Protective action of *Cuminum cyminum* against cisplatin induced nephrotoxicity in rats

Zamzam Elmahdi Burkan,* Balasubramanian Rajkapoor,* Shivalinge Gowda KP,**
Ashok Kumar Gupta,**

Abstract:
Objective: The nephroprotective activity of aqueous extract of *Cuminum cyminum* (AEC) was evaluated on cisplatin (CP) induced nephrotoxicity in rats.
Methods: The rats were divided into four groups of six animals each. Group I treated with normal saline (2 ml/kg, p.o.). The group II treated with cisplatin (12 mg/kg i.p), group III and IV were treated with AEC (100 & 200 m/kg, p.o.). for 10 days, After 24 hrs of last dose of AEC treatment, blood was collected and serum was separated and subjected to serum creatinine and urea analysis. The kidneys were removed and homogenate for estimation of lipid peroxidation, reduced glutathione, superoxide dismutase (SOD) and catalase.
Results: CP treated rats caused nephrotoxicity as evidenced by marked elevation of serum urea, creatinine and lipid peroxidation levels and decreased levels of glutathione, SOD, catalase. Treatment of rats with different doses of AEC (100 and 200 mg/kg) significantly (P<0.001) altered serum urea, creatinine enzymes, lipid peroxidation and antioxidant levels to near normal against cisplatin treated rats. The results shown AEC produce nephroprotective effect on dose dependent manner.
Conclusion: The *Cuminum cyminum* showed the protective effect against CP induced nephrotoxicity in rats.
Keywords: Cisplatin, Nephrotoxicity, Antioxidants, *Cuminum cyminum*

Introduction:
Cisplatin [(cis-diamminedichloroplatinum (II); (CP)], one of the most potent and widely used anticancer drugs containing platinum, is highly effective against many tumors, including testicular, lung, head, neck and bladder carcinomas[1]. CP crosses the plasma membrane and migrates to the nucleus, where it elicits changes in DNA structure by forming intra and inter-chain adducts. However, the clinical use of CP is limited by its adverse effects, of which renal toxicity is the most serious dose-limiting factor. Signs of injury, such as changes in urine volume, osmolality and reduction of the glomerular filtration rate characterize the kidney toxicity in a large number (28–36%) of patients undergoing CP treatment[2].
Till date even though there is tremendous advance in the field of medicine there is no satisfactory treatment or precaution for this toxicity. It’s documented that 80% of the world’s population has faith in traditional medicine particularly herbal drugs for their primary health care[3]. The *Cuminum cyminum* (species: Apiaceae synonyms Cumin, Jira, Jirige, Jirakam, Jiraka, Jila karra) is commonly used species in the daily which is reported for its antioxidant[4], astringent, digestive, carminative, anthelmentic, diuretic[5], antimicrobial[6], anticarcinogenic[7], anticonvulsant[8], hypolipidemic[9], antidiabetic and hepatoprotective [10] etc. It consists of carvone, cuminol, cumin aldehyde, mixture of hydrocarbons, cymene or cymol, terpene, small quantities of α-pinine, β-pinene, phellandrene, cuminic alcohol, hydrated cuminaldehyde hydrocumin[7] and glucosides[11]. In the present study was focussed to evaluate the protective action of *Cuminum cyminum* on cisplatin induced nephrotoxicity in rats.

Materials and methods:
Chemicals
Nitroblue tetrazolium (NBT), riboflavin, reduced glutathione (GSH), 5-5' dithiobis 2-nitro benzoic acid (DTNB) were obtained from Sisco Research Laboratories Pvt Ltd, Mumbai, India and Cisplatin from Samarth Pharma Pvt Ltd, Mumbai, India. Thiobarbituric acid (TBA) was purchased from Hi-media Laboratories, Mumbai, India. All other chemicals and reagents used in this study were of analytical grade.

*) Department of Pharmacology, Faculty of Medicine, Sebha University, Sebha, Libya.
**) Department of Pharmacology, Sree Siddaganga College of Pharmacy, Tumkur, India.

