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Effects of Nanocurcumin Against Cisplatin Induced-Nephrotoxicity in Rats

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Nephrotoxicity is the major limitation for the clinical use of cisplatin as an antitumor. Nanocurcumin, unlike curcumin, is readily dispersed in aqueous media. The purpose of this preliminary study was to investigate the potential of nanocurcumin against cisplatin-induced nephrotoxicity in rats. This study conducted for 9 days treatment, five groups of male Sprague-Dawley rats were examined: normal, cisplatin (CDDP) 7 mg/kgBW, CDDP + curcumin (CMN) 100 mg/kg BW/day, CDDP + nanocurcumin (NC) 50 mg/kg BW/day, and CDDP + NC 100 mg/kg BW/day. After 72 h following injection cisplatin, specimens were collected. Plasma blood urea nitrogen (BUN), plasma creatinine, urinary ureum levels, urinary creatinine levels, MDA levels in kidney, and GSH levels in kidney were investigated. Rats were weighed before and after study. Data were analyzed using one-way analysis of variance (ANOVA). This study resulted a single dose injection of cisplatin caused a significant increase in plasma BUN, plasma creatinine, and MDA levels by 6 fold, 2.4 fold, and 1.4 fold respectively as compared to normal group. Pre-treatment with CMN and NC were reduced plasma BUN levels, plasma creatinine levels, MDA levels in kidney and increased GSH level in kidney compared with CDPP-induced nephrotoxicity rats without treatment. At the end of treatment, the difference of body weight between normal group and CDDP group was statistically significant. CDPP is able to induce nephrotoxicity in rats that mimicked acute kidney injury in human. CMN and NC tend to reduce the CDPP-induced nephrotoxiciy.

Keywords: Cisplatin, Nephrotoxicity, Curcumin, Nanocurcumin.

1. INTRODUCTION

Cisplatin is one of the most effective chemotherapeutic agents, and it is used in the management of a variety of tumors, including testicular cancer, ovarian germ cells tumors, head and neck cancer, non-small cell lung cancer, and adrenocorticol carcinoma.[1] Cisplatin has been used as both alone or a standard component of combination chemotherapy in several cancers. Although platinum derivatives with fewer adverse events, such as carboplatin and oxaliplatin, have been developed more recently, cisplatin still provides better survival rate in some cancers such as lung cancer.[2] Pharmacokinetics studies in humans indicate an initial cisplatin plasma half-life of 25–49 min and a secondary half-life of 58–73 h and protein binding occurs rapidly, up to 90% in 2 h.[3]

The therapeutic activity of cisplatin is dose dependent and the clinical usage limited by its undesirable side effects, including nephrotoxicity, hepatotoxicity, neurotoxicity, ototoxicity.[2] Among these factors, nephrotoxicity has been reported as the major side effect that may restrict the therapeutic use of cisplatin. Nephrotoxicity is found in 28–36% patients who received a single dose (50 mg/m^2) of cisplatin.[4] Although nephrotoxicity is temporary and dose dependent, it can decrease glomerular filtration rate (GFR), which can be clinically evaluated from increased serum creatinine and decreased creatinine clearance.[2]

For several decades, studies measuring the cisplatin content in different target organs have been reported. Human tissues from patients with different tumours and subjected to treatments based on cisplatin were analysed by X-ray Fluorescence. Liver, kidney and prostate were the organs with the highest cisplatin levels. Lower concentrations were found in bladder, muscle, testicles, pancreas and spleen, being the lowest concentrations detected in bowel, adrenal gland, heart, lung, brain and cerebellum.[5] Studies in rats and mice indicate that cisplatin undergoes metabolic activation in the kidney to a more potent toxin.[6]

Cisplatin concentrations within the kidney exceed those in blood suggesting an active accumulation of drug by renal parenchymal cells. The toxic effects occur primarily in the renal proximal tubules, particularly in the epithelial tubular cells of S-3 segment, glomeruli and distal tubules are also affected afterward.[7] Studies in recent years have identified two different membrane transporters capable of transporting cisplatin into cells: Ctr1 and OCT2. Ctr1 is a copper transporter which was also shown to mediate cisplatin uptake into mammalian cells, including ovarian cancer cells.[8] The mechanisms of cisplatin-induced nephrotoxicity are complex and involve multiple pathways and molecules, such as inflammation, oxidative stress, and apoptosis.

