

Protective Effect of Nettle (*Urtica dioica*), Leaves and Seeds on Kidney Disorder in Gentamicin-Induced Rats

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ABSTRACT:

The influence of various concentrations of 2.5 and 5 % of nettle leaves, seeds, and their mixes as powder on gentamicin-induced renal disorder in male rats was investigated. Forty-eight albino rats, weighing about 150 ± 10 g, were used and divided into 8 equal groups; the first one was kept as a negative (-ve) control group, while the other 7 groups were injected with gentamicin at the dose of 100mg/kg body weight for 10 consecutive days. When the experiment ended, renal functions, including serum uric acid, creatinine, and urea, lipid fractions consisting of triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL-c), very low-density lipoprotein (VLDL-c), and high-density lipoprotein (HDL-c) levels, serum glucose, and liver enzyme activities such as ALP, AST, and ALT, which were measured. The data was analyzed using [specific statistical analysis method]. From the obtained results, it may be stated that the best values for protection, improvement of renal functions, liver functions, lipid fractions, and glucose ranges were reported at a 5.0% mix of nettle leaves and seeds powder. In conclusion, we could utilize nettle leaves and seeds and their combined powder in our regular beverages, demonstrating the potent nutraceutical therapeutic advantages of consuming them separately and together for treating renal disorders.

Keywords: Rats, nettle, bitter wood, serum sugar, biochemical analysis

1- INTRODUCTION

In vertebrates, there are two organs called kidneys that resemble beans. They are around 11 centimeters in length in adult humans and are found each on the left as well as right sides of the retroperitoneal region. Blood via the interconnected renal arteries and veins, both passing through the body, each kidney is connected to a ureter, a pipe

that connects the bladder to the excreted urine [1]. Mature humans have kidneys that play several crucial regulatory tasks. They serve an essential phase in the urinary system and perform homeostatic tasks include controlling electrolytes, maintaining the acid-base balance, and controlling blood pressure (by ensuring that salt and water levels are balanced). They act as a natural blood filtration for

the body, removing waste that would otherwise go to the urine bladder [2]. Numerous metabolic waste products are excreted by the kidneys into the urine. A renal cell is a kidney's microscopic structure and functional unit [3]. There are numerous End-stage renal disease (ESRD) reasons, and each patient's experience will vary. One of the main risk factors for chronic kidney disease (CKD) is getting older. Population, type 2 diabetes, hypertension, and medications like the typical long-term medication for pain use those results in analgesic nephropathy and renal damage. Polycystic renal disease is an example of CKD that has a genetic cause [4]. An aminoglycoside known as gentamicin (GM) is recognized for its renal toxicity, and one of the proposed reasons is damage caused by the production of free radicals. GM-induced nephrotoxicity may be caused by ROS/Nitrogen species, which are linked to increased lipid peroxide production and decreased antioxidant enzyme activity [5].

Some herbal medications that contain herbs, nuts, or mushrooms may also have nephrotoxicity built right in. Renal diseases linked to herbs are not simply caused by their natural qualities. Concerns include herb-drug interactions, dosage and identification errors, pollutants in mixes, heavy metal adulteration, and even purposeful adulteration using unlabeled plant extracts [6]. The family Urticaceae consists of the herbaceous perennial flowering

plant recognized as *Urtica dioica*. It is also referred to as the stinging nettle, or ordinary nettle (although not all plants of this species sting), nettle leaves, nettle, or only stinging. Most of subtropical Asia, Western North Africa, and parts of Europe were their original habitats [7]. A perennial plant with a diverse chemical constitution is nettle. According to research, the dry matter content of nettle aerial parts is 2.5-3.6% crude fat, 18-34% crude protein, 9% crude fiber, 16% total ash, and 37% carbohydrates. Vitamins C, B, and K, phenolic compounds, as well as carotenoids are all abundant in the leaves [8]. Nettle extracts significantly reduced kidney disorder, tubular atrophy, brush border loss, reduction in nephron size, hydropic cell epithelial loss, and tubulointerstitial fibrosis. They also showed therapeutic advantages in patients undergoing partial nephrectomy or transplantation of the kidney [9]. Nettle's preventive properties against gentamicin-induced nephrotoxicity may be attributed to its phenolic content. Due to their capacity to scavenge free radicals and active oxygen species such single oxygen, free radicals, and hydroxyl radicals, phenolic compounds exhibit antioxidant characteristics [10]. Nettle's nephroprotective impacts on gentamicin-induced renal disorder can maintain biological systems at intracellular levels. and assist the enhancement of gentamicin excretion at hazardous levels [11]. Nettle contains several

phytochemicals and exhibits significant anti-urolithiasis activity, making it potentially useful as a natural treatment agent for a variety of urological problems [12].