Plant material:
The dried seeds of *Cuminum cyminum* were collected and it was identified and authenticated by Dr. Siddappa, Botanist, A voucher specimen has been deposited at the department of pharmacology, Sree Siddaganga College of Pharmacy, Tumkur, India.

Extraction and preparation of test drug
The seeds were chopped finely using a grinder. The seeds powder was extracted using Soxhlet apparatus for 72 hours. The extract was evaporated under vacuum. The extract was stored in airtight container. The required quantity of extract was dissolved in distilled water and used for the study.

Animals
Male Wistar rat weighing 150-200 g were used for the experiment. The animals were housed in group (six animals in each cage) and fed with standard diet and water *ad libitum.* Animals were kept in central animal house of Sree Siddaganga College of Pharmacy-Tumkur, with maintenance of room temperature (25 ± 2 ^{o}C) and light: dark exposure of 12:12 h. The experiments were carried out after obtaining prior approval from Institutional Animal Ethical Committee (IAEC) approval no. (SSCPT/IAEC/61/2008-09).

Experimental design: The animals were divided into four groups containing 6 rats each. All animals had free access to regular rat chow and drinking water *ad libitum.*
Group I : Controls received the vehicle viz. normal saline (2 ml/kg).
Group II : Received cisplatin (12 mg/kg; i.p) at every 72 h for 10 days.
GroupIII : Received AEC (100 mg/kg[12] p.o) for 10 days and simultaneously administered cisplatin (12 mg/kg; i.p). e(very 72 h.
GroupIV : Received AEC (200 mg/kg p.o) for 10 days and simultaneously administered cisplatin (12 mg/kg; i.p). every 72 h.

At the end of experimental period, all the animals were sacrificed by cervical decapitation. Blood samples were collected, allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for serum urea and serum creatinine levels. A 10% of kidney homogenate was used for antioxidant studies such as lipid peroxidation (LPO)[13] (Devasagayam and Tarachand, 1987), superoxide dismutase (SOD)[14] (Marklund and Marklund, 1974), catalase[15] (Sinha, 1971) and reduced glutathione[16] were estimated.
Serum Creatinine is determined by alkaline picric acid method using a diagnostic kit (Agappe Diagnostic Pvt Ltd, India). Serum urea was determined by diacetylmonoxime (DAM) reagent (modified Berthelot methodology) using a diagnostic kit (Agappe Diagnostic Pvt Ltd, India).

Estimation of lipid peroxidation[13]
The malondialdehyde (MDA) content, a measure of lipidperoxidation, was assayed by TBARS method. The reaction mixture consists of 0.2 ml of 8.1% w/v sodium lauryl sulphate, 1.5 ml of 20% v/v acetic acid solution adjusted to pH 3.5 with NaOH and 1.5 ml of 0.8% w/v aqueous solution of thiobarbituric acid was added to 0.2 ml of 10% w/v of PMS. The mixture was brought up to 4.0 ml with distilled water and heated at 95°C for 60 minutes. After cooling with tap water, 1.0 ml distilled water and 5.0 ml of the mixture of n-butanol & pyridine (15:1 v/v) was added and centrifuged. The organic layer was taken out and its absorbance was measured at 532 nm. TBARS were quantified using an extinction coefficient of 1.56 × 105 M-1/cm-1 and expressed as nmol of TBARS per mg protein. Tissue protein was estimated using Biuret method of protein assay and the renal MDA content expressed as nM of malondialdehyde per mg of protein.

Estimation of superoxide desmutase (SOD)[14]
The assay system consists of EDTA 0.1 mM, sodium carbonate 50 mM and 96 mM of nitro blue tetrazolium (NBT). In the cuvette, 2 ml of above mixture, 0.05 ml hydroxylamine and 0.05 ml of PMS were taken and the auto-oxidation of hydroxylamine was observed by measuring the absorbance at 560 nm.