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There have been studies confirming that cisplatin induces the formation of reactive oxygen species (ROS), which responsible for the life-threatening nephrotoxicity induced cisplatin via apoptosis pathway.[9]

Natural antioxidants as potential nutraceuticals have been studied to reduce severe side effects as well as enhance anticancer activities of antitumor drugs.[10] Curcumin (CMN), a hydrophobic polyphenol derived from the rhizome of the herb Curcuma longa has a wide spectrum of biological and pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic activities. The use of CMN has been reported as a therapeutic agent to mitigate various kinds of toxicity including cardiotoxicity, nephrotoxicity, hepatotoxicity and neurotoxicity.[11] Various animal models or human studies proved that CMN is extremely safe even at very high doses.

The clinical implication of CMN is hindered due to low intrinsic activity, poor absorption, inactivity of metabolic products and/or rapid elimination and clearance from the body, low solubility, physico-chemical instability. However, these issues can be overcome by utilizing an efficient delivery system. In 2005, an active scientific research was initiated to improve CMN's pharmacokinetics, systemic bioavailability, and biological activity by loading CMN into nanoform(s) (nanoformulations).[12] Considering this perspective, this study provides an *in vivo* study to assess the effects of CMN and nanocurcumin especially nephroprotective effect. In addition, if this study of CMN and nanocurcumin nephroprotective proven to have any effect on cisplatin-induced nephrotoxicity, then it can be used for further research in human.

2. METHODS

2.1. Ethics Statement and Experimental Animals

Male Sprague Dawley rats (150–300 g), bred in the Research and Development Institute of Health (LITBANGKES) were used. The rats were acclimatized for a week before start of the experiments. The animals were housed under standard laboratory condition at temperature 22 ± 2 °C with relative humidity at $65 \pm 10\%$. Standard pellet rodent diet and water were provided to the animals ad libitum. The experimental protocols were approved by the Institutional Animal Ethics Committee of Universitas Indonesia.

2.2. Drugs and Chemicals

Cisplatin were purchased from Sigma Aldrich (St. Louis, MO, USA). CMN and nanocurcumin (NC) were purchased from PT Plamed green Science Limited (China). Cisplatin (CDDP) was dissolved in normal saline (0.9%) and administered intraperitoneally. CMN and NC was suspended in 0.5% carboxymethylcellulose (CMC) and administered orally. Reduced glutathione (GSH) was purchased from PT AAT Bioquest (Mercury Drive, Sunnyvale), MDA was purchased from PT Merck Millipore (Indonesia), urea and creatinine kit were purchased from PT DiaSys Diagnostic System GmbH (Holzheim, Germany).