Through this research, we examined the effects of various nettle leaf and seed powder concentrations on the biological and biochemical alterations of renal disorder in rats.

2- MATERIALS & METHODS

MATERIALS

The leaves and seeds of nettle (*Urtica dioica*, L.) were purchased in 2022 at Shebin El-Kom City, Menoufia Governorate, Egypt, from a herbalist.

Experimental rats

Adult forty-eight normal male albino Sprague Dawley strain rats weighing 150 ± 10 g were obtained by the Vaccine and Immunity Organization, Ministry of Health, Helwan Farm, Cairo, Egypt for this study.

Gentamicin

Memphis Company in Cairo, Egypt, bought antibiotics namely name gentamicin (GM), chemical name known as aminoglycosides.

The kits

For use in the measurement of the following tests: TC, TG, HDL-c, ALT, AST, ALP, urea, creatinine, uric acid, and glucose, chemical kits had been acquired from Al-Gomhoria Company for Trading Drug, Chemicals, and Medical Instruments, Cairo, Egypt.

METHODS

Preparing of nettle leaves and seeds

A nearby herbalist sold the dried nettle seeds and leaves, then we ground nettle samples in a machine that grinds (Broun grinder, model FX3030, Germany) to get a fine powder.

Induction of kidney damage

According to [13], rats had been given gentamicin (GM) (aminoglycosides antibiotics), acquired from Memphis Company, Cairo, Egypt, intraperitoneally in rats at the dose of 10mg/kg body weight for 10 consecutive days.

Experimental design

The research was once conducted in Animal House at the University of Menoufia in Egypt, which has been authorized, Department of Nutrition and Food Science, Faculty of Home Economics according to Ethical approval of the Science Research Ethics Committee of Faculty of Home Economics cleared the study protocol #7-SREC-05-2021.

In this study, 48 grown-up male white rats, ten weeks old, and averring weighed 150gm, were utilized. For seven days straight, all rats received a regular diet in this test in accordance with [14]. Rats are then placed into eight groups with six rats each after the time of adaptation, as follows: Group (1): Rats given a regular diet and served as negative control group. Group (2): A nephrotoxic group rats were given a regular diet and served as a positive control group. Group (3): A

nephrotoxic group rats were given a regular diet and nettle leaves as powder by 2.5% of the weight of the diet. Group (4): A nephrotoxic group rats were given a regular diet and nettle leaves as powder by 5% of the weight of the diet. Group (5): A nephrotoxic group rats were given a regular diet and nettle seeds as powder by 2.5% of the weight of the diet. Group (6): A nephrotoxic group rats were given a regular diet and nettle seeds as powder by 5% of the weight of the diet. Group (7): A nephrotoxic group rats were given a regular diet and mixture of nettle leaves and seeds (1:1) as powder by 2.5% of the weight of the diet. Group (8): A nephrotoxic group rats were given a regular diet and mixture of nettle leaves and seeds (1:1) as powder by 5% of the weight of the diet.

The research study lasted for twenty-eight days throughout the time for the investigation. Each rat is weighed independently at the finish of the experiment before being slaughtered and having blood samples collected.

Blood tests:

After a 12-hour fast, blood samples of each rat were acquired from the hepatic portal vein at the finish line of each trial. The serum was taken away from the blood samples by centrifuging them for 10 min. at 4000 rpm after they had been drawn into dry, clean centrifuge tubes and allowed to clot for 30 minutes in a water bath (37°C). After gently collecting the serum into clean cuvette tubes, it was

then frozen until analysis according to Schermer [15].

Biochemical analysis

Renal functions

According to [16, 17 and 18], respectively, the serum concentrations of urea, uric acid, and creatinine have been measured with an enzymatic approach.

