Studies on effects of celery leaves on lipids profile and nephrotoxicity in rats induced by gentamicin

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ABSTRACT

The protective effects of celery leaves extract (CLE) to nephrotoxicity induced by gentamicin (GM) and lipids profile in rats were evaluated. Forty adult male rats were randomly distributed into 5 groups, 8 animals each Group. Group A control negative with no treatment, Group B was daily injected with GM (80 mg/kg, i.p.) during the last 8 days of the experiment to induce nephrotoxicity positive control (C+ve). Groups C, D and E were orally pretreated with celery leaves extracts in the doses at 200, 400 and 600 mg/kg respectively for 6 weeks and intoxicated with gentamicin in the last 8 days. Blood samples at last 24 hr of the experiment were collected for biochemical analyses. Rats were sacrificed and both kidneys were taken for estimating lipid peroxidation, antioxidant enzymes activity and histopathology. The results showed that oral pretreatments with celery leaves extracts in gentamicin intoxicated rats for 6 weeks induced significant (P< 0.05) decreases in serum urea nitrogen, uric acid and creatinine when compared with GM-intoxicated rats. Pretreatments with Oral intake of 200, 400 and 600 mg/kg b. wt., of celery leaves extract were significantly (P<0.05) reduced serum Total cholesterol levels by 12.86, 22.99 and 27.29 % respectively. On the other hand, triglycerides were significantly decreased by 18.35, 22.43 and 28.60 % respectively when compared to Group (B) positive control (C+ve). The extracts decreased tissue malondialdehyde (MDA) and increased activity of antioxidant enzymes. The nephroprotective mechanisms of both extracts could be attributed to inhibition of lipid peroxidation and enhancement of antioxidant enzymes activity. These results affirm the traditional use of these herbs in folk medicine for the prevention of kidney diseases.

Key words: Celery leaves extract, Lipids profile. Nephrotoxicity, Gentamicin

Introduction

Celery is a biennial plant, belongs to the Umbelliferae family. The celery plant cultivated in the Mediterranean region and its Arabic name is Karafs. It is slender and stands about two to three feet tall. It has 3 - 5 segmented leaves and flowers with small white petals. Celery extract was found to be effective in lowering cholesterol in hypercholesterolaemic rats, prevent tumor cell growth in various animal studies and help lower blood pressure and cholesterol, as well as protect the liver from damaging substances (Baek et al., 2003 and Sultana et al., 2005).

Celery (Apium Graveolense) is a medical herb used as a food and also in traditional medicine. Celery contains aromatic substances in the stem, leaves and roots. The healing properties of celery are due to its essential oil and flavonoids (Li et al., 2014). Among the copious effects of celery, its antifungal, antibacterial, antioxidant, antidiabetic (Kolarovic et al., 2010; Popovic et al., 2006), However, the effect of celery leaves extract on lipid profile and fructoseinduced hypertension has not been shown yet.

Celery is an edible vegetable, was firstly described by the Greeks and was popular in the Middle Ages for curing ailments. Celery boasts of a very pleasant and distinctive odor, the reason why it is used as an ingredient in stews, in salads, in soups, as mix in cocktail drinks, etc. Celery has been used in traditional medicine and aromatherapy due to its many health benefits (Jung et al., 2011). Celery is used as an effective remedy for various ailments such as lower blood pressure, bronchitis, liver and spleen disease, arthritic pain and this natural holistic approach to health is becoming more

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and more popular now a days. Celery stimulates healthy and normal functioning of kidney by helping in the elimination of the body toxins. It also prevents kidney stones (Kolarovic et al., 2010). Celery (Apiumgraveolens; family, Apiaceae) is used in Indian system of medicine owing to its richness in flavonoids and antioxidant property for the treatment for liver ailments. Studies have indicated that celery lowers blood pressure, regulates heart function and reduces complications of diabetes (Shivashri et al., 2013). The healing property of celery is due to its bioactive compounds like rutein, quercetin, luteolin, kaempferol, apigenin and myricetin. (Mimica-Dukic and Popovic, 2007). However, no reports have been found on the protective effect of A. graveolens on APAP-induced hepatotoxicity. Thus, we hypothesized that flavonoids rich in celery would prevent APAP-linked abnormalities.

Nephrotoxicity is a major complication characterized by functional alterations including protein losses, reduced glutathione depletion, lipid peroxidation and mitochondrial damage. Oxidative damage is thought to be one of the main mechanisms involved in nearly all chronic renal pathologies. Certain drugs may induce oxidative stress by forming drug-derived radicals that can not only deplete the antioxidant defenses but can also react directly with biomolecules (Abd El-Ghany et al., 2012). Gentamicin has been used clinically due to its wide spectrum of activities against Gram-negative bacterial infections. In experimental research, Gentamicin used for study of acute kidney failure as it generates free oxygen radicals, leading to tissue injury such as nephrotoxicity and otoxicity (Laurent et al., 1990). In the present study, we investigated the effects of celery leaves on lipids profile and nephrotoxicity in rats induced by gentamicin.