Estimation of catalase[15]
The assay mixture consists of 1.95 ml phosphate buffer (0.05 M, pH 7.0), 1.0 ml hydrogen peroxide (0.019 M) and 0.05 ml PMS (10%) in a final volume of 3.0 ml. Changes in absorbance were recorded in spectrophotometer (LAB INDIA) at 240 nm. The molar extinction coefficient of H_2O_2, 43.6 M cm-1 was used for the catalase activity estimation. One unit of activity is equal to 1mM of H_2O_2 degraded /min and is expressed as units/mg of protein.

Estimation of reduced glutathione[16]

For the glutathione estimation 1.0 ml of PMS (10%) was precipitated with 1.0 ml of sulphosalicylic acid (4%). The samples were kept at 4°C for at least 1 hour and then subjected to centrifugation at 1200 g for 15 minutes at 4°C. The assay mixture contained 0.1 ml filtered aliquot and 2.7 ml phosphate buffer (0.1 M, pH 7.4) in a total volume of 3.0 ml. The yellow colour developed was read immediately at 412 nm on a spectrophotometer. The amount of glutathione was determined using molar extinction coefficient 1.36x104 and was expressed in µg/mg protein.

Statistical analysis:

The values were expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison test. P values < 0.05 were considered as significant.

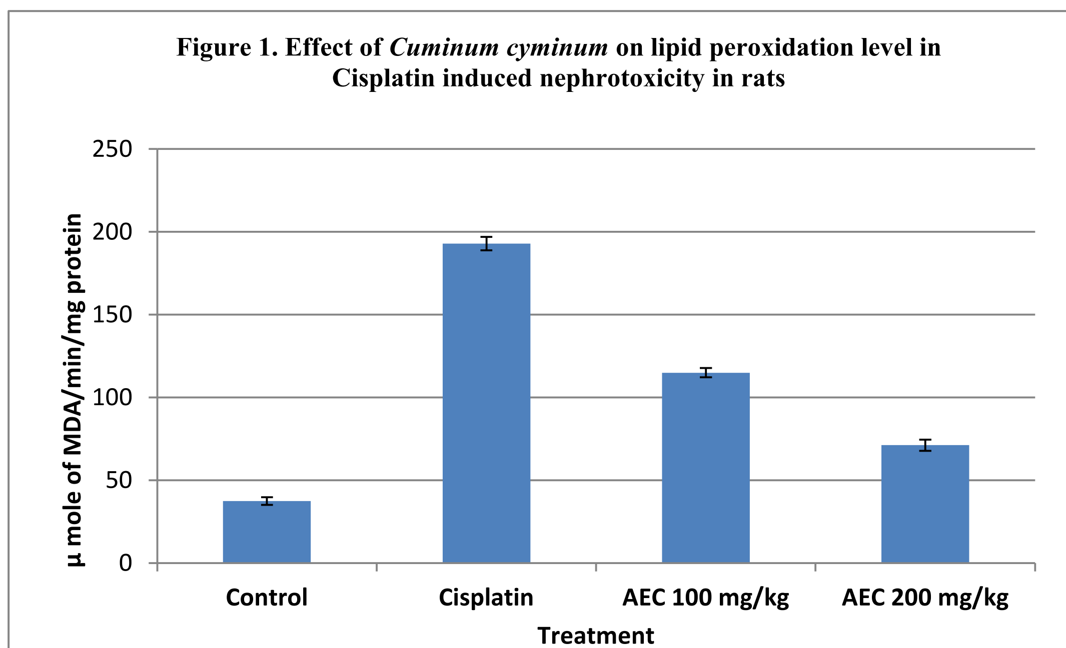
Results:

The data presented in [Table 1] reveals that the values of serum urea and creatinine were significantly (P<0.001) elevated in the cisplatin treated group. Cisplatin treatment resulted in a two-fold increase in the values of serum urea and creatinine levels as compared to that of the untreated control group. The administration of AEC to cisplatin treated rat could restore the elevated levels of urea and creatinine to that of the untreated control levels.