Determination of blood glucose

Serum glucose was once measured using a calorimetric enzyme method in accordance with [19].

Liver enzymes

Using the techniques described in [20, 21 and 22], respectively, the serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) have been measured.

Lipids fractions

Using Thomas [23] colorimetric procedure, the cholesterol was measured. According to Young [24] and Fossati & Pricipe [25], serum triglycerides were measured using enzymatic techniques. The method described by [26] was used to calculate HDL-c. According to Lee and Nieman [27], very low density-lipoprotein was estimated in mg/dl applying the formula below: Triglycerides (mg/dl) = very low density-lipoprotein cholesterol / 5. According to Lee and Nieman [27], low density-lipoprotein cholesterol was estimated in mg/dl as below: Low density-lipoprotein cholesterol = Total cholesterol - High density-lipoprotein cholesterol - Low density-lipoprotein cholesterol.

Statistical analysis

The data were analyzed using a completely randomized factorial design according to SAS, [28] when a significant main effect was detected; the means were separated with the Student-Newman-Keuls Test. Differences between treatments of ($P \leq 0.05$) were considered significant using SPSS program. Biological results were analyzed by One Way ANOVA.

3- RESULTS AND DISCUSSION

The information in Table (1) demonstrates the impacts of nettle leaves, seeds, and their powdered mixes on the serum levels of urea, uric acid, and creatinine in nephrotoxic rat models. The results exhibited a substantial variance between the lower value for serum urea levels obtained using the control negative group and the higher value obtained using the positive control group, which were 52.00 and 22.20 mg/dl, respectively. However, there appeared to be a substantial variance between the average values for the treated groups (nephrotoxic groups), and the smallest number for the 4% nettle mixes and the greatest value for the 2% nettle leaves, which were 38.13 and 34.20 mg/dl, respectively.

Regarding uric acid, the findings indicated that the control positive group had greater serum uric acid ranges whereas the control negative group had fewer levels with significant variations, were the relative readings of 9.80 and

6.25 mg/dl. On the other direction, in the groups that were treated (nephrotoxic groups), there was once a substantial variance between the smallest number for the 4% nettle combination and the greatest number for the 2% nettle leaves. The mean values were 8.11 and 6.85 mg/dl. Serum creatinine ranges varied extensively between the positive control group and the negative control group, with greater values being documented in the positive control group. The averages for the relative means have been 1.47 and 0.76 mg/dl. Contrarily, 4% nettle mixes provided the smallest value, whilst 2% nettle leaves created the best creatinine ranges amongst treated groups (nephrotoxic groups), with variants that had been statistically significant. The values for the relative means have been 1.12 and 0.78 mg/dl. These findings assist [29], who mentioned that a medical test using nettle seed extracts for the remedy of renal failure had yielded excellent results. For superior levels of renal failure, seeds are suggested as a restorative kidney stimulant.

Furthermore, every group of rats that were kidney-damaging given various diets revealed a significant decrease in mean values of urea, uric acid and creatinine concentrations as compared to control positive group. The lowest values were recorded for the group fed on 5% plant mixture with no significant difference when compared with the negative group [30].

Additionally, nettle remedy following gentamicin nephrotoxicity induction shielded the rabbits in opposition to changes in serum urea and creatinine percentages. The nettle group receiving remedy confirmed an excessive antioxidant activity with the aid of a rise in glutathione ranges and a reduction in

malondialdehyde contents. Resulting it is anticipated that the renal-protective influence of nettle in gentamicin-induced kidney injury will keep intracellular degrees related with biological activity whilst allowing elevated gentamicin clearance [31].

