 mg/dl in rats in the control, couch grass, gentamicin, and gentamicin+couch grass groups, respectively. The highest value was found in gentamicin (65.67±3.8 mg/ dl), the lowest value was found in the couch grass group (42.08±1.5 mg/ dl). Statistical significance was determined between the groups (p<0.05).

Plasma ALP levels were determined as 191.25±33.72, 181.4±28.23, 183.9±27.27, 158.9±18.24 U/ L in rats in the control, couch grass, gentamicin, and gentamicin + couch grass groups, respectively. It was determined that the mean ALP levels of the rats in the gentamicin and couch grass group were close to each other, the gentamicin + couch grass group was lower and the control group was the highest. There was no statistical significance in the difference between the groups (p>0.05).

Plasma cystatin C levels were determined as 1.23±0.12, 1.15±0.27, 1.36±0.02, 1.29±0.07 mg/ dL in rats in the control, couch grass, gentamicin and gentamicin + couch grass groups, respectively. The mean cystatin C levels of the groups were found to be close to each other. There was no statistical significance in the difference between the groups ($p>0.05$).

Discussion

The pharmacokinetics, pathology, and clinical pattern of gentamicin-induced kidney injury have been extensively studied in both humans and animals. However, there is no consensus in the literature regarding their possible mechanisms of action or factors that can modulate nephrotoxicity. If a way can be found to protect the kidneys from these undesirable side effects, the value of aminoglycosides, including gentamicin, in clinical practice will be greatly increased. Over the past two decades, many review articles have been published addressing various aspects of gentamicin nephrotoxicity in humans and animals (17, 18). In another study, it was revealed that fucoidan has a protective effect in nephrotoxicity induced by gentamicin (19).

Gentamicin is considered an "obligatory nephrotoxin". It has been reported by some authors that even small doses cause nephrotoxicity in humans and animals. It has been suggested that aminoglycoside-induced nephrotoxicity may be unavoidable to some degree in every patient, and that only in its most severe form, damage to the kidney reaches a level that permits clinical diagnosis (18).

In cortical homogenates obtained from rats chronically treated with gentamicin, Na, K, ATPase activity is reduced, and this change is considered as a mediator in gentamicin nephrotoxicity. However, the site of action for inhibition of Na-K ATPase is not localized. (20, 21).

It has been found that couch grass has no effect on urolithiasis risk factors when given to rats in combination with different diets (standard, high glycosidic and high protein) (16).

The sugar mannitol, found in large quantities in couch grass, is known as a standard 'osmotic diuretic', meaning it is completely absorbed from the gut and is largely excreted by the renal tubules. Its presence in the tubules means that extra water must be retained to maintain the osmotic pressure. The saponins and vanillin in its structure also have diuretic properties. Couch grass has been used to clear the urinary tract during infections due to its diuretic and antimicrobial effects (15).

Data from 313 patients with urinary tract infections or irritable bladders were analyzed in a study designed to investigate the efficacy and tolerability of couch grass liquid extract in patients with urinary tract infections or irritable bladders. Patients were given 50-60 drops 3 times a day for an average of twelve days. Its primary effect was the change of urological symptoms during treatment. Initially documented urological symptoms changed positively in 69% to 91%. Depending on the established urologic diagnosis, 32% to 53% of patients have complete resolution of symptoms following treatment. The investigators observed no adverse drug reactions (15).

BUN and creatinine increase or creatinine clearance are mostly used parameters to monitor the possible nephrotoxic effects of substances that may cause nephrotoxicity, such as antibiotics and analgesics. Since urea and creatinine are cleared from the blood by glomerular filtration, creatinine and BUN are important parameters used to evaluate GFR (22, 23).

Pınar et al., in their study, showed that the BUN value increased in nephrotoxicity induced by gentamicin in rats (24). In the presented study, urea, BUN and creatinine levels, which are nephrotoxicity assessment parameters, were examined. The highest plasma urea level was found in the Gentamicin group. The result is compatible with the literature. It was observed that urea decreased in the group that was given couch grass with gentamicin and approached the control group. The lowest amount of urea was found in the couch grass group. It was observed that the administration of couch grass together with gentamicin significantly reduced the amount of serum urea. The difference was statistically significant ($p<0.05$) (Table 1).

While the highest plasma creatinine level was found in the gentamicin group (0.62±0.6 mg/dl), it was observed that it decreased to 0.56±0.05 mg/ dl in the gentamicin + couch grass group, approaching the control group. The decrease in the amount of serum creatinine was statistically significant in the group given couch grass together with gentamicin ($p<0.05$). The data obtained clearly reveal that the application of couch grass reduces the serum creatinine level.

In line with the findings obtained in this study, it was observed that the plasma urea, BUN and

creatinine levels in the group that was given couch grass with gentamicin decreased significantly and approached the levels of the control group. The decreases were statistically significant.

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