Protective Effect of *Apium graveolens* Against Cisplatin Induced Nephrotoxicity in Male Mice.

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التأثير الوقائي لنبات الكرفس ضد التأثير السمي لعقار السيسبلاتين (Cisplatin) على الكلية في ذكور الفئران.

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الخلاصة

المقدمة: إن السيسبلاتين (Cisplatin) عقار كثير الاستعمال وفعّال جداً في علاج السرطان . تعتبر سمية الكلية واحده من أهم الآثار الجانبية المُحدّدةِ لاستعمال السيسبلاتين، إن التأثير السمي للسيسبلاتين على الكلية يُرتَبطُ مباشرةً بزيادةِ أكسدة الدهون (lipid peroxidation) في الكليةِ . حيث وجد إن السيسبلاتين يقلل من نشاطات إلانزيماتِ المضادة للأكسدة كما انه يسبب نضوب الكلوتاثايون (GSH). لقد أجريت العديد مِنْ الدِراساتِ لمعرفة فاعلية بَعْض النباتاتِ كمضادّ لسمية بَعْض الأدوية بضمنها أدوية السّرطانِ . إن هذه الدراسةِ صُمّمتْ لمعرفة فاعلية نبات الكرفس (*Apium graveolens*) كمضادّ لسمية السيسبلاتين على الكلية في ذكور الفئران

المواد وطرائق العمل : لقد تم استخدام ٢٨ ذكرا من الفئران البالغة لإجراء هذه الدراسة والتي قسمت عشوائيا إلى أربعة مجاميع حيث تم حقن المجموعة الأولى داخل البريتون ب ٠,٤٥مل من الماء المقطر أما المجموعة الثانية فقد تم حقنها داخل البريتون ب ٠,٤٥ مل (٧,٥ ملغم\كغم) من عقار السيسبلاتين كجرعة مفردة،.إما المجموعة الثالثة فقد تم إضافة أوراق وسيقان نبات الكرفس إلى طعامها يوميا بجرعة ٧,٥ غم\كغم لمدة ٧ أيام قبل وبعد حقنها داخل البريتون بجرعة مفردة (٧,٥ ملغم\كغم) من عقار السيسبلاتين، فيما تم أضافت أوراق وسيقان الكرفس بجرعة (١٥ غم\كغم) يوميا إلى طعام المجموعة الرابعة لمدة ١٤ يوما قبل وبعد حقنها داخل البريتون بجرعة مفردة (٧,٥ ملغم\كغم) من عقار السيسبلاتين. تم سحب الدم عند اليوم ١٥من حقن الماء المقطر في المجموعة الأولى و الأيام ٨ ، ٨ و ١٥ من حقن السيسبلاتين في المجاميع الثانية ، الثالثة والرابعة بالترتيب حيث تم قياس مستوى اليوريا والكرياتنين في مصل الدم . كما تم تقطيع كلى الفئران بعد قتلها وتحضير المقاطع النسيجية وصبغها بصبغة الهيماتوكسلين والايوسين حيث فحصت تحت المجهر الضوئي للكشف عن أي تغيرات نسيجية.

النتائج :لقد بينت نتائج هذه الدراسة حصول انخفاض معنوي في مستوى اليوريا والكرياتنين في مصل الدم للمجموعة الرابعة مقارنة بالمجموعة ا لثانية في حين لم يكن هناك فرق معنوي بين المجموعتين الثالثة والثانية . أما الفحص النسيجي فقد أوضح وجود تهديم واسع الانتشار في النبيبات الكلوية في

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المجموعتين الثانية والثالثة بينما كان التهديم بؤري مع وجود نبيبات كلوية سليمة في المناطق المجاورة في ٥ حالات فقط من المجموعة الرابعة.

الاستنتاجات: لقد استنتج من هذه الدراسة إن نبات الكرفس المعطى بجرعة ١٥ غم /كغم لمدة ١٤ يوم قبل وبعد حقن السيسبلاتين كان لها دور في حماية الكلية من التأثير السمي لعقار السيسبلاتين.

مفتاح الكلمات: السيسبلاتين، الكرفس، سمية الكلية، الفئران.

Summary:

Background: Cisplatin (CIS) is a widely used and highly effective cancer chemotherapeutic agent. Nephrotoxicity is a major complication and dose limiting factor for CIS therapy. The CIS- induced nephrotoxicity is closely associated with an increase in lipid peroxidation in the kidney. It has been found that CIS lower antioxidant enzymes activities, also it induced depletion of GSH. Many studies were conducted to investigate the activity of some plants against the toxicity of some drugs including anticancer agents. This study was designed to investigate the activity of *Apium graveolens* (*A. graveolens*) against CIS-induced nephrotoxicity.