Table (1): Effect of nettle leaves, seeds, and their mixture powder on kidney functions of nephrotoxic rats

Groups \ Parameters	Urea mg/dl	Uric acid mg/dl	Creatinine mg/dl
G1 C (-)	20.20g ± 1.10	6.25c± 0.13	0.76b± 0.04
G2 C (+)	52.00a ±1.20	9.80a ± 0.90	1.47a± 0.10
G3 (2% Nettle seeds)	43.96c± 1.00	7.47b± 0.60	1.02b± 0.03
G4 (4% Nettle seeds)	40.27d ± 0.93	7.10b± 0.40	0.88b± 0.01
G5 (2% Nettle leaves)	48.13b± 1.20	8.11a ± 0.60	1.12a± 0.11
G6 (4% Nettle leaves)	45.40c± 0.45	7.75b± 1.20	0.96b± 0.05
G7 Mixture 2%	38.25e± 0.60	7.00b± 1.30	1.01b± 0.04
G8 Mixture 4%	34.20f± 0.70	6.85c± 1.10	0.78b± 0.02
LSD (P≤0.05)	1.980	1.005	0.372

Each value corresponds to the mean of standard deviation of three replicates.

Means in the same column that have different letters are significantly different (P≤0.05).

The impact of nettle leaves, seeds, and their mixes on the glucose ranges of nephrotoxic rats are proven in Table (2). It is clear to observe that the control negative group recorded a decrease value with a statistically significant difference, whereas the control positive group mentioned a greater glucose degree. The corresponding averages had been 103.30 and 176.73 mg/dl.

In comparison to the positive control group, all renal toxic rats fed nettle leaves, seeds, or a combination of the two showed significant reductions in indicated values. The group of rats who acquired 2% nettle leaves had the biggest glucose level, whilst the group that acquired 4%

nettle combo had the smallest value; their respective averages had been 164.89 and 135.49mg/dl. These findings are in harmony with Altamimi [32], who suggested that the nettle originally had the ability to inhibit the disaccharides involved in the breakdown of carbohydrates. Nettle has been utilized in traditional medicine due to its anti-disaccharidase and glucose transport inhibiting properties. This shows that it might possibly be a potent plant remedy for diabetes. Nettle also demonstrated anti-diabetic properties in a laboratory experiment.

Additionally, nettle leaves had been observed to considerably decrease

patients' fasting blood sugar ranges in eight randomized medical trials that concerned diabetic mulitas kind two patients. Determining the active elements and cellular mechanism of motion of the nettle extract is a vital first step in finding out its function in controlling blood glucose degrees in the diabetes situation [33].

Table (2) Influence of nettle leaves, seeds, and their mixture powder on glucose level of nephrotoxic rats

Groups \ Parameters	Glucose mg/dl
G1 C (-)	103.30g± 1.12
G2 C (+)	176.73a± 1.60
G3 (2% Nettle seeds)	156.46c± 1.60
G4 (4% Nettle seeds)	147.54d± 1.41
G5 (2% Nettle leaves)	164.89b± 1.55
G6 (4% Nettle leaves)	160.70b± 1.50
G7 Mixture 2%	142.19e± 1.40
G8 Mixture 4%	135.49f± 1.30
LSD (P≤005]	4.152

Each value corresponds to the mean of standard deviation of three replicates. Means in the same column that have different letters are significantly different ($P\leq 0.05$).

Data in Table (3) confirmed the impacts of nettle leaves, seeds, and their mixes as powders on liver enzymes ranges (ALT, AST, and ALP) of nephrotoxic rats. It is clear to point out that the greater ALT liver enzyme ranges recorded for control positive group, whilst control negative group recorded the decrease value with a substantial difference. The average numbers were 150.82 and 68.20 U/L, respectively. But on the other side, the ALT liver enzyme showed a substantial variation value of the treated groups (nephrotoxic groups), with the greatest

value being recorded for 2% nettle leaves and the smallest value being reported for 4% nettle combination which were 121.79 and 84.10 U/L, respectively.

In the instance of the liver enzyme AST, the control positive group had greater levels than the control negative group, who had a significantly lower number. The average values were, respectively, 76.24 and 19.42 U/L. On the other side, 2% nettle leaves recorded the best AST liver enzyme value of nephrotoxic groups, whereas 4% nettle mixed recorded the lowest value, with significant variations. The corresponding mean values were 64.89 and 39.78 U/L.