The major antioxidant enzymes such as GSH, SOD and Catalase were found to be decreased significantly (P<0.001) in cisplatin treated animals and per oral administration of AEC could elevate these levels on dose dependent manner, as can be realized from the data presented in [Table 1].

From [Figure 1] it can be seen cisplatin treatment resulted in increased peroxidation of lipids in the kidney tissues of rat and administration of AEC resulted in inhibition of cisplatin-induced peroxidation of lipids in these tissues. The protection against cisplatin induced toxicity could seeds from the potent antioxidant activity of AEC. Inhibition of LPO in biomembranes has been caused by antioxidants present in the plant extract. From the above results, it can be inferred that the AEC offers significant (P<0.001) protection against cisplatin induced oxidative stress in renal tissues.

Figure 1. Effect of *Cuminum cyminum* on lipid peroxidation level in Cisplatin induced nephrotoxicity in rats



N=6 animals in each group; Values are expressed as mean ± SEM.
[a]P<0.001 Vs Control
[b]P<0.001 Vs Cisplatin
Data were analyzed by One way ANOVA followed by Tukey multiple comparison test.

Table 1: The effect of AEC on serum creatinine, urea, SOD, catalase and GSH on CP induced nephrotoxicity.

Treatment	Serum creatinine mg/dl	Serum urea mg/dl	SOD	Catalase	GSH
Control	0.90 ± 0.03	44.89 ± 2.02	37.25 ± 2.73	30.37 ± 2.15	1.61 ± 0.09
Cisplatin	1.23 ± 0.07[a]	117.2 ± 4.59[a]	20.26 ± 1.57[a]	16.69 ± 1.37[a]	0.67 ± 0.05[a]
AEC 100 mg/kg	1.14 ± 0.02[b]	91.11 ± 3.28[a,e]	29.33 ± 1.98[c,f]	24.24 ± 1.91	0.72 ± 0.047[a]
AEC 200 mg/kg	1.01 ± 0.05[f]	65.58 ± 2.93[c,d]	33.99 ± 1.12[d]	28.36 ± 2.51[e]	1.48 ± 0.03[d]

N=6 animals in each group; Values are expressed as mean ± SEM.
[a]P<0.001; [b]P<0.01; [c]P<0.05 Vs Control
[d]P<0.001; [e]P<0.01; [f]P<0.05 Vs Cisplatin
Data were analyzed by One way ANOVA followed by Tukey multiple comparison test.
SOD - Unit/min/mg protein
Catalase - mole of H_2O_2 consumed/min/mg protein
GSH - µg/mg protein

Discussion:

Nephrotoxicity is one of the major side effects of cisplatin. Although several studies have been performed to elucidate the molecular mechanisms that cause cisplatin nephrotoxicity, the factors responsible for this are not fully understood. Recently, induction of oxidative free radicals has been implicated in this process[17]. Different strategies have been proposed to inhibit cisplatin induced toxicity. The development of therapies designed to prevent the damaging actions of free radicals may influence the progression of oxidative renal damage induced by cisplatin.

The major antioxidant enzymes such as GSH, SOD and Catalase were found to be decreased in cisplatin treated animals and per oral administration of AEC could elevate these levels [Table 1]. Reactive oxygen species (ROS) such as hydrogen peroxide, the superoxide anion, and hydroxyl radicals are generated under normal cellular conditions and are immediately detoxified by endogenous antioxidants, like GSH, catalase and superoxide dismutase, but excessive ROS accumulation by cisplatin causes an antioxidant status imbalance and leads to lipid peroxidation and GSH depletion[18].

