Original Article

Protective Effect of Curcumin and Cerium Oxid Nanoparticles on Carboplatin Induced Myelotoxicity and Hepatotoxicity in Adult Male Wistar Rats

Marwa AbdEl-Moniem Amer [1], Dena Mohamed Naguib Abdel Moawed [1*], Reham Sameh [2], Rasha Ahmed Agaga [3] Nisreen E. Elwany [4], Amira Mohamed Abdelhamid [4]

[1]Department of Forensic Medicine & Clinical toxicology, Faculty of medicine, Zagazig University, Zagazig, Egypt. [2]Department of Pathology, Faculty of medicine, Zagazig University, Zagazig, Egypt. [3]Department of human anatomy and embryology, Faculty of medicine, Zagazig University, Zagazig, Egypt. [4]Department of clinical Pharmacology, Faculty of medicine, Zagazig University, Zagazig, Egypt.

[*]Corresponding Author
Dena Mohamed Naguib Abdel Moawed, M.D
Email:
Dena@zu.edu.eg
Denaforensic@gmail.com

ABSTRACT

Background: Carboplatin is a chemotherapeutic agent used in many types of cancers. Carboplatin adversely affects multiple organs like the bone marrow, liver, gastrointestinal tract, and kidney. **Aim of the study:** The aim of this work is to evaluate the potential protective role of curcumin and cerium oxide nanoparticles (CONPs) on carboplatin induced myelotoxicity and hepatotoxicity**. Methods:** Forty-eight adult male Wistar rats were classified into seven groups*;* ***I.*** Control (Subdivided into negative and positive control groups), ***II.*** Curcumin-treated, ***III.*** CONPs-treated, ***IV.*** Carboplatin intoxicated, ***V.*** Curcumin-treated carboplatin intoxicated, ***VI.*** CONPs-treated carboplatin intoxicated & ***VII.*** Curcumin & CONPs-treated carboplatin intoxicated groups. Curcumin was administered orally, once daily for 4 consecutive weeks. CONPs, suspended in saline, and carboplatin were administered intraperitoneally once a week for 4 consecutive weeks. After 4 weeks, blood samples were collected after anesthesia of rats. The animals were then sacrificed, with bone marrow samples & livers collected for biochemical, histopathological and immunohistochemical studies. **Results:** Carboplatin treatment decreased blood cells count and elevated liver enzymes and bone marrow & hepatic malondialdehyde (MDA). It decreased GSH levels and causes DNA damage. Carboplatin induced hypocellularity in bone marrow samples and showed Strong 8-OHdG immunoreactivity. It also induced septal fibrosis with architectural distortion in liver tissue. Administration of either curcumin or CONPs ameliorated these toxic effects of carboplatin. Meanwhile the concurrent administration of curcumin and CONPs revealed much better improvement than each of them used alone. **Conclusions:** Combined administration of curcumin and CONPs ameliorated carboplatin-induced myelotoxicity and hepatotoxicity through antioxidant mechanism.

KEYWORDS: Curcumin; Cerium oxide; Nanoparticles; Carboplatin; Myelotoxicity; Hepatotoxicity.

I. INTRODUCTION

Carboplatin, a second-generation platinum-based chemotherapeutic drug, is used to treat numerous types of cancer such as ovarian, lung, head, and neck cancers. It has better anti-cancer effectiveness and fewer adverse effects when compared to cisplatin, the first-generation drug (Dalian et al. 2012).

The clinical use of carboplatin has been linked to many toxicities such as bone marrow suppression, hepatotoxicity, nephrotoxicity, ototoxicity, and peripheral neurotoxicity (Stojanovska et al. 2015; Cheng et al. 2017; Stevens et al. 2018).

Patients receiving carboplatin experience myelosuppression, which is the major complication of this drug. It is in the form of anemia, leucopenia, and thrombocytopenia (Pujari and Bandawane 2019).

Another adverse effect to carboplatin is hepatotoxicity, which is in the form of cholestasis, aspartate aminotransferase (AST) elevations and steatosis. These adverse effects are produced by oxidative stress, inflammation, tissue necrosis and apoptosis (Zhang et al. 2015).

Curcumin is the main active constituent of turmeric. For centuries, it has been used as a dietary ingredient. It has numerous pharmacologic actions including antioxidant activity as it inhibits the formation of reactive oxygen species (ROS), anti-inflammatory and anti-cancer properties (Joe et al. 2004; Kuhad and Chopra 2007; Du et al. 2011).

Curcumin has been used in the management of many diseases such as hyperlipidemia, metabolic syndrome, chronic inflammation, arthritis, cardiovascular disease, and anxiety (Hewlings and Kalman 2017; Sundar Dhilip Kumar et al. 2018).

Nanotechnology is one of the crucial technologies today. CONPS were tested in various studies and showed hopeful results as potent antioxidant (Corsi et al. 2018). CONPs are effective agents used in many diseases caused by oxidative stress and inflammation (Das et al. 2013).

