Copper (I)-Nicotinate Complex may have a protective effect against Cisplatin Toxicity on kidney in Albino Rats

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Abstract:

Cisplatin (CP) is one of the most widely used and most effective cytotoxic drugs in the treatment of different epithelial tumors but it has several side effects. Oxidative stress has been proven to be involved in CP-induced toxicity and Glutathione (GSH) depletion is an important mechanism of CP toxicity.

This experiment was carried out to study the possible protective effect of Copper (I)-nicotinate complex (CNC), the strong antioxidant as it has superoxide dismutase-mimetic activity against the side effects of CP on experimental rat models. For this purpose, forty male albino rats were divided into four groups: Control-ve group, CNC group, CP group and CP&CNC group. At the end of experiment, the whole rats were sacrificed and serum samples for kidney function and kidney tissue samples for light and transmission electron microscopies.

The results showed that, Cisplatin induced adverse effects on blood picture, kidney function and renal tissue in CP group, but CP & CNC group showed a marked decrease of these changes observed in CP group.

In conclusion, CNC seems to have a protective role against CP induced toxicities.

Introduction:

Cisplatin is one of the most effective chemotherapeutic agents which has been used in treatment of many tumors including testicular cancer, metastatic ovarian cancer, head & neck cancers, bladder cancer, cervical cancer, osteosarcoma, metastatic melanoma, penile cancer and pancreatic cancer [1]. Unfortunately, it has several side effects as myelosuppression, nephrotoxicity, ototoxicity, neurotoxicity and bone marrow suppression, but its chief dose limiting side effect is cumulative nephrotoxicity [2]. Glutathione depletion is an important mechanism of CP toxicity. This is due to the intracellular binding to sulfhydryl (SH) groups leading to lipid peroxidation (LPO) and mitochondrial damage so, new measures have been tried in clinical studies to prevent GSH depletion and scavenge the intracellular free oxygen radicals [3]. Copper (I) nicotinate complex, the possible protective compound in the present experiment was firstly synthesized by Gohar and Dratovsky, (1975) and known to be a strong antioxidant. It is one of copper complexes which have the ability to scavenge the oxygen-free radicals either by enhancing the synthesis of superoxide dismutase (SOD) or by SOD-mimetic activity. Actually, this complex has amazing properties that have been

confirmed by numerous previous studies such as the ability to treat gastric ulceration in rats [4]. Also, it regulated the levels of Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (GGT) in addition to nitric oxide (NO) and lipid peroxidation in rats [5]. Accordingly, this study was carried out to evaluate the efficacy of this complex to reduce the side effects induced by Cisplatin in male albino rats.

Materials and Methods:

Animals:

Forty male Sprague-Dawley albino rats of 175-200g were obtained from VACSERA, Helwan, Egypt. Rats were housed in cages (five per cage) and kept under the standard laboratory conditions (room temperature and normal light/dark cycle) in the laboratory of Pathology and Clinical Pathology Department, Faculty of Veterinary Medicine, Assiut University. They were acclimatized to the laboratory conditions for two weeks before the experiment and they had ad libitum access to commercial pellets and fresh drinking tap water throughout the study.

Chemicals and kits:

Cisplatin was obtained from Mylan. Copper (I)-nicotinate complex was prepared by Dr. Ahmed Yassin Ahmed Nassar, Professor of Biochemistry, Department of Biochemistry, Faculty of Medicine, Assiut University. Creatinine kit was purchased from Spectrum Diagnostics, Egypt and urea kit from Biodiagnostic Co. (Giza, Egypt).

Experimental design

After the two weeks of adaptation, rats were divided into four groups, ten rats for each group. G1, the Control -ve group was injected intraperitonially with normal saline, G2, CNC group was injected intraperitonially with CNC (400µg/kg B.W.) [6] three times weekly for six weeks, G3, CP group was injected intraperitonially with CP (2mg/kg B.W.) once a week for five weeks [7] and G4, CP&CNC group was injected intraperitonially with CP (2mg/kg B.W.) once a week for five weeks & CNC (400µg/kg B.W.) three times weekly for six weeks. All experimental protocols held on animals were done according to regulations set by the Institutional Animal Care and approved by Assiut University.