Materials and methods: Twenty eight albino mice were randomly divided in to 4 groups . Group 1(negative control) injected intraperitoneally (i.p.) with 0.45 ml distill water (D.W). Group 2 (positive control) was injected with CIS (7.5 mg/kg) only. Group 3 received 7.5 g/kg *A. graveolens* with their food daily for 7 days before and after CIS (7.5 mg/kg) injection (at day 8), whereas group 4 received 15 g/kg *A. graveolens* with their food daily for 14 days before and after CIS (7.5 mg/kg) injection (at day 15). Blood samples were collected at day 15 of D.W injection from group 1 and at days 8, 8 and 15 of CIS injection from groups 2, 3 and 4 respectively. Serum urea and creatinine were measured, after that the animals were sacrificed and their kidneys were sectioned and stained with haematoxiline and eosin stain and examined under light microscope to detect the histopathological changes.

Results: Levels of serum urea and creatinine were significantly decreased in group 4 in comparison to group 2 (86.43 vs. 134.29 and 1.29 vs. 3.04 respectively), whereas no significant changes were observed in group 3 in comparison to group 2 (126.29 vs. 134.29 and 2.78 vs. 3.04 respectively). The examination of the histopathological sections of kidneys showed diffuse renal tubular necrosis in 7 cases (100%) in group 3 and 7 cases (100%) in group 2, whereas focal tubular necrosis with surrounding normal tubules were found in 5 (71.43%) cases and diffuse tubular necrosis in 2 (25.57%) cases in group 4.

Conclusion: The dose of 15 g/kg *A. graveolens* given 2 weeks before and after CIS injection can provide a protection against CIS-induced nephrotoxicity.

Key words: cisplatin, *Apium graveolens*, nephrotoxicity, mice.

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Introduction

Drug induced kidney disease is recognized as a main cause of mortality and morbidity (Dolin and Himmelfarb, 2008). Several common mechanisms have been proposed for Drug-induced nephrotoxicity, including altered intra-glomerular hemodynamics, tubular cell toxicity, inflammation (i.e., glomerulonephritis, acute and chronic interstitial nephritis), crystal nephropathy, rhabdomyolysis and microangiopathy. In addition, patient-specific or drug-related factors may predispose certain patients to drug-induced kidney injury (Cynthia, 2008; Dolin and Himmelfarb, 2008).

Cisplatin (CIS)

Cisplatin or cis-diamminedichloroplatinum (II) (CDDP) (Yonezawa *et al.*, 2006) is highly effective chemotherapeutic agent that has been widely used in the treatment of solid tumors such as testicular, ovarian and breast cancers and is also widely employed for treating bladder, cervical, head and neck, oesophageal and small cell lung cancer . (Giaccone, 2000; Dolin and Himmelfarb, 2008). Despite its success, nephrotoxicity is a major and dose limiting side effect of CIS, with an incidence reported as 6-13% (Dolin and Himmelfarb, 2008).

Mechanism of action of cisplatin

Numerous laboratory studies have provided considerable information on the molecular mechanisms of antitumor action of CIS (Fuertes *et al*., 2003). It has emerged from these studies, that the biochemical mechanisms of CIS cytotoxicity involve the binding of the drug to DNA and non- DNA targets and further induction of cell death through apoptosis, necrosis or both within the heterogeneous population of cells that forms a tumoral mass (Cvitkovic, 1998).

It is generally accepted that binding of *cis*-DDP to genomic DNA (gDNA) in the cell nucleus is the main event responsible for its antitumor properties (González *et al*., 2001) Thus, the damage induced upon binding of CIS to gDNA may inhibit transcription, and/or DNA replication mechanisms. Subsequently, these alterations in DNA processing would trigger cytotoxic processes that lead to cancer cell death. Although gDNA is generally accepted as the critical target of CIS mediated apoptosis, there are evidences that other cellular components that have nucleophilic sites may also be involved in the cytotoxicity of the CIS. These actions of CIS involve (1) Binding to mitochondrial DNA causing the release of mitochondrial cytochrome-c and caspase-3 activation and leading to apoptosis.(2) Interacting with phospholipid and phosphatidylserine in cell membranes.(3) Disrupting the cytoskeleton microfilaments. (4) Affecting the polymerization of actin (Gonzalez *et al.,* 2001).

Cisplatin adverse effects:

Cisplatin has several disadvantages, which include severe side effects (Giaccone, 2000): (1) The major limiting toxicity is dose-related nephrotoxicity.(2) Neurotoxicity characterized by paresthesia and loss of proprioception. (3) Ototoxicity with hearing loss and tinnitus. (4) Nausea and vomiting. (5) Mild bone marrow suppression. (6) Earlier studies also reported cardiotoxicity with CIS treatment (Florea and Büsselberg, 2011). (7) Hypersensitivity reactions ranging from skin rashes to anaphylaxis (Finkel *et al*., 2009).