CONPs have selective cytotoxicity. They are toxic to tumor cells and sensitize them to chemotherapy and radiotherapy. In contrast, CONPs defend normal tissues from the serious effect of ROS so it can be used as anticancer agent (Gao et al. 2014).

This research aims to evaluate the potential protective effect of curcumin and CONPs on carboplatin induced

myelotoxicity and hepatotoxicity in adult male Wistar rats.

II. MATERIAL AND METHODS

II.1 Chemicals and drugs:

Carboplatin solution was purchased as pharmaceutical preparation (Mylan, Sky pharma Company, Mumbai, Maharashtra, India; 450 mg / 45 ml vial). Curcumin yellow powder from Curcuma longa (Turmeric) was purchased from Sigma-Aldrich chemical company (St. Louis, USA).

Cerium oxide white powder with particulate size <25 and purity 99.95% was purchased from Sigma/Aldrich chemical company, USA (St. Louis, USA).

II.2 Characterization of CONPs:

Characterization of CONPs was done in the Electron microscopic unit, Faculty of Agriculture, Cairo University, by a transmission electron microscope (JEM-1400 TEM) JEOL Ltd., Tokyo, Japan. Operating at an acceleration voltage 80 kV. The primary particle size and shape of CONPs are assessed by preparing a sample of CONPs by suspending the nanopowder in distilled water the sonicating it. A drop of suspension the put on a 400 mesh Copper grid coated with a thin layer of carbon and allowed to dry in air the examined by TEM.

II.3 Animals:

Forty-eight adult male Wistar rats, aged 8- 10 weeks with a weight 180-200 grams/rat, were obtained from the Faculty of Medicine animal house, Zagazig University, Egypt. They were left for acclimatization for 10 days before beginning the experiment. The room was properly prepared with 12-h light/dark cycle. Rats were allowed free access to water and food.

II.4 Ethical Statement:

The current study protocols including animal handling and experiments were approved by Ethical Committee for Animal Handling at Zagazig University (ECAHZU) on 27th of September 2020 with approval number (ZU-IACUC/3/F/97/2020), and this is in accordance with the recommendations of the Weather all report and National Institutes of Health guide for the care and use of Laboratory animals.

II.5 Study design:

Rats were classified into seven groups after the period of acclimatization. ***Group I (Control group):*** It is subdivided into:

Group IA (Negative control group): 6 rats were administered only water and ordinary food without any stress.

Group IB (Positive control group): 6 rats were administered normal saline (0.5 ml) by intraperitoneal (IP) injection once a week and 0.5 ml corn oil orally every day for 4 consecutive weeks; ***Group II (Curcumin-treated group):*** 6 rats were orally administered Curcumin, once daily for 4 consecutive weeks via oral gavage at a dose of (100 mg/kg body weight) as an emulsion in corn oil **(Avci et al. 2017)**; ***Group III (CONPs-treated group):*** 6 rats were IP injected with CONPs suspended in saline once a week, at a dose of (60 mg/kg body weight) for 4 consecutive weeks (Nigjeh et al. 2012); ***Group IV (Carboplatin intoxicated group):*** rats received one IP injection of carboplatin (20 mg/kg body weight), once a week for 4 consecutive weeks **(Kaplan et al. 2016)**; ***Group V (Carboplatin + Curcumin treated group):*** 6 rats were received Curcumin by oral gavage combined with IP injection with carboplatin in the same previously mentioned doses and duration; ***Group VI (Carboplatin + CONPs treated group):*** 6 rats were injected IP with CONPs suspended in saline and three hours later, carboplatin was injected IP at the same previously mentioned doses and duration; ***Group VII (Carboplatin + Curcumin + CONPs treated group):*** 6 rats were received Curcumin by oral gavage; injected IP with CONPs suspended in saline and three hours later, carboplatin was injected IP at the same previously mentioned doses and duration.

II.6 Samples collection:

After 4 weeks, rats were sacrificed after anaesthetization by 50 mg/kg Sodium Pentobarbital (IP) then blood samples, liver, two femurs and right tibia were collected from rats of all groups.

Blood samples were obtained by means of capillary glass tubing from rats' retro-orbital plexus for biochemical studies. Some blood was taken on EDTA tube for measurement of total WBCs, RBCs and Platelets count and the other sample of blood was incubated at 37 °C till clotted and then centrifuged (4000 Xg for 15 min) to isolate the sera for the measurement of liver enzyme markers: serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Then, a midline incision was performed, and the liver was dissected and cleaned by normal saline. The liver was divided into two Parts. One part was homogenized, centrifuged and the supernatant was used for biochemical evaluation of malondialdehyde (MDA) and reduced glutathione (GSH) levels in the liver tissues. The other part of

the liver was kept in 10% formalin solution for fixation & preparation for histopathological study.

Femurs were dissected and their ends were cut using ophthalmic scissors. The bone marrow of the right femur was extracted, fixed, and then resuspended in the standard phosphate buffered saline (PH 7.4) for Comet assay. The left femur was rinsed with 1 ml pre-warmed 1×PBS (pH=7.4) for 5 times to collect the marrow suspension, followed by a centrifugation for 3 min to collect the precipitated bone marrow cells for biochemical assessments of MDA and GSH. The right tibia was dissected and fixed in 10% formalin solution then used for histopathological and immunohistochemical studies.