Sampling:

At the end of the experiment, the rats were sacrificed and samples were collected from each animal. Blood samples were collected from the medial canthus of the eye before the sacrificing into sterilized blood tubes containing anticoagulant “EDTA” for blood picture analysis. For measurement of kidney function parameters, other blood samples were collected into sterilized plain tubes (without anticoagulant), left to clot in a slop position at room temperature and centrifuged then sera were separated by micropipette and collected into epindorf tubes. After those sera were kept frozen at -20 °C till the time of determination of creatinine and urea levels. In addition, kidneys were removed, washed by normal saline and divided into two parts. The first part was fixed in 10 % phosphate-buffered formalin till processing for paraffin sections and light microscopic study. The second part was fixed in 5 % cold buffered glutaraldehyde immediately after dissecting the animals and kept in the refrigerator at at 8 °C till processing for semithin sections and transmission electron microscopic study in Electron Microscope Unit of Assiut University.

Kidney function:

This test was measured in the Central Laboratory of Pathology & Clinical Pathology Department, Faculty of Veterinary Medicine, Assiut University by using of 6705 UV |Vis Spectrophotometer (JENWAY) to detect the levels of creatinine and urea according to [8&9].

Histopathological examination:

Light microscopic study:

After good fixation, samples were washed by running tap water, dehydrated in ascending ethyl alcohol grades, cleared with xylene, infiltrated with paraffin and embedded in paraffin wax. Sections of 4 µm were cut from these blocks, mounted on slides, dried overnight at 37 °C and then processed for hematoxylin and eosin staining according to [10]. Finally, the stained sections were examined under light microscope (Olympus CX31, Japan) and photographed using digital camera (Olympus, Camedia C-5060, Japan) in the Photmicrograph Lab. of Pathology & Clinical Pathology Department, Faculty of Veterinary Medicine, Assiut University.

Transmission electron microscopic study:

Four blocks of 1x2 mm were taken from each sample and fixed in 5 % cold buffered glutaraldehyde immediately after dissecting the animals for 24-48 hours. The specimens were then washed in cacodylate buffer (pH 7.2) 3-4 times for 20 minutes every time and post fixed in 1% osmium tetraoxide for 2 hours. After that, samples were washed in the same buffer for four times and dehydrated by ascending grades of ethyl alcohol (3050-70-90 and 100% 2hours) of each were done. Then, the samples were embedded in epon-araldite mixture according to the protocol of Electron Microscope Unit, Assiut University [11]. From the embedded blocks, semithin sections were prepared by LKB ultramicrotom in thickness of 0.5-1 µm for orientation of the tissue then photographed by sc30 Olympus camera. Then, ultrathin sections of 500 – 700 Ao thickness were made using leica AG ultramicrotome and contrasted in uranyl acetate and lead citrate, as usual and examined by transmission electron microscope 100 CXII electron microscope at 80 kv and photographed by CCD digital camera model XR-41.

Statistical analysis:

Statistical analysis was done using analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test as post-Test by using Prism program, version 5.01 (GraphPad Prism). The level of

significance was set at P<0.05. Data were presented as mean ± S.E.

Results:

Kidney function parameters (creatinine and urea levels)

Creatinine level was non-significantly decreased in CNC group, but it was increased in CP group when compared with control group. While in CP&CNC group, creatinine level was decreased in comparison with CP treated group. In addition, the level of urea showed non-significant increase in CNC group and there was a significant increase in its level in CP treated group when compared with the control group. By contrast, the level was decreased in CP&CNC group in comparison with CP group (Table 1 and Fig. 1).