Cisplatin induced nephrotoxicity:

Cisplatin-induced nephrotoxicity is mainly mediated through drug transport into renal epithelial cells, which subsequently causes injury to nuclear and mitochondrial DNA, activation of cell apoptosis and necrosis, and stimulation of inflammatory responses (Ronald *et al.,* 2010; Pabla and Dong, 2008; Fumie *et al*., 2000).

Renal side effects of CIS include acute kidney injury, Fanconi-like syndrome, distal renal tubular acidosis, hypomagnesemia, hypocalcemia, renal salt wasting, renal concentrating defect, hyperuricemia, transient proteinuria, erythropoietin deficiency, thrombotic microangiopathy and chronic renal failure (Ronald *et al.,* 2010). CIS

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induced production of reactive oxygen species (ROS) has also been implicated in its nephrotoxicity (Christopher *et al.*, 2001).

Cisplatin induced nephrotoxicity and antioxidant defense mechanisms

It has been hypothesized that the oxidative stress mechanism of CIS induced nephrotoxicity is attributed to its ability to cause significant decrease in the levels of the antioxidants (Glutathione reductase) GSH, bilirubin, albumin, vitamin A, C and E and the ratio of vitamin E/cholesterol + triglycerides that can results in a depletion of the antioxidant defense system (Weijl *et al.*, 1998 ; Silva *et al.*, 2001).

The increase in free radical generation and the decrease in antioxidant defense system may result in an increase in renal lipid peroxidation and malondialdehyde (MDA) production in renal tissue. (Greggi *et al*., 2001; Silva *et al*., 2001 ; Mora *et al*., 2003). It has been suggested that nitric oxide (NO) could play a role in CIS-induced nephrotoxicity. A previous report showed that several anti-tumor drugs stimulate NO production (Linds *et al*., 1997). It has been reported that CIS treatment results in a significant increase in the activity of calcium-independent nitric oxide synthase in kidney and liver leading to enhanced NO formation. (Srivastava *et al*., 1996). NO is known to react with the superoxide radical, forming more potent oxidizing agent ,peroxynitrite (Ischiropoulos *et al*., 1992) which can react directly with sulfhydryl residues in the cell membrane leading to lipid peroxidation or with DNA resulting in cytotoxicity (Radi *et al*., 1991).

The disturbance of the balance between the production of ROS including oxygen free radical and non-radical oxygen and antioxidant defenses against them produces oxidative stress which then amplifies tissue damage.

Free radicals initiate autocatalytic reactions, whereby molecules with which they react are themselves converted into free radicals, thus propagating the chain of damage. Proteins, lipids, carbohydrates, and nucleic acids are major targets of free radical damage. ROS are produced normally in cells during mitochondrial respiration and energy generation, but they are degraded and removed by cellular defense systems. Thus, cells are able to maintain a steady state in which free radicals may be present transiently at low concentrations but do not cause damage. When the production of ROS increases or the scavenging systems are ineffective, the result is an excess of these free radicals, leading to a oxidative stress (Gutteridge, 1995; Kumer *et al.*,2010).

Medicinal plants are increasingly being used as herbs in most part of the world today (Abolaji *et al.*, 2007), and the interests on antioxidants from natural sources that are contained in vegetables and fruits, continuously increases (Wolski and Dyduch, 2000). Flavonoids have attracted the interest of researchers because they show promise of being powerful anti-oxidants that can protect the body from free radicals and against oxidative stress (Bors *et al.*, 1996). Flavonoids cannot be produced by the human body and are taken in through the daily diet. The evidence reported that flavornoids play a vital biological role, including the function of scavenging ROS (Pietta and Simonetti, 1998). On the other hand, it has been shown that phenolics from edible fruits and vegetables are also effective antioxidants (Karadeniz *et al.*, 2005). The antioxidative properties of phenolics arose from their high reactivity as hydrogen or electron donors and from the ability of polyphenol-derived radicals to stabilize and

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delocalize the unpaired electron or from their ability to chelate transition metal ions (Rice-Evans *et al*., 1997).

Apium graveolens (*A. graveolens*) is classified as a member of the *Apiaceae* family and commonly known as Celery (Rizzo *et al*., 2011). It is an annual or biennial herb that can be found throughout Europe, the Mediterranean, and Asia. *A. graveolens* has been cultivated for the last 3.000 years and has been used as food and as medicine (Mimica-Dukic and Popovic, 2007; Baananou *et al*., 2012). *A. graveolens* has many beneficial health effects, ranging from cardioprotective to anticancer properties (Guerrero, 2005; Sultana *et al*., 2005). Some of these benefits are attributed to the potent antioxidant effects of flavonoids which are found in different parts of *A. graveolens* including the leaves, stems, roots, flowers and seeds and are among the most popular anti-cancer candidates worldwide (Heim *et al*., 2002; Amin and Buratovich, 2007; Gates *et al*., 2007). Phytochemical investigations of celery seeds revealed the presence of terpenes like limonene, flavonoids like apigenin and phthalide glycosides. Apigenin is an antioxidant that was documented as one of the major celery's active principals in *A. graveolens* (Miean and Mohammed, 2001).