2.3. Experimental Design

The CDPP dose used in this experiment was similar to that previously described by Wada et al., (2014). The dose of CMN was based on previously published reports Zhongfa et al., (2012). Thirty animals were randomly divided into 5 groups containing 6 rats in each. Group 1 (Normal), rats were administered with 0.5% CMC-Na once daily orally for 9 consecutive days and a single intraperitoneally (i.p.) injection of 0.9% normal saline on 7th day. Group 2 (CDPP), rats were administered with 0.5% CMC-Na once daily orally for 9 consecutive days and a single i.p. injection of CDPP (7 mg/kg dissolved in 0.9% normal saline) on 7th day. Group 3 (CDPP + CMN), rats were administered CMN (100 mg/kg/day dissolved in 0.5% CMC-Na) orally for 9 consecutive days and a single i.p. injection of CDPP (7 mg/kg dissolved in 0.9% normal saline) on 7th day. Group 4 (CDPP + NC50), rats were administered NC (50 mg/kg/day dissolved in 0.5% CMC-Na) orally for 9 consecutive days and a single i.p. injection of CDPP (7 mg/kg dissolved in 0.9% normal saline) on 7th day. Group 5 (CDPP + NC100), rats were administered NC (100 mg/kg/day dissolved in 0.5% CMC-Na) orally for 9 consecutive days and a single i.p. injection of CDPP (7 mg/kg dissolved in 0.9% normal saline) on 7th day. Animals were weighed regularly during the experiment. Twenty-four hours after CDPP injection, all rats were placed individually in the metabolic cage for urine collection. At the end of the experiment (i.e., on 10th day), the animals were anesthetized with ether and sacrificed by cervical decapitation. Blood was collected from all the experimental rats and plasma was separated. The left and right kidneys were isolated, and a midline incision was performed. The kidney samples were stored at −80 °C for further biochemical analysis. Plasma samples were stored at −80 °C and assayed for blood urea nitrogen (BUN) and creatinine using standard diagnostic kits.

2.4. Preparation of Tissue Homogenates

Kidney tissue was homogenized with ice-cold saline (0.9% sodium chloride) using a rotorstator homogenized, and the mixture was centrifuged at 3000× g for 10 min at 4 °C. The supernatant was separated and stored at −80 °C until analyzed.

2.5. Measurement of Levels BUN and Creatinine in Urine and Plasma

Urine samples were centrifuged at 3000× g (10 min, 4 °C) and the supernatant was collected. Levels of BUN in urine and plasma were measured using urease-GLDH: UV enzymatic test with DiaSys kit. Levels of creatinine in urine and plasma were measured using Jaffe method with DiaSys kit.

2.6. Measurement of MDA in Homogenate

MDA standard solution was prepared with concentrations of 0, 10, 20, 40, 80, 160, and 320 nmol/mL. A total of 200 mL sample was added to 1800 mL of distilled water, and added in 1 mL of 20% TCA and 2 mL of 0.67% TBA, mixed and heated at 100 °C for 10 minutes. The mixture was centrifuged and the supernatant was collected. The absorbance was read at 530 nm wavelength.

2.7. Measurement of GSH in Homogenate

GSH standard solution prepared with concentration 0; 0.0781; 0.1563; 0.3125; 0.625; 1.25; 2.5; dan 5 μM. GSH concentration were measured using Amplite Rapid Flourimetric Glutathione GSH/GSSG Ratio Assay Kit. Absorbance was read by a fluorescence microplate reader at $E_x/E_m = 490/520$ nm.

2.8. Statistical Analysis

Data were shown as mean ± SEM and were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test

as post-hoc analysis. Values of $p < 0.05$ were considered as significant. All the statistical analyses were performed using IBM SPSS software.

3. RESULT

3.1. Body Weight Changes

For calculation of body weight gain or loss, we considered the day of CDPP injection as day 0. In CDPP alone administered group of rats, we observed a 2%–5.7% body weight loss when compared to other groups at day 7th (i.e., the day of CDPP injection). The loss of body weight of CDPP alone group was even more lower (5%–7.9%) at day 9th of the experiment. CMN and both doses of NC treatment along with CDPP attenuated the body weight loss when compared to CDPP alone treated rats (Fig. 1(A)). The relative weight of kidney to body weight in CDPP alone treated rats were increased compared to that of normal group. Nanocurcumin treatment at both doses and curcumin attenuated the increase of ratio kidney weight to body weight compared to CDPP alone treated rats (Fig. 1(B)).