Histopathological findings

Light and ultrastructure microscopy of kidneys of control and CNC groups showed nearly the same morphological appearance (Fig. 2A&B). While, there were several pathological findings in the renal tubules, glomeruli and interstitium of CP treated group. The tubular changes were in form of severe necrobiosis, presence of deeply stained lysosomes and vacuolation of their epithelium. Cystic dilatation and presence of renal casts also were seen in some tubules. The glomerular changes were in form of thickening of Bowman's capsule and some of them appeared corrugated, the glomerular capillary tufts also showed a prominent thickening of their basement membrane (B.M.). In addition, atrophied glomeruli, decrease of mesangial cells and matrix and dilatation of the urinary space as well as periglomerular and interstitial cellular reaction were observed in most cases. As well, the ultrastructure of kidney tubules of this group revealed vacuolation with presence of numerous intracytoplasmic variable size and shape electron dense lysosomes and large amount of electron dense lipofuscin granules in the cells. Also, sthere was necrosis of epithelial cells manifested by increase of electron density of mitochondria with disintegration of their cristae. In addition to thickening of tubular basement membrane, the lumen appeared containing renal casts in form of proteineous material and cellular debris. The glomerular changes were in form of dilatation of urinary space, atrophic changes of the podocytes and mesangial cells and matrix as well as a prominent thickening of Bowman's capsule and the basement membrane of glomerular tufts. Cellular reaction of macrophages, fibroblasts, lymphoid and plasma cells as well as edema in form of fine, light electron dense granules were also noticed in the interstitium (Fig. 2C-F). On the other hand, light microscopy of kidneys of CP&CNC group revealed marked decrease of the pathological changes which observed in CP group. These changes were manifested by presence of mild necrobiotic changes in the tubular epithelium, cystic dilatation in few tubules and renal casts in the lumen of few tubules as well as mild interstitial cellular reaction. Also, there was a slight thickening of Bowman's capsule, however, the normal morphological appearance of the glomerular mesangial cells and matrix and the basement membrane of glomerular capillary tufts was observed. Transmission electron microscopy of kidneys of this group revealed that the basement membrane of glomerular tufts and the mesangial cells and matrix appeared with their normal morpholgical appearance while podocytes appeared swollen. Also, there was a slight thickening of Bowman's capsule and tubular basement membrane. Cytoplasmic vacuolation with presence of moderate amount of electron dense lipofuscin granules and lysosomes in the tubular epithelium in addition to periglomerular fibrosis were also detected

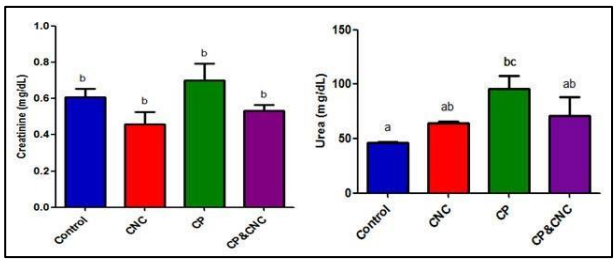

Figure 1: Creatinine and urea levels (mg/dL) in the four experimental groups. Means with different superscripts were significantly different at P< 0.05. Data were expressed as the mean ± S.E

Table 1: Parameters of kidney function in the four experimental groups

	Control	CNC	CP	CP&CNC
Creatinine (mg/dL)	0.6±0.05^{b}	0.46±0.07^{b}	0.7±0.09^{b}	0.5±0.03^{b}
Urea (mg/dL)	46±1.1^{a}	64.6±1.3^{ab}	95.8±12.3^{bc}	70.8±17.3^{ab}

Means within the same row with different superscripts were significantly different at P< 0.05. Data were expressed as the mean ± S.E.

Table 2: Scoring of histopathological findings in renal tissue of the different groups

Pathological findings	The four experimental groups and the severity scoring of findings among them			
	Control	CNC	CP	CP & CNC
Necrobiotic changes in the tubules	-ve	-ve	+++	-
Cystic tubular dilatation	-ve	-ve	++	
Renal casts	-ve	-ve	++	-
Interstitial cellular reaction	-ve	-ve	++	-
Thickening of Bowman's capsule	-ve	-ve	+++	-
Atrophy of mesangial cell and podocytes	-ve	-ve	++	-ve
Decrease of mesangial matrix and dilatation of urinary space	-ve	-ve	++	-ve