Material and Methods

Animals:

Forty male albino rats of Wistar strain weighed 180-200g each, were obtained from the experimental Animal Unit of King Fahd Medical Research Center, King Abdul-Aziz University, Jeddah, Saudi Arabia.

Gentamicin drug and Kits for biochemical analyses were purchased from The Gamma Trade Company for Pharmaceutical and Chemicals, in Riyadh.

Methods:

Preparation of celery leaves Extract: Two hundred grams of dried celery leaves powder were soaked in one liter of 90% ethyl alcohol and kept in a refrigerator with daily shaking for 5 days. This was followed by percolation for 5 times till complete exhaustion. The liquid ethanolic extracts were concentrated using vacuum rotatory evaporator at 50 C under reduced pressure. The administered doses of celery extract were 200, 400 and 600 mg/kg b.wt.

Preparation of the Basal Diet:

The basal diet for rats was prepared using AIN-93 according to Reeves et al. (1993). The basal diet consists of the following: Protein (Casein) 20%; Sucrose 10%; Corn Oil 4%; Choline Chloride 0.2%; Vitamin mixture 1%; Salt mixture 3.5%; Fibers (Cellulose) 5% and the remainder is Corn Starch up to 100%.

Experimental Design and Animal Groups: Forty rats were randomized into 5 equal groups each of 8 animals. Group A was injected intraperitoneally with sterile normal saline (0.2 ml) and keep as normal (negative) control. Group B was daily injected with GM in a dose of 10 mg/kg b.wt during the last 8 days of experiment to induce nephrotoxicity and kept as a nephrotoxic (positive) control. Groups C, D and E were orally pretreated with celery leaves extract in 200, 400 and 600 mg/kg for 6 weeks, respectively and intoxicated with GM. In the last 8th days of experiment, the rats were placed individually in metabolic cages for collection of 24 hr urine and the urine volume was measured using a graduated cylinder.

At the end of the experimental period, all rats were fasted overnight then sacrificed. Blood samples were immediately collected from the retro orbital plexus with capillary tubes under mild
ether anesthesia, into clean dried centrifuge tubes. The tubes were then centrifuged at 3000 rpm for 15 minutes. Clear serum samples were carefully separated using Pasteur pipettes, and frozen at -20°C until biochemical analysis (Margoni et al., 2011).

**Histological examination:**

Kidney specimens were taken and preserved in 10% neutral formalin solution. The fixed specimens were trimmed, dehydrated in ascending grades of alcohol, cleared in xylene. The specimens were embedded in paraffin boxes, sectioned at 4-6 microns thickness and stained with Hematoxylin and Eosin (H&E) and then examined microscopically (Carleton, 1976).

Serum concentrations of urea nitrogen was estimated by the method of Patton and Crouch (1977). Uric acid was estimated by the method of Fossati et al., (1980). Creatinine was determined by the method of Husdan and Rapoport (1969). Total protein was determined according to Sonnenwirth and Jaret, (1980) and Albumin was determined as described by Drupt (1974).

Total cholesterol (TC) was assessed by using enzymatic colorimetric kit as described by Allain et al. (1974). Enzymatic colorimetric GPO-PAP kit was used for measured triglycerides (TG) as described by Fossati and Prenape (1982). An enzymatic colorimetric kit was used for the determination of high density lipoprotein cholesterol (HDL-c) as described by Lopes-Virella et al. (1977).

Statistical analysis:

Statistical analysis was done by using (SPSS) Statistical Package for the Social Sciences for Windows, version 22 (SPSS Inc., Chicago, IL, USA). Collected data was presented as mean± standard error (SE). Analysis of Variance (ANOVA) test was used for determining the significances among different groups according to Armitage and Berry, (1987). All differences were considered significant if P < 0.05.

**Results and Discussion**

The effect of different concentrations from celery leaves extracts on serum urea nitrogen, uric acid and creatinine in gentamicin-nephrotoxic rats are presented in Fig (1) and Fig (2). The nephrotoxicity rats Group (B) positive control(C+ve) were increased in serum levels of urea nitrogen, uric acid and creatinine when compared with Group (A) control negative (C-ve). Oral intake of 200,400 and 600 mg/kg b. wt., of celery leaves extract in nephrotoxic rats were induced decreased in high serum levels of urea nitrogen, uric acid and creatinine when compared with Group (B) positive control(C+ve). Duke, (1997) recorded that the seeds and stalks of celery are known to reduce uric acid levels, relieving symptoms of joint pain and immobility. Celery is rich in B-Complex vitamins, adding to its stress reducing and sedative qualities. It is rich in vitamins A and C, and is indicated in arthritis and kidney problems.