3.2. BUN Levels in Urine and Plasma

The urine BUN levels decreased in CDPP alone group compared to that of normal group. Treatment with CMN and NC at both doses could increase the urine BUN levels, although there was no statistically significant difference in all groups ($p > 0.05$) (Fig. 2(A)). As shown in Figure 2(B), the plasma BUN concentration increased significantly in the CDPP alone group as

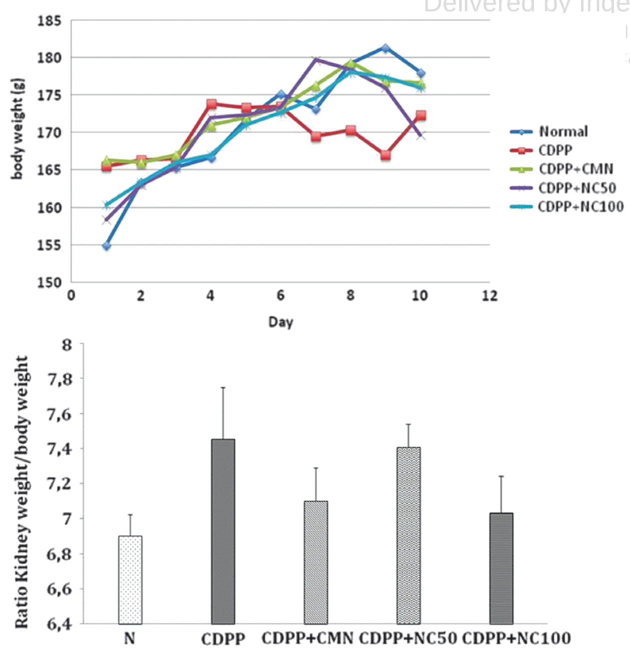

Fig. 1. (A) Effect of curcumin and nanocurcumin on the body weight of rats during the study period. (B) Effect of curcumin and nanocurcumin on the kidney weight to body weight ratio. Results are expressed as mean ± SEM. $n = 6$ per group. Normal, age-matched normal rats; CDPP, acute kidney injury rats induced by cisplatin administered with vehicle; CDPP + CMN, acute kidney injury rats induced by cisplatin administered with curcumin 100 mg/kg/day; CDPP + NC50, acute kidney injury rats induced by cisplatin administered with nanocurcumin 50 mg/kg/day; CDPP + NC100, acute kidney injury rats induced by cisplatin administered with nanocurcumin 50 mg/kg/day. $^{*}p <$ 0.05 versus CDPP, $^{\#}p < 0.05$ versus CDPP.

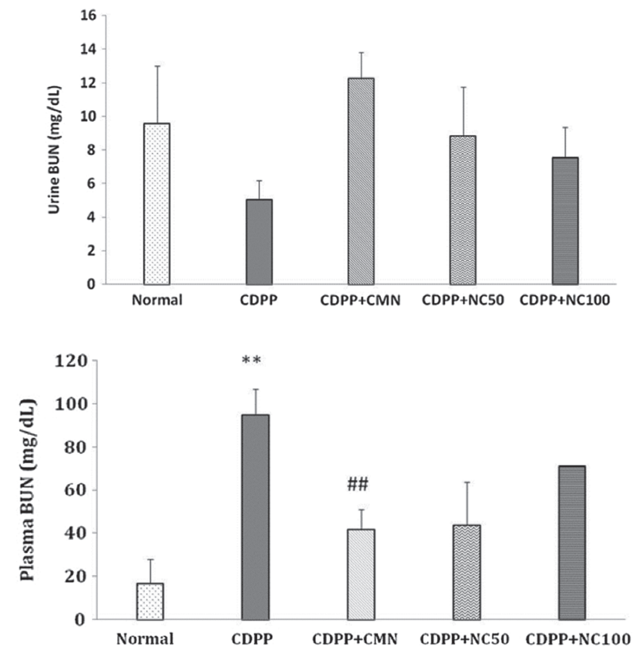

Fig. 2. Effects of CDDP, CMN, and NC on urine and plasma BUN levels were determined by specific kit. Values were expressed as mg/dL. Each value represented as mean ($n = 6$). Significant differences were indicated by $^{*}p < 0.05$ versus normal group, $^{\#}p < 0.05$ versus CDDP group.

compared to that of the normal group. Treatment with CMN could decrease the plasma BUN concentration significantly to that of CDPP alone group, treatment with NC at both doses also decrease the concentration of plasma BUN, though it did not reach a statistically significant as compared to that of CDPP alone group.