The basic effect of cisplatin induced toxicity on the cellular membrane is believed to be peroxidation of membrane lipids. The depletion of glutathione at early intervals in treated animals may be due to its utilization in large amounts to combat the acute cisplatin induced free radical damage, as glutathione is a major nonenzymatic antioxidant. The measurement of lipid peroxidation as thiobarbituric acid reacting substances (TBARS) is a convenient method to monitor oxidative damage in tissues. Reactive oxygen species cause peroxidation of membrane lipids with devastating effect on functional states. The preservation of cellular membrane integrity depends on protection or repair mechanisms capable of neutralizing oxidative reactions. Our data show that cisplatin induced malondialdehyde (MDA) production was significantly decreased by the p.o. administration of AEC in vivo and it also attenuated cisplatin induced GSH depletion in rat. It has been suggested that cisplatin is able to generate ROS and that it inhibits the activities of antioxidant enzymes in renal tissues[19]. In the present study the reduced activities of GSH, SOD and Catalase in kidneys of rat treated with cisplatin were restored by administration of AEC to a considerable extent indicating the ability of AEC to eliminate oxidative stress.

Cisplatin has been thought to bind to the renal base transport system. Cisplatin induces hypomagnesemia through its renal toxicity possibly by a direct injury to mechanisms of magnesium reabsorption in the ascending limb of the loop of Henle as well as the distal tubule. Cisplatin preferentially accumulates in cells of the S3 segment of the renal proximal tubules and is toxified to form a reactive metabolite intracellularly by hydration. The primary symptoms of cisplatin nephrotoxicity are inhibition of protein synthesis and

intracellular GSH and protein-SH depletion, resulting in lipid peroxidation and mitochondrial damage[18]. The peroxidation of membrane lipids may account for its nephrotoxicity[20]. Available evidence suggests that cisplatin exerts its nephrotoxic effects by the generation of free radicals[21-23]. GSH and protein-SH form the major cellular anti-oxidant defense systems, which control lipid peroxidation. From these pathomechanisms of cisplatin nephrotoxicity, it is clear that the nephrotoxicity of cisplatin involves reactive radicals. Thus the reasonable cellular-protective agents against cisplatin toxicity may have at least some antioxidant properties to prevent GSH depletion and/or scavenge the intracellular reactive oxygen species.

The present observations support the hypothesis that the mechanism of nephrotoxicity is related to the depletion of the antioxidant defense system. Cisplatin treatment has been shown to induce loss of copper and zinc in the kidneys. The decrease in SOD activity in renal tissues following cisplatin administration might be due to the loss of copper and zinc[24]. The activity of Catalase and GSH is also found to decrease after cisplatin administration resulting in the decreased ability of the kidney to scavenge toxic hydrogen peroxide and lipid peroxides. The results from the present study indicate that the extract (AEC) significantly reduced the depletion of GSH levels and antioxidant enzyme activity in the renal cortex of rat treated with cisplatin.

Numerous studies have shown that cisplatin induces renal damage by free radical generation. Hence antioxidants and free radical scavengers of natural and synthetic origin might provide nephroprotection in cisplatin induced renal injury[25].

The present study demonstrates the potent antioxidant properties of the extract. Hence, it may be concluded that the mechanism of nephroprotection by *Cuminum cyminum* extract in cisplatin treated rat could be due to the antioxidant and free radical scavenging activity.

References:

1. Al-Majed AA, Abd-Allah ARA, Al-Rikabi AC, Al-Shabanah OA, Mostafa AM. Effect of oral administration of Arabic gum on cisplatin induced nephrotoxicity in rats. *J Biochem Mol Toxicol.* 2003; 17: 146-53.
2. Sayed AAR. Proanthocyanidin protects against cisplatin induced nephrotoxicity. *Phytotherapy Res.* 2009; 23: 1738–41.
3. Dubey NK, Kumar R, Tripathi P. Global promotion of herbal medicine: India’s opportunity. *Current Sci.* 2004; 86: 37-41.
4. Lado CM, Then, Varga IES, Szentminalyi K. Antioxidant Property of Volatile Oils Determined by the Ferric Reducing Ability. *Z. Naturforsch* 2004; 59c, 354-358.
5. Indian medicinal plants, Vol. II. A compendium of 500 species. Orient Longman, Chennai.2005; p.241.
6. Iacobellis NS, Cantore PL, Capasso F, Senatore F. Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. *J. Agri. Food Chem.* 2005; 53: 57-61.
7. Aruna K, Sivaramakrishnan VM. Anticarcinogenic effects of some Indian plant products. *Food Chem. Toxicol.* 1992; 30: 953-956.
8. Sayyah M, Mahboubi A, Kamalinejad M. Anticonvulsant effect of the fruit essential oil of *Cuminum cyminum* in rat. *Pharm. Biol.* 2002; 40: 478-480.
9. Dhandapani S, Subramanian VR, Rajagopal S, Namasivayam N. Hypolipidemic effect of *Cuminum cyminum* L. on alloxan induced diabetic rats. *Pharmacol Res.* 2002; 46: 251-255.
10. Willatgamuwa SA, Patel K, Saraswathi G, Srinivasan K. Antidiabetic influence of dietary cumin seeds in streptozotocin induced diabetic rats. *Nutrition Res.* 1998; 18: 131-142.
11. Gachkar L, Yadegari D, Rezaei MB, Taghizadeh M, Astaneh SA, Rasooli I. Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils. *Food Chem.* 2007; 102: 898-904.
12. Hosseinzadeh H, Mohammad R, Basirat FM. Anticonvulsant effects of *Cuminium cyminum* L.seeds extracts and essential oil in micet. *J . Med. Plants.,* 2002;1, 28-30.
13. Devasagayam, T.P.A., Tarachand, U., 1987. Decreased lipid peroxidation in the rat kidney during gestation. Biochemical and Biophysical Research Communication 56, 836 – 842.
14. Marklund, S., Marklund, G., 1974. Involvement of superoxide anion radical in

the auto oxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry 47, 469-474.

15. Sinha, A.K., 1972. Colorimetric assay of catalase. Analytical Biochemistry 47, 389-394.
16. Griffith OW. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal. Biochem*. 1980; 106: 207–12.
17. Baldew GS, McVie JG, van der Valk MA, Los G, de Goeij JJ, Vermeulen NP. Selective reduction of cis-diammine-dichloroplatinum(II) nephrotoxicity by ebselen. Cancer Res 1990; 50: 7031-7036
18. Kim YH, Kim YW, Oh YJ, Back NI, Chung SA, Chung HG. Protective effect of the ethanolic extract of the roots of Brassica rapa on cisplatin induce nephrotoxicity in LLC-PK1 cells and rats. Biol Pharm Bull 2006; 29: 2436-2441.
19. Naziroglu M, Karaogl A, Aksoy AO. Selenium and high dose vitamin E administration protects cisplatin induced oxidative damage to renal, liver and lens tissues in rats. Toxicology 2004; 195: 221-320
20. Safirstein R, Miller P, Guttenplan JB. Uptake and metabolism of cisplatin by rat kidney. Kidney Int 1984; 25: 753-758.
21. Trakshel GM, Maines MD. Characterization of glutathione S-transferases in rat kidney: Alteration of composition by cis-platinum. Biochem J 1988; 252: 127-136
22. Ishikawa M, Takayanagi Y, Sasaki K. Enhancement of cisplatin toxicity by buthionine sulfoximine, a glutathione depleting agent in rat. Res Commun Chem Pathol Pharmacol 1990; 67: 131-141
23. Uslu R, Bonavida B. Involvment of the mitochondrion respiratory chain in the synergy achieved by treatment of human ovarian carcinoma cell lines with both tumor necrosis factor-alpha and cis-diamminedichloroplatinum. Cancer 1996; 77: 725-732
24. Sharma RP. Interactions of cisplatin with cellular zinc and copper in liver and kidney tissues. Pharmacol Res Commun 1985; 17: 197-206
25. Maliakel DM, Kagiya TV, Nair CK. Prevention of cisplatin-induced nephrotoxicity by glucosides of ascorbic acid and alpha-tocopherol. Exp Toxicol Pathol 2008; 60: 521-527.