Aim of the study

This study was designed to investigate the activity of *A. graveolens* against CIS-induced nephrotoxicity

Materials and Methods

Animals

Twenty eight mature male mice have been used in the experiment. This study was conducted from April to July 2013 in the animal house of medical collage at Babylon university, Iraq. Before beginning of the experiment animals were acclimatized for two weeks. Room temperature was maintained at 23± 2°C, the light-dark cycle was on a 12:12 h throughout the experimental period. Animals were fed on the standard chow and drinking water *ad libitum* throughout the experiment.

Experimental design

Male mice were randomly divided into 4 equal groups (7 mice in each) as follows: Group (1): (negative control), injected intraperitoneally (i.p.) with 0.45ml of distill water (D.W). After 14 days of D.W injection blood samples were withdrawn, then the animal were sacrificed after being anesthetized with diethyl ether.

Group (2): CIS treated (positive control), injected i.p. with 0.45 ml of CIS (7.5 mg/kg) as a single dose. After 7 days of CIS injection blood samples were withdrawn, then the animal were sacrificed.

Group (3): received 7.5g/kg fresh leaves and stalks of *A. graveolens* with food daily for 7 days. At day 8, they were injected i.p. with 0.45 ml of CIS (7.5 mg/kg) as a single dose. The fresh *A. graveolens* (15g/kg) was given to the animal for another 7 days, then blood samples were withdrawn, after that the animal were sacrificed.

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Group (4): received 15g/kg fresh leaves and stalks of *A. graveolens* with food daily for 14 days. At day 15, they were injected i.p. with 4.5 ml of CIS (7.5 mg/kg) as a single dose. The fresh *A. graveolens* (15g/kg) was given to the animal for another 14 days, then blood samples were withdrawn, after that the animal were sacrificed.

Note: another 7 mice were injected i.p. with 0.45 ml of CIS (7.5 mg/kg) as a single dose CIS, we wanted them to serve as a positive control group that should be sacrificed after 14 days of CIS injection, but they all died between day 8-10 after CIS injection.

Preparation of blood samples:

The blood was aspirated through intracardiac puncture at day 8 of D.W injection in group 1 and at day 8 of CIS injection in group 2 and 3, and at day 15 from the mice in group 4. The blood was collected in disposable plastic syringes and immediately transferred into plastic test tubes without anticoagulant and then centrifuged for 15-20 minutes at 2000 rpm and the serum was used to estimate the levels of blood creatinine and urea.

Measurement of serum creatinine:

In an alkaline media, creatinine reacts with picrate to form a coloured (yellow-orange) complex which absorbs light at 510 nm. The rate of color formation is proportional to the creatinine concentration in the sample (Henry, 1974). Kit used is spinreact sa kit.

Measurement of serum urea:

According to the modified urease-Berthelot method, the salicylate and hypochlorite in the reagent react with the ammonium ions to form a coloured (green) complex, that can measured by spectrophotometer at 580 nm to determine the level of urea in the serum (Fawcett and Scott, 1960). Kit used is biomerieux sa kit.

Preparation of histopathological slides:

The kidneys of mice were sectioned and stained with haematoxiline and eosin (H&E) stain and examined under light microscope to detect the histopathological changes.

Statistical analysis

The data expressed as mean ± SD, SPSS version 17.0 was used for the statistical analysis, ANOVA test was used is this study for the measured creatinine and urea, while chi-square test was used for histopathological changes . P- values less than 0.05, 0.01 and 0.001 were considered as statistically significant, high significant and extremely significant respectively (Daniel, 1999).

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Results

1. Biochemical-serum investigation

In group 2 (received a single dose of 7.5 mg/kg CIS only) the serum levels of both creatinine and urea were high significantly increased, as compared to group 1 (3.04±0.47 vs. 0.69±0.12 and 134.29 vs. 47.94±3.96 respectively). In group 4 (received 15g/kg *A. graveolens* for 14 days before and 14 days after CIS injection), the *A. graveolens* treatment resulted in a extreme significant reduction ($p< 0.001$) in serum levels of both creatinine and urea, as compared to group 2 (1.29 vs. 3.04 and 86.43±6.18 vs. 134.29 respectively), while in the group 3 (received 7.5g/kg *A. graveolens* for 7 days before and 7 days after CIS injection), the *A. graveolens* treatment showed no significant changes in serum levels of both creatinine and urea as compared to group 2 (2.78±0.38 vs. 3.04±0.47 and 126.29±5.37 vs. 134.29±11.7 respectively) and to group 4 (2.78±0.38 vs. 1.29±0.26 and 126.29±5.37 vs. 86.43±6.18 respectively) as shown in (figure 1, 2).