The effect of different concentrations from celery leaves extracts on Total cholesterol and Triglyceride in gentamicin-injected rats are presented in Table (2) and Fig (4). The results revealed that the total cholesterol was significantly increased in nephrotoxic rats Group (B) positive control(C+ve) compared to Group (A) control negative (C-ve). Oral intake of 200,400 and 600 mg/kg b. wt., of celery leaves extract in nephrotoxic rats were significantly (P<0.05) reduced serum Total cholesterol levels by 12.86, 22.99 and 27.29 % respectively when compared to Group (B) positive control(C+ve). On the other hand, triglycerides was significantly decreased in Group (B) positive...
control(C+ve) compared to Group (A) control negative (C-ve). Oral intake of 200, 400 and 600 mg/kg b. wt., of celery leaves extract in gentamicin-nephrotoxic rats were significantly (P<0.05) reduced serum triglyceride levels by 18.35, 22.43 and 28.60 % respectively when compared to Group (B) positive control(C+ve).

**Fig 1:** The effect of different concentrations from celery leaves extracts on serum urea nitrogen in gentamicin-nephrotoxic rats.

**Fig 2:** The effect of different concentrations from celery leaves extracts on serum uric acid and creatinine in gentamicin-nephrotoxic rats.

**Table 2:** The effect of different concentrations from celery leaves extracts on lipid profile in gentamicin-injected rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total cholesterol mg/dL</th>
<th>Triglyceride mg/dL</th>
</tr>
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<tbody>
<tr>
<td>Group (A)</td>
<td>93.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.39&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group (B)</td>
<td>130.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.52&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group (C)</td>
<td>113.68&lt;sup&gt;c&lt;/sup&gt;</td>
<td>42.88&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group (D)</td>
<td>100.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40.74&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group (E)</td>
<td>94.87&lt;sup&gt;d&lt;/sup&gt;</td>
<td>37.50&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values with different superscripts within the column are significantly different at P< 0.05.

Group (A) control negative (C-ve), Group (B) positive control(C+ve), (C) celery extract 200 mg /kg.b.w., Group (D) celery extract 400 mg / kg.b.w., Group (E) celery extract 600 mg / kg.b.w.
Effect of different doses from celery leaves extract on the serum level of high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) in gentamicin-injected rats are illustrated in Table (3) and Fig (5). The nephrotoxic rats Group (B) positive control(C+ve) had a significant (P< 0.05) decrease in HDL-c serum when compared with Group (A) control negative (C-ve) by 36.70 %. Oral intake of 200, 400 and 600 mg/kg b. wt., of celery leaves extract to nephrotoxic rats Group (B) positive control(C+ve) were significantly (P<0.05) increases serum HDL-c level by 9.34, 27.25 and 39.44 % respectively when compared to nephrotoxic rats Group (B) positive control(C+ve).

Concerning serum levels of very low density lipoprotein cholesterol (VLDL-c), the results revealed that Group (B) positive control(C+ve) had a significant (P<0.05) decreased in serum level of VLDL-c when compared to Group (A) control negative (C-ve) by 35.10 %.

Oral intake of 200, 400 and 600 mg/kg b. wt., of celery leaves extract to nephrotoxic rats were significantly (P<0.05) decrease in serum levels of VLDL-c by 17.41, 27.63 and 30.75 % respectively, when compared to the Group (B) positive control(C+ve). Nephrotoxic rats Group (B) positive control(C+ve) had a significant (P<0.05) increased in serum level of low density lipoprotein cholesterol (LDL-c) when compared to Group (A) control negative (C-ve) by 150.45 %. Oral intake of celery leaves extract at three dosage levels significantly (P<0.05) decreased serum levels of LDL-c when compared to Group (B) positive control(C+ve). The decreases in serum levels of LDL-c in rats given celery leaves extract at doses 200, 400 and 600 mg/kg b. wt., were 31.82, 42.67 and 55.63 % respectively.

Table 3: The effect of different concentrations from celery leaves extracts on cholesterol profile in gentamicin-injected rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>LDL-c mg/dL</th>
<th>VLDL-c mg/dL</th>
<th>HDL-c mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (A)</td>
<td>35.44^a</td>
<td>14.28^a</td>
<td>45.72^a</td>
</tr>
<tr>
<td>Group (B)</td>
<td>88.76^a</td>
<td>10.57^a</td>
<td>28.95^a</td>
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<tr>
<td>Group (C)</td>
<td>60.52^b</td>
<td>8.73^c</td>
<td>31.68^d</td>
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<tr>
<td>Group (D)</td>
<td>50.89^c</td>
<td>7.65^d</td>
<td>36.84^e</td>
</tr>
<tr>
<td>Group (E)</td>
<td>39.38^d</td>
<td>7.32^d</td>
<td>40.37^b</td>
</tr>
</tbody>
</table>