3.3. Creatinine Levels in Urine and Plasma

Similarly with the result of urine BUN levels, the urine creatinine level decreased in the CDPP alone group. Treatment with CMN and NC at both doses could increase the level of urine BUN (Fig. 3). The level of plasma creatinine was increased significantly in the CDPP alone group compared to that of normal group. Curcumin treatment could decrease the plasma creatinne level significantly as compared to that of CDPP alone group.

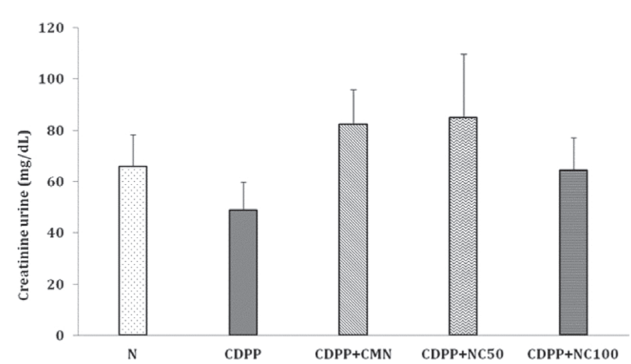

Fig. 3. Effect of CDDP, CMN, and NC on the urine creatinine levels were determined by specific kit. Values were expressed as mg/dL. Each value represented as mean ($n = 6$).

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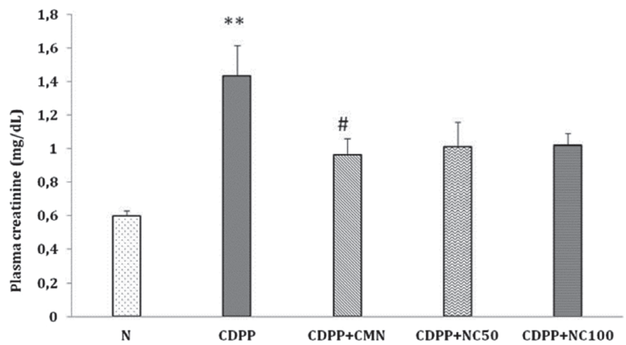

Fig. 4. Effect of CDDP, CMN, and NC on the plasma creatinine level were determined by specific kit. Values were expressed as mg/dl. Each value represented as mean ($n = 6$). Significant differences were indicated by $^{**}p < 0.001$ compared with normal group, $^{\#}p < 0.05$ compared to CDPP group.

Nanocurcumin treatment at both doses, also decrease the plasma creatinine level compared to that of CDPP alone group (Fig. 4).

3.4. MDA and GSH Levels in Kidney

The CDPP administration produced a reduction in the kidney GSH content and an elevation in the kidney MDA production in comparison with the normal group. CDPP plus CMN treatment and NC treatment at both doses increased the kidney GSH and reduces the kidney MDA production but not significantly.

4. DISCUSSION

Nephrotoxicity is a frequent disturbing adverse effect of cisplatin chemotherapy. The distinctive pharmacological profile of curcumin has attracted considerable attention in the field of cancer research. Unfortunately, curcumin has a poor bioavailability that hindered its utility in the cancer treatment. In this study, we used nanocurcumin that is more readily dispersed in aqueous solution and better physical-chemical properties compared to that of curcumin.