2. Histopathological investigation (Table 1)

1- Histopathological examination of sections taken from kidneys of mice in group 1 (control group) showed normal renal proximal tubules in 7(25%) (Figure 3).

2- Histopathological examination of sections taken from kidneys of mice that received single dose of CIS (7.5mg/kg) showed diffuse renal tubular necrosis and inflammatory cells infiltrate in 7 (100%) cases of this group (Figure 4).

3-. Histopathological examination of sections which were taken from kidneys of mice received 7.5g/kg *A. graveolens* for 7 days showed diffuse renal tubular necrosis and inflammatory cells infiltrate 7 (100%) similar to the second group (Figure 4).

4- Histopathological examination of sections taken from kidneys of mice received 15g/kg *A. graveolens* for 14 days showed focal area of renal tubular necrosis and inflammatory cells infiltrate in 5 (71.43%) (Figure 5) and diffuse renal tubular necrosis with inflammatory cells infiltration in 2 (25.75) cases (Figure 4).

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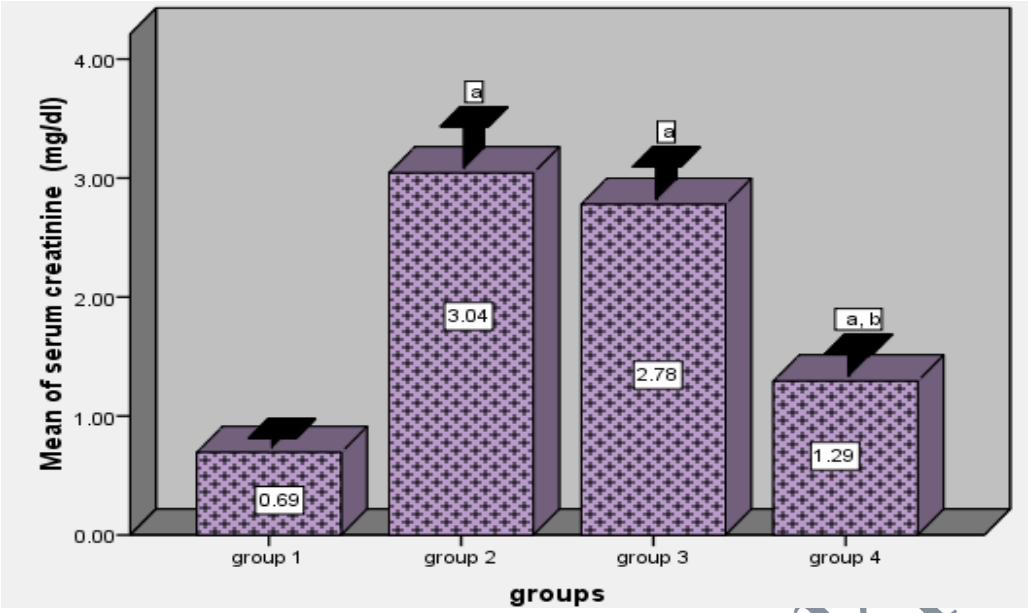

Figure (1) Effects of *A. graveolens* treatment on the serum creatinine in the kidney of mice exposed to CIS nephrotoxicity. Values are expressed as mean ±S.E. (a= P< 0.001 vs. control group.b= P< 0.001 vs. CIS group).