Values with different superscripts within the column are significantly different at P< 0.05.
Group (A) control negative (C-ve), Group (B) positive control(C+ve), (C) celery extract 200 mg /kg.b.w., Group (D) celery extract 400 mg /kg.b.w., Group (E) celery extract 600 mg /kg.b.w.
Effect of extracts of celery leaves extract on kidney contents of reduced glutathione (GSH) and malondialdehyde (MDA) in gentamicin-nephrotoxic rats are presented in Fig (6) and Fig (7). Data showed that the significant (P<0.05) decrease in reduced glutathione (GSH) content and increase in malondialdehyde (MDA) in kidney tissues when compared with Group (A) control negative (C-ve). Oral intake of 200, 400 and 600 mg/kg b. wt., of celery leaves extract to nephrotoxic rats were significantly (P<0.05) increased GSH and decreased MDA contents in renal tissues as compared to the Group (B) positive control (C+ve). These results agreement with Nabila et al., (2015) and Elkhamisy (2015). MDA a degradation product from lipid peroxide, provides an index of the peroxidation of lipids in biological tissue. It was observed by Kuhad et al. (2007) that an increased production of MDA measured as TBARS in the kidney under the effect of nephrotoxicity. Also, it was obviously that the antioxidant activity of the antioxidant compound mainly depend on the phenolic OH-group, although a small fraction may be due to the >CH2 site.
Effect of celery leaves extracts on the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) antioxidant enzymes in kidney tissues of gentamicin-nephrotoxic rats are illustrated in Fig (8) and Fig (9). Nephrotoxic rats Group (B) positive control (C+ve) had a decrease in activities of renal superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) enzymes compared with Group (A) control negative (C-ve). Oral intake of 200, 400 and 600 mg/kg b. wt., of celery leaves extract to nephrotoxic rats were increased the activities of SOD, GPx and CAT enzymes as compared to the Group (B) positive control (C+ve). Cao et al., (2012) recorded that the activities of superoxide dismutase, glutathione peroxidase, catalase in the dichlorvos induced oxidative stress were declined significantly and MDA was significantly increased when compared with the flavonoid extracts group and flavonoid extracts from celery. The observations showed the protective role of flavonoids of celery in minimizing the oxidative stress induced by dichlorvos in rats. It may be concluded that celery has antioxidant effects which can be used as natural antioxidant in food industries and also in nutritional planning for patients suffering from renal disease.

**Fig 7:** The effect of different concentrations from celery leaves extracts on kidney contents of malondialdehyde (MDA) in gentamicin-nephrotoxic rats.

**Fig 8:** The effect of different concentrations from celery leaves extracts on the activity of superoxide dismutase (SOD) in kidney tissues of gentamicin-nephrotoxic rats.
Fig. 9: The effect of different concentrations from celery leaves extracts on the activity of glutathione peroxidase (GPx) and catalase (CAT) enzymes in kidney tissues of gentamicin-nephrotic rats.

**Histopathological examination of kidneys:**

Histological examination of kidneys of normal rats showed normal histological structure of renal glomeruli and tubules. Kidney of rat from group A control negative (C-ve) showing the normal histological structure of renal parenchyma Photo (1). Kidneys of rats intoxicated with GM (10 mg/kg every 24 hr. for eight days to induce nephrotoxicity, group B positive control (C+ve) photo (2). Examination of kidneys of nephrotoxic rats pre-treated with 200 mg/kg.b.w. Celery leaves extract showed mild necrosis in renal tubules with protein casts in their lumen photo (3). In nephrotoxic rats given the dose (400 mg/kg) of celery leaves extract, the examinations showed only mild congestion of inter tubular blood vessels photo (4). Kidneys of nephrotoxic rats pretreated orally with the (600 mg/kg) of celery leaves extract showed almost normal histological architecture of renal glomeruli and tubules photo (5).

**Photo 1:** Kidney of rat from group A control negative (C-ve) showing the normal histological structure of renal parenchyma (H & E X 400).
Photo 2: Kidney of rat from group B positive control (C+ve). showing congestion of renal blood vessel and focal mononuclear interstitial inflammatory cells infiltration (H & E X 400).

Photo 3: Kidney of rat from group C (treated with 200 mg /Kg.b.w. celery leaves extract) showing mild necrosis in renal tubules with protein casts in their lumen (H & E X 400).

Photo 4: Kidney of rat from group D (treated with 400 mg /Kg.b.w. celery leaves extract) showed only mild congestion of inter tubular blood vessels (H & E X 400)
Photo 5: Kidney of rat from group E (treated with 200 mg/kg.b.w. celery leaves extract) showed almost normal histological architecture of renal glomeruli and tubules (H & E X 400).

References