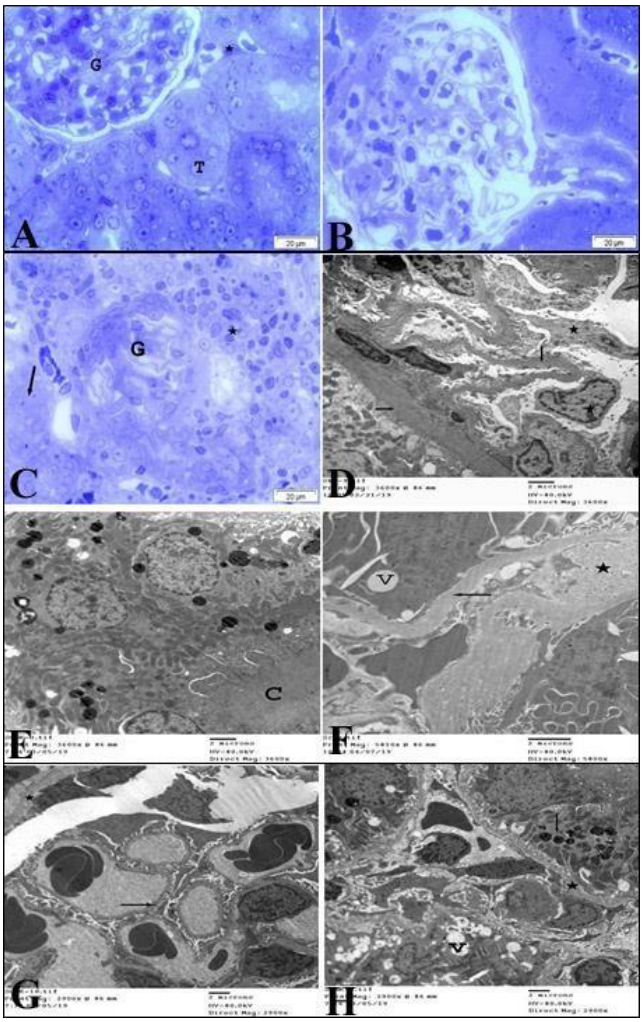

Figure 2: Histopathological findings of kidney sections of the four experimental groups: (A) Semithin section of kidney from control group showing the normal morphological appearance of renal glomerulus (G), renal tubular epithelium (T) and interstitium (star). (T.B. stain). (B) Semithin section of kidney from CNC treated group showing normal morphological appearance of the glomerulus, tubular epithelium and interstitium. (T.B. stain). (C) Semithin section of kidney from CP treated group showing atrophied glomerulus (G), thickening of the basement membrane of glomerular tufts, thickening of Bowman's capsule, decrease of mesangial cells and matrix and prominent periglomerular and interstitial cellular reaction (star). Notice: necrobiosis of the tubular epithelium (arrow). (T.B. stain). (D) T.E. micrograph of kidney glomerulus from CP treated group showing thickening of Bowman's capsule (arrow) and the basement membrane of glomerular tufts (forked arrow) and atrophic changes of the podocytes and mesangial cells and matrix (stars). (E) T.E. micrograph of kidney from CP treated group showing presence of large amount of electron dense lipofuscin granules in the tubular epithelium and the tubular lumen contains renal casts (C). (F) T.E. micrograph of kidney from CP treated group showing thickening of the tubular basement membrane (arrow), vacuolation of the tubular epithelium (v) and interstitial edema in form of fine, light electron dense granules (star). (G) T.E. micrograph of kidney from CP&CNC treated group showing normal basement membrane of glomerular tufts (arrow) and slight thickening of Bowman's capsule (star). (H) T.E. micrograph of kidney from CP&CNC treated group showing slight thickening of the tubular basement membrane (star) with presence of moderate amount of electron dense lipofuscin granules in the tubular epithelium (arrow). Notice: presence of numerous cytoplasmic vacuoles in the tubular epithelium (v).

Discussion:

Cisplatin is one of the most commonly used potent antineoplastic agents for treatment of wide range of cancers [12]. Despite its excellent anticancer activity, its clinical use is often limited by its undesirable severe toxic effects that interfere with its therapeutical efficacy [13]. This experiment was suggested to study the toxic effects of CP on experimental rats, study whether these effects could be prevented by CNC or not and determine the degree of this protection.

