

Renal Protective Effect of Cardamom Against Nephrotoxicity Induced by Gentamicin in Rats

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Abstract

This study was conducted to evaluate the renal protective effect of cardamom against experimentally-induced gentamicin toxicity in rats. Forty adult male Wistar albino rats were randomly grouped into four groups (each of 10 rats). Rats in group (1) were orally administered saline (the vehicle) daily for 8 consecutive days and served as control group. Group 2: were given gentamicin (80 mg/kg. b.wt. i.p.) daily for 8 consecutive days. Group 3: were given gentamicin (80 mg/kg. b.wt. i.p.) daily for 8 consecutive days, then cardamom extract (100 mg/kg. b.wt. p.o.) daily from 9th to 21th days. Group 4: were given gentamicin (80 mg/kg. b.wt. i.p.) daily for 8 consecutive days, then cardamom extract (200 mg/kg. b.wt. p.o.) daily from 9th to 21th days. Gentamicin-treated rats showed significant renal damage as it the creatinine, urea, glucose and total protein showed a significant decrease. While in groups (gentamicin+cardamom 100 mg/kg & gentamicin+cardamom 200 mg/kg), showed significant protection to rat's kidney from structural and functional changes associated with gentamicin. Gentamicin which induced nephrotoxicity can be prevented by co-administration with cardamom.

Keywords: Cardamom , Gentamicin, Nephrotoxicity.

1. Introduction

Herbs and spices are being continuously used since ancient time as flavoring agent, food preservatives and remedies. Spices are rich in antioxidants [5] and are potent inhibitors of tissue damage and inflammation. Spices and herbs in regular diet may improve to health and lower the risk of many diseases [30]. Herbs and spices have antioxidant and anti-inflammatory activities [32]

Cardamom, *Elettaria cardamomum* maton (Fam. Zingiberaceae) known as true or lesser cardamom is the widely-cultivated variety and important in the world trade. It occupies a high second or third place in world trade, being a high priced spice. It belongs to the sweet spices group and is used predominantly to flavor sweets, baked goods, and coffee, particularly in the Arab countries [8]. Cardamom is a herbaceous perennial belonging to the family Zingiberaceae. Its dried fruit is one of the highly priced spices in the world. The dried fruit is used either whole or in ground form as a flavouring agent and also in the medicinal preparations for ingestion for flatulence. The most functionally important constituent of cardamom is its volatile oil [17]. In addition to its culinary uses, cardamom has been employed in traditional medicinal plants used against kidney and urinary disorders [1]

Some studies showed that extracts and its constituents from cardamom also possess antimicrobial [29,11,9] gastroprotective effects[20], antidiabetic effect [36], hepatoprotective activity [12,2] anti-hyperlipidemic[28, 34,27]and antioxidative effects [4].

Aminoglycoside antibiotics are employed clinically because of their potent bactericidal activities, less bacterial resistance, post-antibiotic effects and low cost. However, drugs belong to this class are well-known to cause nephrotoxicity,

which limits their frequent clinical exploitation. Gentamicin, a commonly used aminoglycoside, is associated with an induction of tubular necrosis, epithelial edema of proximal tubules, cellular desquamation, tubular fibrosis, glomerular congestion, perivascular edema and inflammation, which ultimately show the way to renal dysfunction [21]. It is a matter of debate whether we have promising agents to prevent the incidence of gentamicin-induced nephrotoxicity. The aim of the present study was to determine the possible therapeutic approach of cardamom to prevent gentamicin-induced nephrotoxicity in rats.

2. Materials and methods

2.1 Animals

Forty adult male Wistar albino rats (160-190 g) were obtained from the Animal House, Faculty of Veterinary Medicine, Benha University, Egypt. They were maintained on standard pellet diet and tap water ad libitum and were kept in plastic cages under a 12 hr light/dark cycle and room temperature 22-24°C. Rats were acclimatized to the environment for two week prior to experimental use. This investigation was approved by the Animal Research Ethics Committee.

2.2 Drugs and medicinal plant used

Gentamicin: (Garamycin® injection), is one of aminoglycoside antibiotics. It was obtained from Memphis Company for Pharmaceutical and Chemical Industries, Cairo, Egypt. It is dispensed in the form of ampoules; each containing 40 mg/ml. Rats were intra-peritoneally administered with gentamicin in a dose of 80 mg/kg to induce acute nephrotoxicity and hepatotoxicity [21].

Cardamom: (*Elettaria cardamomum*) fruits were purchased from the local market of Agricultural Herbs, Spices and Medicinal plants, Cairo, Egypt.

The dried plant materials were grinded into a fine powder and kept till the preparation of aqueous extracts.

2.3 Preparation of Aqueous Extract

Air-dried powder (10 g) of the respective plant seeds was mixed well in 100 ml sterilized distilled water and kept at room temperature for 24 h on an orbital shaker with 150 rpm. The solution was further filtered using muslin cloth. The filtrate was centrifuged at 5000 rpm for 15 min. The supernatant thus obtained was filtered through Whatman filter No. 1 under strict aseptic conditions and the filtrate was collected in a pre-weighed sterilized test tube. Aqueous extracts were prepared in final concentration of 100 mg/ml. Test tubes were cotton plugged and stored in refrigerator until further used [14].