In this preliminary study, we provide evidence that protection against the development of cisplatin-induced nephrotoxicity that is mimicked acute kidney injury in human, by nanocurcumin treatment involves changes in the urine and plasma BUN and creatinine levels, kidney MDA levels, as well as activity of GSH levels. We here showed that single cisplatin injection produced loss of body weight. Nanocurcumin and curcumin treatment prevented the development of nephrotoxicity induced by cisplatin injection by significantly lowering the plasma BUN and creatinine levels. Furthermore, nanocurcumin and curcumin treatment maintained the body weight of animals throughout the study period. We also found that pretreatment with nanocurcumin and curcumin could reduce MDA levels and could increase the activity of GSH.

Cisplatin (cis-diamminedichloroplatinum (II)) is an inorganic complex formed by an atom of platinum surrounded by chlorine and ammonia atoms in cis position of a horizontal plane.[3] Cisplatin is cleared by the kidney by both glomerular filtration and tubular secretion. Cisplatin undergoes biotransformation in the kidney to highly reactive thiols.[13] Typically, the onset of renal insufficiency begins several days after the dose cisplatin, as revealed by increase in the plasma creatinine and blood urea nitrogen (BUN) concentration.[13] Nephrotoxicity increases with an increase in dosage and frequency of administration cisplatin.

The BUN and creatinine are screening test of renal function, because they are handled primarily by glomerular filtration with little or no renal regulation or adaptation in the course of declining renal function, they essentially reflect GFR.[14] BUN is the mass of nitrogen within urea, not the mass of urea. Urea and creatinine are nitrogenous end products of metabolism. Urea is the primary metabolite derived from dietary protein and tissue protein turnover. Creatinine is the product of muscle creatine catabolism. Small changes in kidney function can produce large increments in BUN and creatinine.[14]

Several investigation have shown that cisplatin-induced nephrotoxicity is associated with elevated ROS that damages cell membranes by peroxidation lipid in radical-mediated chain reaction and the inhibition of this process by curcumin is mainly attributed to the ability of scavenger free radicals.[15] Malondialdehyde (MDA) is a degradation product from lipid hydroperoxide, provides an index of the peroxidation of lipids in biological tissue. It is well documented that cisplatin cause lipid peroxidation in the kidneys via ROS generation.[16]

In this study, we found an increased production of MDA as measured by TBARS in the kidney of cisplatin treated rats. MDA levels in cisplatin group significantly increased compared to that of normal group, the administration of curcumin and nanocurcumin reduced the levels of MDA, however it did not reach a statistically significant. We suggest that nanocurcumin and curcumin act as antioxidant to scavenge ROS, eventually abrogating oxidative stress and improve kidney functions.

Oxidative damage induced by cisplatin has been associated with the depletion of enzymatic antioxidant defense system (SOD, catalase, glutathione peroxidase, glutathione transferase and glutathione reductase) and non-enzymatic (GSH and NADPH) in the rats kidneys.[7] GSH is one of the essential compounds for maintaining cell integrity against ROS and being non-enzymatic free radical scavenger, it participates in the detoxification of ROS.[9] In this study, we have shown that the activity of GSH was significantly decreased in the kidney of cisplatin treated rats compared to that of normal group. Nanocurcumin and curcumin administration to the cisplatin-induced rats could increase the activity of GSH which suggests that curcumin and nanocurcumin could maintain the non-enzymatic antioxidant defense system. Furthermore, it has been reported that curcumin can increase the synthesis and concentration of reduced glutathione (GSH). Curcumin is a bifunctional antioxidant, it applies antioxidant activity in a direct and an indirect way through scavenging reaction oxygen species and provoking an antioxidant response.[17]

5. CONCLUSION

Single intraperitoneal injection of cisplatin at the dose of 7 mg/kgBW in rats could induce nephrotoxicity that mimicked acute kidney injury in human. The administration of nanocurcumin and curcumin protect against the development of acute kidney injury that is induced by cisplatin injection, which involves abrogation of oxidative stress. Moreover, the antioxidant effects of nanocurcumin and curcumin are responsible for the reduction of lipid peroxidation and the increase of antioxidant activity. The antioxidant effects of nanocurcumin did not show in a dose-dependent manner.

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