The recent experiment showed that, Copper nicotinate complex had no or little side effects on renal tissue. This was manifested by the non-significant change of creatinine and urea levels in CNC group when compared with control one. This result could be confirmed by the light and transmission electron microscopic examination of the kidneys of this group which showed the same appearance of control kidneys. In contrast, there was an impairment of kidney function in Cisplatin treated group expressed by an increase of serum levels of creatinine and urea when compared with control group. In this aspect, [7,14; 15&16] reported similar results. Microscopic examination of kidneys of this group in our study confirmed this nephrotoxicity induced by CP as light microscopical and ultrastructural findings of kidneys of CP treated rats revealed several pathological changes in the renal tubules, glomeruli and interstitium. These findings were compatible with the findings of [7,16, 17&18]

Mitochondrial damage observed in CP group may be due to the intracellular binding of CP to SH groups leads to GSH depletion, resulting in LPO and eventually mitochondrial damage [3]. Presence of necrotic debris in the tubular lumen with tubular damage and appearance of lysosomal bodies in any cell might be an indication to the degenerative activity and the onset of necrosis [19]. The glomerular changes were in consistent with [19&20] .where the later reported that CP caused severe atrophy of glomerulus, which was apparent due to the reduction in its size. The alterations in glomerular function may be secondary to ROS generation [21] which induce mesangial cells contraction, altering the filtration surface area and modifying the ultrafiltration coefficient factors that decrease the glomerular filtration rate [22]. Also, [19,23; 24&25] reported similar findings. Experimental studies showed that free radical formation through an oxidative stress pathway was the basic factor for CP nephrotoxicity. Free radicals impair the reabsorption action of the proximal convoluted tubule (PCT) for water, ions (Na+) and glucose [19]. On the other hand, CP nephrotoxicity may be attributed to the fact that kidney is an essential organ for metabolism and elimination of toxic agents and drugs such as CP, via conjugating these agents to glutathione [26]. This conjugation of glutathione and CP is the first step in the enzymatic pathway, which converts some intermediate compounds to potent nephrotoxin. CP is conjugated to GSH and then metabolized through GGT and cysteine S conjugate- β-lyase-dependent pathways to a reactive thiol, a potent nephrotoxin [27]. The S3 segment of the long-looped nephron of the corticomedullary region shows the highest degree of cell injury in CP-induced renal toxicity, although the loop of Henle and the distal tubular segment can also be affected [26]. Other study stated that, it seems that inflammatory cells and inflammatory cytokines are a portion of the mechanistic pathway in cisplatin-induced acute renal failure. The results of Fouble and coworkers showed that in cisplatin-induced acute renal failure, the extent of neutrophils as well as some inflammatory cytokines such as interleukin 1-β, interleukin 18 and interleukin 6 were increased in the renal tissue [28].

However, the pre-administration of CNC in CP&CNC group caused a marked decrease of creatinine and urea levels in comparison with CP group. This was similar to [29] whose results revealed that Copper (I)-nicotinate complex improved the renal dysfunction induced by Glycerol by recovering the levels of urea and creatinine. These results were consistent with our microscopic findings of CP&CNC group as there was a marked decrease of the pathological changes which observed in CP group. Similarly, [6] concluded that Copper nicotinate complex could be used as a biologically active agent against aflatoxicosis. Also, [29] reported that CNC reduced the effect of Glycerol on the kidney by decreasing the fibrosis, degenerative changes and necrotic changes in the renal tubules by its effect as an anti-oxidant.

Conclusion and recommendations:

Cisplatin induced harmful side effects on the kidney function parameters besides the harmful pathological effect on renal glomeruli and renal tubules which was attributed to the oxidative stress induced by Cisplatin. The use of copper nicotinate complex is a promising protective agent and this protective activity may be due to its antioxidant activity. This was manifested by its protective effect against the side effects on kidney function and histopathological findings of the studied animals injected with Cisplatin. Finally, authors recommended that further studies on the protective role of CNC against CP induced adverse effects must be carried out to determine the most suitable dose for human being.

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