Regarding the ALP liver enzyme, control positive group levels were greater, while control negative group levels were lower, with a significant difference. The average values were, respectively, 41.62 and 17.18 U/L. On the other hand, there was a substantial difference between the ALP liver enzyme levels of the nephrotoxic groups, with the highest value being recorded for 2% nettle leaves and the lowest value being reported for 4% nettle mixture, the corresponding mean values were 35.91 and 14.60 U/L. Our findings corroborated those of [34], who claimed that the nettle has powerful antioxidant fraction which supports the highest level of hepatoprotective potential because of its capacity to operate as a free radical scavenger, as demonstrated by in-vitro and in-vivo antioxidant potential. The findings indicated that the nettle has

hepatoprotective properties which were brought by the presence of phenolic

substances like ferulic acid, which function as antioxidants.

Table (3): Influence of nettle leaves, seeds, and their mixture powder on liver enzymes of nephrotoxic rats

Groups \ Parameters	ALT U/L	AST U/L	ALP U/L
G1 C (-)	68.20h± 0.93	19.42g± 0.50	17.18f± 0.20
G2 C (+)	150.82a± 2.60	76.24a± 1.60	41.62a± 0.70
G3 (2% Nettle seeds)	118.25c± 1.50	60.88c± 1.40	32.87c± 0.60
G4 (4% Nettle seeds)	10513 e±1.20	54.57e± 1.20	30.57d±0.40
G5 (2% Nettle leaves)	121.79b± 1.60	64.89b± 1.40	35.91b± 0.60
G6 (4% Nettle leaves)	113.23d± 1.30	58.32d± 1.50	32.67c± 0.50
G7 Mixture 2%	90.67f± 0.11	53.06e± 1.30	25.83e± 0.30
G8 Mixture 4%	84.10g± 0.10	39.78f± 0.60	14.60g± 0.10
LSD (P≤0.05)	4.020	1.690	1.403

ALT=Alanine amino-transferase, AST=Aspartate amino-transferase ALP= Alkaline phosphatase. Each value corresponds to the mean of standard deviation of three replicates. Means in the same column that have different letters are significantly different (P≤0.05).

The impacts of nettle leaves, seeds, and their powdered mixes on the serum ranges of total cholesterol and triglycerides in nephrotoxic rats were demonstrated by the data in Table (4). The acquired results showed a significant difference between the smaller value recorded by the control negative group and the greater value recorded by the control positive group for serum total cholesterol levels. The relative mean values were 175.00 and 80.75 mg/dl. While 2% nettle leaves were associated with the best serum total cholesterol levels in the nephrotoxic groups, 4% nettle mixture was associated with the littlest value, with a significant difference. The relative mean values were 147.00 and 97.25 mg/dl.

In terms of serum triglyceride levels, it was discovered that the control positive group had greater triglyceride ranges than the

control negative group, with significant differences. The corresponding mean values were 140.75 and 66.50 mg/dl. On the other hand, there was a substantial difference between the serum triglyceride ranges of the nephrotoxic groups, with the greatest levels being found for 2% nettle leaves and the smallest for 4% nettle mixes. The corresponding mean values were 128.75 and 89.25 mg/dl. These findings guide the findings of [35] who advised that the nettle leaves' very excessive polyphenol content material can also extensively contribute to fitness benefits such as lowering TC, and TG levels.

Additionally, as in distinction to the positive control group, rats given the Jew's stone diet and the Jew's stone with nettle, Juniper, and corn silk proven the great possible reduction in total cholesterol ranges [36].

Table (4) Influence of nettle leaves, seeds, and their mixes powder on serum total cholesterol, and triglycerides of nephrotoxic

Groups	Parameters	T.C mg/dl	TG mg/dl
G1 C (-)		80.75g± 1.20	66.50g± 1.45
G2 C (+)		175.00a± 2.60	140.75a± 2.50
G3 (2% Nettle seeds)		144.25b± 2.40	121.60c± 2.30
G4 (4% Nettle seeds)		139.25d± 2.21	116.50d± 2.10
G5 (2% Nettle leaves)		147.00b± 2.50	128.75b± 2.40
G6 (4% Nettle leaves)		143.50c± 2.20	120.31c± 2.30
G7 Mixture 2%		113.00e± 2.20	102.00e± 2.10
G8 Mixture 4%		97.25f± 1.73	89.25f± 1.54
LSD (P≤0.05)		3.460	3.351

TC= Total cholesterol. TG= Triglycerides. Each value corresponds to the mean of standard deviation of three replicates. Means in the same column that have different letters differ substantially different (P≤0.05).