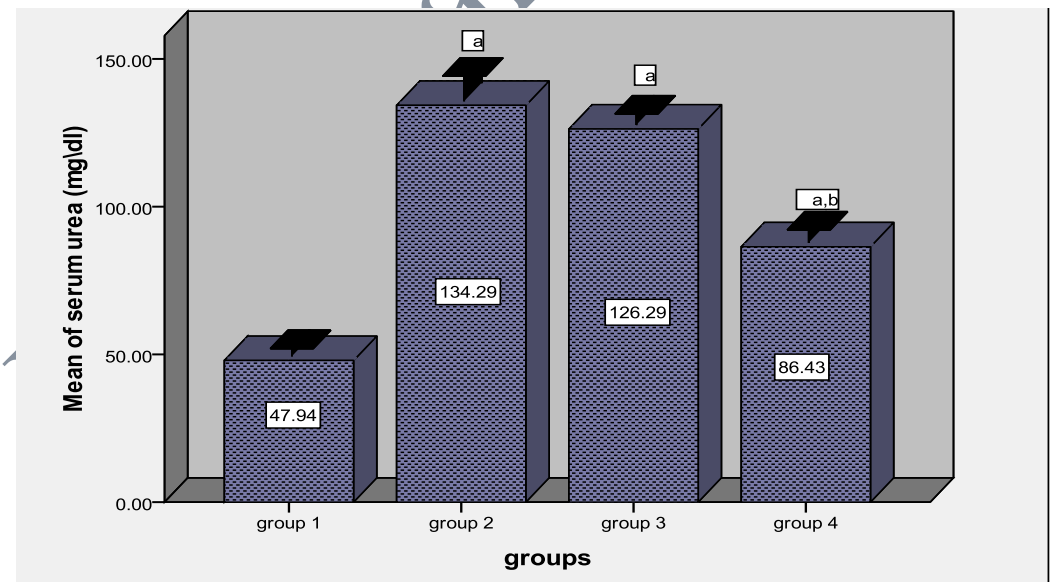

Figure (2) Effects of *A. graveolens* treatment on the serum urea in the kidney of mice exposed to acute CIS nephrotoxicity. Values are expressed as mean ±S.E. n= 7 in each group. (a= P< 0.001 vs. control group. b= P< 0.001 vs. CIS group).

Table 1: Shows the different histopathological changes in each experimental group.

Experimental groups	Normal Renal histology	Focal tubular necrosis + inflammation	Diffuse tubular necrosis + inflammation	Total
- ve control	7 (100%)	/	/	7 (25%)
+ve control	/	/	7 (100%)[a]	7 (25%)
CIS+ *A. graveolens* 7 days (before and after CIS)	/	/	7 (100%)[a]	7(25%)
CIS+ *A. graveolens* 14 days (before and after CIS)	/	5 (71.43%)	2 (25.57%)[a, b]	7 (25%)
Total	7(25%)	5(17.85%)	16(57.14%)	28(100%)

(a= P< 0.01 vs. control group. b= P< 0.01 vs. CIS group).

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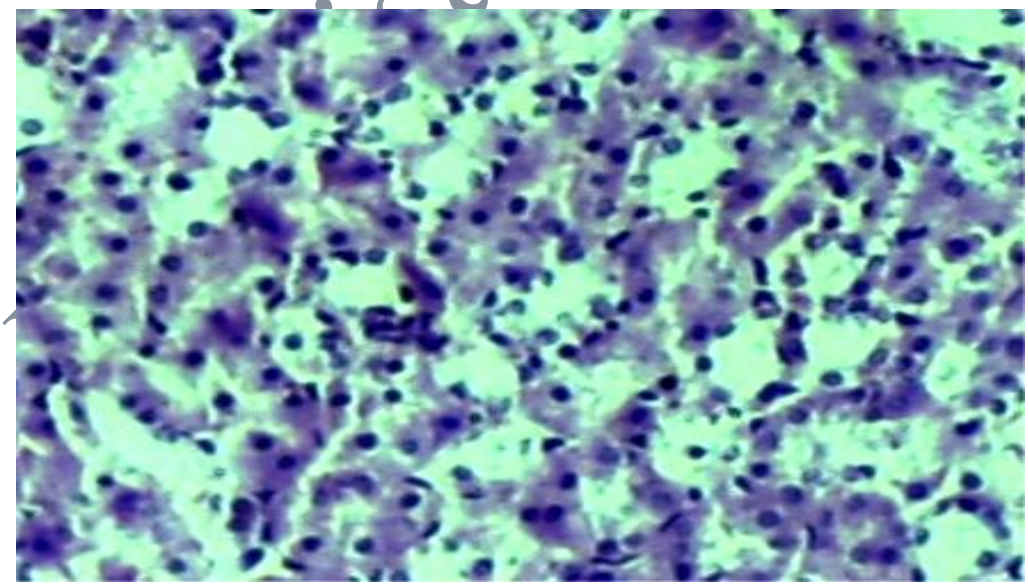

Figure 3: Show normal renal proximal tubule H&E stain. (magnification x100).