2.4 Experimental design

The rats were randomly grouped into four groups (each of 10 rats). Group 1: Rats which served as the control was orally administered saline (the vehicle) daily for 8 consecutive days. Group 2: Rats in this group were given gentamicin (80 mg/kg. b.wt. i.p.) daily for 8 consecutive days. Group 3: Rats in this group were given gentamicin (80 mg/kg. b.wt. i.p.) daily for 8 consecutive days, then Cardamom extract (100 mg/kg. b.wt. p.o.) daily from 9th to 21th days. Group 4: Rats in this group were given gentamicin (80 mg/kg. b.wt. i.p.) daily for 8 consecutive days, then Cardamom extract (200 mg/kg. b.wt. p.o.) daily from 9th to 21th days. At the end of the 3 week, blood samples were taken from all rats which underwent laparotomy, and experimental parameters were measured.

2.5 Blood samples

Blood samples were collected 24 h after the end of the 21th day. Blood was collected by retro-orbital sinus puncture from each anesthetized rats with ether. The collected blood samples were allowed to clot and serum samples were obtained by centrifugation at 3000 rpm for 10 min, the serum was separated immediately and stored at -20°C until determination of biochemical parameters.

2.6 Biochemical assay

Serum was obtained for determination of chemical parameters: creatinine, urea, glucose and total protein. Creatinine concentration in serum was determined calorimetrically by the method described by [19] using kits from Diamond Diagnostic Company (Egypt). Urea concentration in serum was determined calorimetrically by the method described by [13] using kits from Diamond Company. Glucose concentration in serum was determined colorimetrically by the method described by [31] using kits from Diamond Diagnostic Company (Egypt). Total protein

concentration in serum was determined calorimetrically by the method described by [16] using kits from Diamond Diagnostic Company (Egypt).

2.7 Statistical analysis

The data were expressed as (mean \pm SEM) and analyzed using SPSS (16) software (SPSS Inc., Chicago, USA) and differences between the averages were examined by Duncan's multiple range test. Mean values within a row with different superscript letters are significantly different ($P \leq 0.05$) [6].

3. Results

The present study showed that the administration of gentamicin to rats once daily for 8 days reduces glomerular function. As reflected by increased serum creatinine concentrations as well as urea and glucose and decrease in total protein Table (1)

Effect of cardamom against gentamicin treatment on the previous parameters: serum urea, creatinine and glucose were decreased significantly, while serum total protein increased significantly after cardamom treatment following nephrotoxicity induced by gentamicin Table (1).

4. Discussion

Gentamicin enhanced the production of superoxide anion, hydrogen peroxide and hydroxyl radicals by mitochondria [37]. Free radicals cause Peroxidation of phospholipids membrane, DNA strand breakage, protein denaturation. Most significant biological damage of active metabolites is their reaction with unsaturated lipid and so their peroxidation. This effect induces changes in membrane fluidity, thus the membrane gets permeable even to molecules as large as enzymes [18]

This study evaluated kidney function by measuring serum creatinine and urea values. Gentamicin treatment is found to elevate creatinine and urea levels in serum, suggesting an impairment of kidney functions. These effects could also be attributed to the aminoglycoside induced nephrotoxicity is characterized by a decrease in the glomerular filtration rate and direct tubular injury. These observations are generally in agreement with other studies[24,26]. Creatinine and urea are waste products of protein metabolism that need to be excreted by the kidney, therefore a marked increase of these parameters, as observed in this study, confirms an indication of functional damage to the kidney[22]. Urea level can be increased by many other factors such as dehydration, antidiuretic drugs and diet, while creatinine is more specific to the kidney, since kidney damage is the only significant factor that increases the serum creatinine level[3]. However, administration of cardamom along with

gentamicin caused significant decrease in urea and creatinine suggested the protective effects of cardamom. This results agreed with [35] who reported a significant reduction in blood urea nitrogen and serum creatinine after beneficial effect of alcoholic seed extract of *A. cardamomum* in Alloxan induced diabetic rats due to improvement on glomerular function of kidney and maintained positive nitrogen balance [25]

Also there was a significant increase in levels of serum glucose levels in gentamicin treated group as compared to control group. However, administration of cardamom following gentamicin caused significant decrease in levels of serum of glucose as compared to gentamicin groups suggested the prophylactic roles of cardamom. The lowering effect of cardamom on glucose level was observed by [7], who studied the hypoglycemic effect of cardamom as one of spices that possess antioxidant compounds.

In the present study, there was a significant decrease in the concentration of serum total protein in gentamicin group as compared to control group, might be depressed as a result of defective protein synthesis. However, administration of cardamom along with gentamicin caused significant increase in total protein suggested the protective effects of cardamom. [35] reported an increase in total protein concentrations after oral administration of alcoholic seed extracts of *A. cardamomum* due to stimulation of number of m-RNA molecule the attachment with ribosome [23].

5. Conclusion

Oral administration of cardamom watery extract produces significant renal protective effect. Its antioxidant properties reduce kidney damage caused by gentamicin Treated.

Table (1) Nephroprotective effect of cardamom on some serum parameters in rats (n=10)

Parameters	Control	Gentamicin	Gentamicin + Cardamom (100 mg)	Gentamicin + Cardamom (200 mg)
Urea (mg/dl)	18.49±0.75 ^c	30.59±1.62 ^a	26.71±1.12 ^b	22.02±1.34 ^f
Creatinine (mg/dl)	1.02±0.05 ^c	2.21±0.12 ^a	1.73±0.08 ^b	1.48±0.07 ^f
Glucose (mg/dl)	142.81±4.18 ^d	212.03±5.17 ^a	179.18±5.23 ^b	159.83±5.08 ^e
Total protein (g/dl)	6.99±0.31 ^a	5.11±0.27 ^d	6.24±0.32 ^b	5.84±0.31 ^e

^{a, b, c, d} Mean values having different letters in the same row differ significantly (P<0.05).

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