The data shown in Table (5) display the impact of nettle leaves, seeds, as well as their powdered blends on the concentrations of the lipid fractions that is serum lipids high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and very low-density lipoprotein cholesterol (VLDL-c). The obtained results showed a significant difference between the lower value recorded by the control positive group and the higher value reported by the control negative group for high density lipoprotein cholesterol, which were 49.77 and 33.80 mg/dl on average, respectively. While nephrotoxic groups' greatest high-density lipoprotein cholesterol levels were found in 4% nettle combination, they were significantly different from the lowest value found in 2% nettle leaves. The corresponding mean readings were 47.16 and 39.39 mg/dl.

The data also showed a significant difference between the lower value

recorded by the control negative group and the higher value recorded by the control positive group for low density lipoprotein cholesterol. The corresponding mean readings were 113.05 and 17.68 mg/dl. While 2% nettle leaves had the greatest amounts of low-density lipoprotein cholesterol in the nephrotoxic groups, 4% nettle combination had the lowest levels, which was a significant difference, which were the corresponding mean values 92.70 and 35.70 mg/dl.

It is possible to draw from this finding that the control positive group had greater levels of very low-density lipoprotein cholesterol than the control negative group, with a significant difference which were 28.15 and 13.30 mg/dl, respectively. While 2% nettle leaves had the greatest levels of very low-density lipoprotein cholesterol (VLDL-c) in nephrotoxic groups, 4% nettle combination had the lowest levels with statistically significant differences. The relative mean values

were 29.00 and 17.90 mg/dl. These findings support the findings of [37], who claimed that the powdered nettle leaves had reduced blood lipid and lipoprotein levels. Lower levels of low-density lipoprotein cholesterol and plasma total apo-protein B were found to have an important effect on the lipid profile, including total cholesterol, cholesterol fractions, and LDL/HDL ratios.

Additionally, rats with hypercholesterolemia and diabetes have

showed a significant reduction in total cholesterol and low-density lipoprotein cholesterol levels when given an organic extract of the nettle at doses of 100 and 300 mg/kg [38].

Likewise, studied plant leaves consist of a quantity of chemical substances that may also reduce the negative effects of a hypercholesterolemic diet. The advantages of which includes such plants in our diets in small amounts are greater, in accordance with the data of [39].

Table (5): Impact of nettle leaves, seeds, and their mixture powder on serum lipid fraction of nephrotoxic rats

Groups \ Parameters	HDL-C mg/dl	LDL-c mg/dl	VLDL-c mg/dl
G1 C (-)	49.77a± 1.78	17.68g± 1.13	13.30f± 1.10
G2 C (+)	33.80f± 1.13	113.05a± 1.70	28.15a± 1.62
G3 (2% Nettle seeds)	42.11d± 1.45	77.82c± 1.40	24.32c± 1.53
G4 (4% Nettle seeds)	45.00c± 1.52	70.95d± 1.33	23.30c± 1.40
G5 (2% Nettle leaves)	39.39e± 1.14	81.86b± 1.50	25.75b± 1.55
G6 (4% Nettle leaves)	39.51e± 1.30	79.93b± 1.53	24.06c± 1.46
G7 Mixture 2%	45.83b± 1.50	46.77e± 1.26	20.40d± 1.31
G8 Mixture 4%	47.16b± 1.65	32.24f± 1.20	17.85e± 1.23
LSD (P≤0.05)	2.050	2.351	1.500

HDL-c =High-density lipoprotein cholesterol. LDL-c = Low-density lipoprotein cholesterol. VLDL-c =Very low-density lipoprotein cholesterol. Each value corresponds to the mean of standard deviation of three replicates. Means in the same column that have different letters differ substantially different (P≤0.05).

4. CONCLUSION

Nettle leaves, seeds and their mixes powder can be used in our ordinary drinks, that demonstrates the powerful nutraceutical therapeutic benefits of consuming nettle leaves and seeds, and each separately and together for treating renal disorder.

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التأثير الوقائي لأوراق وبذور القراض في الفئران المصابة بخلل في الكلى بواسطة الجنتميسين

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