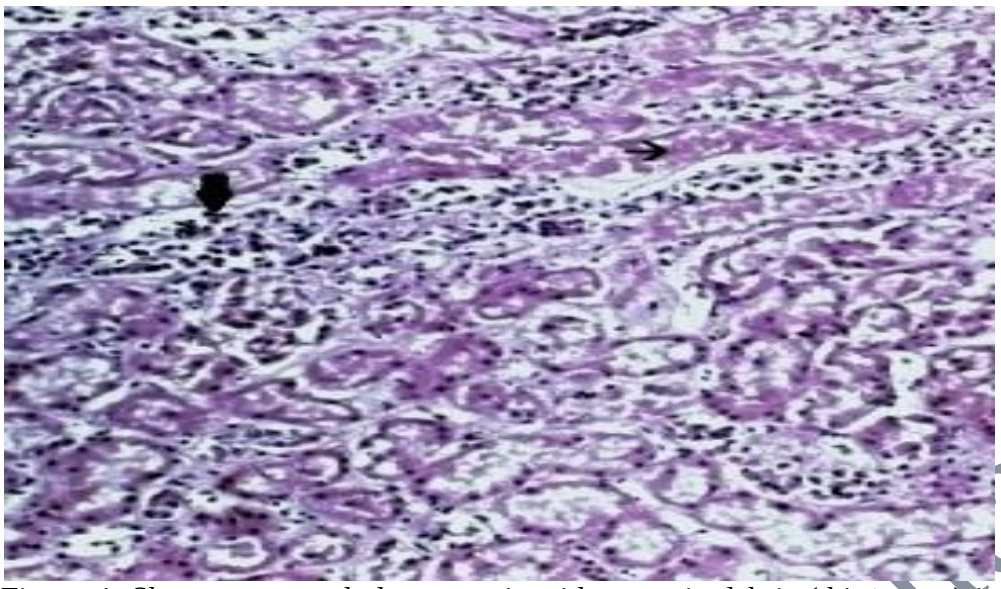

Figure 4: Shows severe tubular necrosis with necrotic debris (thin arrow) inside and inflammatory cells infiltration (bold arrow) H&E stain. (magnification x100).

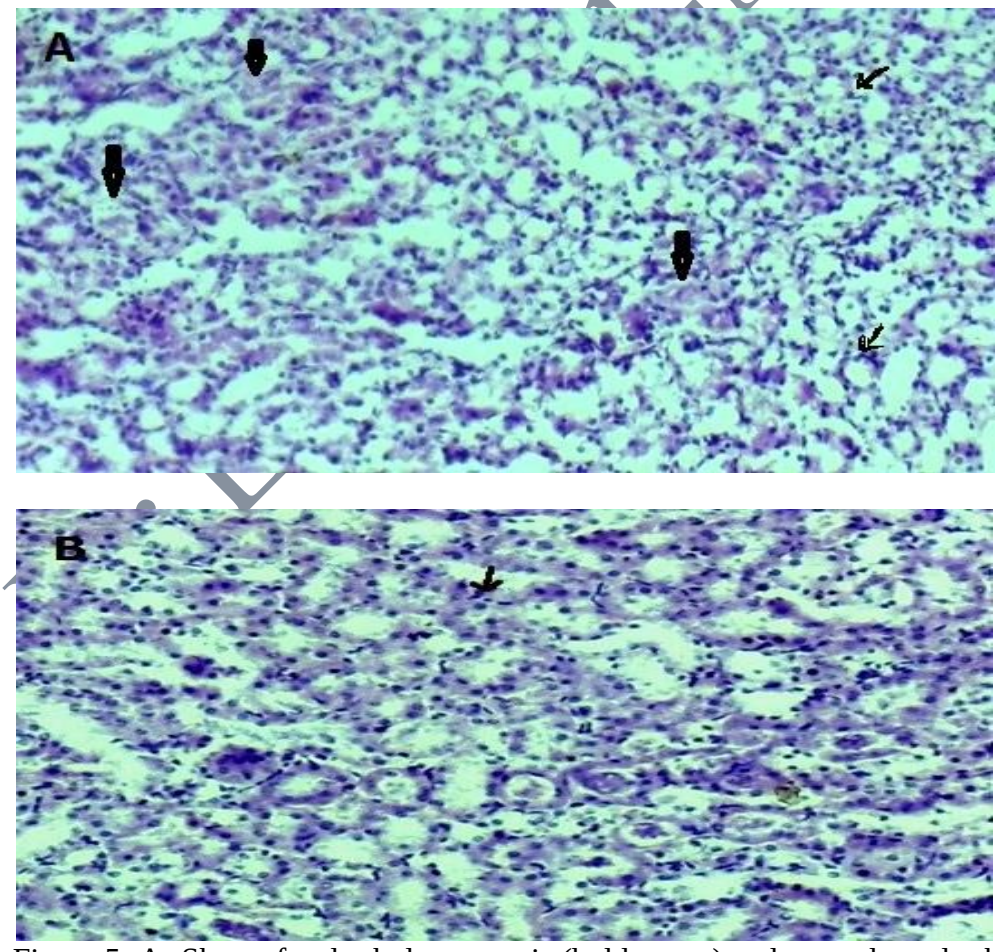

Figure 5: A- Shows focal tubular necrosis (bold arrow) and normal renal tubules (thin arrow) with inflammatory cells infiltration. B- Other field show normal tubules (thin arrow) H&E stain (magnification x100).

Discussion

Nephrotoxicity is a major complication and dose limiting factor for the use of CIS therapy (Kuhad, 2007). Lipid peroxidation and free radical generation due to oxidative stress cause renal tubular cell necrosis (Maliakel *et al*., 2008). It has been reported that CIS induced nephrotoxicity is closely associated with an increase in lipid peroxidation in the kidney (Joy and Nair, 2008). CIS rapidly reacts with proteins in the renal tubules resulting in nephrotoxicity (Montine and Borch, 1990) that can occurs to early within 1 hour after CIS administration (Rao and Rao, 1992). This renal damage characterized by destruction of intracellular organelles, cellular necrosis and lipid peroxidation (Kuhlmann *et al*., 1998). In addition, CIS has been found to lower the activities of antioxidant enzymes and to induce depletion of GSH (Joy and Nair, 2008).

Celery has antioxidant effect (Popovic *et al*., 2006), it contains a large amount of vitamin C which is antioxidant to prevent the free radical damage that triggers the inflammatory cascade. Thus, it helps reduce the severity of inflammatory conditions (Sultana *et al*., 2005). It has been found that the depletion of antioxidant such as glutathione reductase (GSH), vitamin A, C and E (Weijl *et al*., 1998 ; Silva *et al*., 2001), or inhibition of antioxidant enzymes activities such as catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) (Yildirim *et al*., 2003) are the main mechanisms for CIS induced nephrotoxicity. In the present study, the high significant increase in serum creatinine and urea levels in CIS only treated group as compared to D.W treated group is compatible with those observed by many others (Gamal el-Din and Al-Bekairi 2006; Joy and Nair 2008; Kim *et al*., 2010).

In the present study the significant reduction in the serum levels of both urea and creatinine after treatment with 15g/kg *A. graveolens* for 14 days before and after CIS injection as approved by histopathological examination (which showed that renal tubular necrosis were in focal area with surrounding normal tubular structure) as compared to that found in CIS only treated group (in whom the renal tubular necrosis were widely spread with no residual normal tubules), this result could be attributed to the antioxidant effect of *A. graveolens* as it represent a good source for some constituents with antioxidant activity such as luteolin which is naturally occurring flavonoid that can scavenge a wide range of ROS (Richard *et al*., 2002) and reactive nitrogen species and chelate transition metal ions, often decreasing the pro-oxidant activity of metal ions.(Shirai *et al*., 2006).

Also *A. graveolens* contains alpha-tocopherol (Richard *et al*., 2002) and glucosides (Ching and Mohammed, 2001) which also have antioxidant effect (Momin and Nair, 2002). It has been found that consumption of roots and leaves juices of *A. graveolens* resulted in a significant elevation in GSH content (Kolarovic *et al*., 2009). It has been found that platinum sulphydryl group complexes are taken up by renal cells and stabilized by intracellular GSH for several hours. GSH depletion in turn results in lipid peroxidation and this seems to be the prime factor that permits lipid peroxidation and impaired antioxidant enzyme activities (Sheena *et al*., 2003). Thus *A. graveolens* may antagonized the CIS-induced GSH depletion.

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The insignificant reduction in the serum levels of both creatinine and urea after treatment with 7.5g/kg *A. graveolens* for 7 days before and after CIS injection as approved by histopathological examination (renal tubular necrosis was widely spread with no residual normal tubules) as compared to that found in CIS only treated group, may be related to the low dose of *A. graveolens* and /or short duration of its intake that could not provide a good protection against CIS induced nephrotoxicity. Also it may be related to the CIS dose (7.5 mg/kg) used in this study which is high as several studies have shown that administration of 5 mg/kg CIS elevated MDA level by 29-33% 24 hours after treatment (Silva *et al*., 2001 ; Mora *et al*., 2003) and by 51% 3 days after treatment (Greggi *et al*., 2001). Also Yildirim *et al*. (2003) observed a decrease in the activities of catalase (CAT), glutathione peroxidase (GSH-Px) and super oxide dismutase (SOD) in the rat kidney 5 days after 7 mg/kg CIS injection (Yildirim *et al*., 2003).

Up to knowledge this is the first study deals with the protective effect of *A. graveolens* against CIS-induced nephrotoxicity, thus we could not compare results found by this study with other studies.

Conclusions

According to the results of the present study, we can conclude that *A. graveolens* at the dose 15 g/kg given for 2 weeks before and after CIS administration, provided good protection against CIS-induced nephrotoxocity, whereas this protection did not produced by the dose 7.5 g/kg given for 1 weeks before and after CIS administration.

Recommendation

Further studies are needed with the use of higher doses of *A. graveolens* and/or for longer duration of administration. Also clinical studies are necessary as *A. graveolens* is cheap, safe and available all over the year.

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