II.7 Biochemical studies:

- **Complete Blood Count**

Total WBCs, RBCs and Platelets count was measured using the automated method (Impedance technology) based on Coulter principle (1956) for the analysis of WBC, RBC, and platelets (Graham 2013).

- **Liver enzymes**

Serum ALT and AST levels were measured by colorimetric method according to the method described by Moss (1982) and Zilva & Pannall (1979) respectively.

- **Oxidative stress markers**

Liver tissue homogenates and bone marrow were used to measure MDA and GSH by colorimetric method according to the method of Ohkawa et al. (1979) and Beutler et al. (1963) respectively. Oxidative stress parameters were measured using kits MDA and GSH kits (Bio diagnostic chemical company, Cairo).

II.8 Comet assay:

Single cell gel electrophoresis (Comet) assay was performed in Animal Reproductive Research Institute (ARRI) of Agricultural Research Centre of Ministry of Agriculture and Land Reclamation (Elharam, Giza). According to the method of Singh et al. (1988), insertion of a small number of cells in a thin agarose layer was performed. Lysis, exposure to electrophoresis, and then staining with a fluorescent DNA intercalating dye (Ethidium bromide) were followed. DNA fragments resulting from DNA damage migrate faster than undamaged DNA. A comet-like structure with a head (undamaged DNA) and a tail (DNA fragments) were formed. Alkaline lysis is desired as it identifies single-strand breaks,

double-strand breaks and alkali labile sites.

II.9 Histopathological and immunohistochemical studies:

The part of the liver which was collected for histopathological examination and the right tibia were fixed in 10% neutral buffered formalin solution, embedded in paraffin, then 4–5 µm thick sections were cut and then stained with hematoxylin and eosin for examination under light microscope.

Liver tissue histopathological changes were described according to Metavir score (Shiha and Zalata 2001): Stage 0: No fibrosis; ***Stage 1:*** Fibrous expansion of some portal areas with or without short fibrous septa; ***Stage 2:*** Fibrous expansion of most portal areas with or without short fibrous septa; ***Stage 3:*** Fibrous expansion of most portal areas with occasional portal to portal bridging; ***Stage 4:*** Fibrous expansion of portal areas with marked bridging; ***Stage 5:*** Marked bridging with occasional nodules (incomplete cirrhosis); and ***Stage 6:*** cirrhosis, probable or definite.

Immunohistochemical examination of the bone marrow biopsy for detection of 8-hydroxy-2-deoxy guanosine (8-OHdG), a biomarker of oxidative stress (Karihtala and Soini 2007). It was done according to the method of Toyokuni et al. (1997). Bone marrow biopsy was fixed with neutral10% formaldehyde solution. Formalin-fixed, paraffin-embedded tissues were cut into 4 µm thick sections. Then, sections were subjected to dewaxing, rehydration and blocking with hydrogen peroxide. The antigen retrieval was performed with microwave in a 10 mm citrate buffer (pH 6.0) for 10 min and cooled to room temperature. After being blocked with 1% goat serum albumin, sections were incubated with the antibody; 8-OHdG antibody (mouse anti-8-OHdG antibody, monoclonal 15A3; Santa Cruz Biotechnology [AQ2] (sc-66036)) overnight at 4^{o} C, followed with horseradish peroxidase–labeled secondary antibodies for 30 min at room temperature.

The sections were incubated with diaminobenzidine tetrahydrochloride and counterstained with hematoxylin. The intensity of the 8-OHdG immunoreactivity for the nuclei of marrow cells was evaluated by dividing the staining reaction into four grades according to the scoring system described by Toyokuni et al. (1997): ***negative*** immunoreactivity (<5% of cells showing nuclear positivity); ***weak*** immunoreactivity

(5%–20% of cells showing nuclear positivity); ***moderate*** immunoreactivity (21%–80% of cells showing nuclear positivity) and ***strong*** immunoreactivity (>80% of cells showing nuclear positivity).

II.10 Statistical analysis:

Statistical Package for Social Sciences (SPSS version 20.0) was used for analysis. One Way Analysis of Variance (ANOVA), followed by Post Hoc test (Least Significance Difference test "LSD") for multiple comparisons between groups were done for Quantitative data analysis. Chi square test (X2) was used for Qualitative data analysis. T test was used for comparison between positive and negative control groups. Probability (P value) was set as $P > 0.05$ indicates non-significant results, and values of $p < 0.05$ were considered significant.

III RESULTS

The results of all measured parameters between positive and negative control groups were approximate with non-significant difference ($P > 0.05$), so the negative control group was used for comparison with the rest of the study groups.

III.1 Characterization of CONPs:

TEM examination showed that CONPs were mixture of octahedrons and cubes with some agglomeration. The shape of the particles was non-spherical so, the biggest dimension of each particle was chosen to be measured. Size observed from TEM was approximately in the range < 25 nm (Figure 1).

III.2 Biochemical results:

The present work showed no significant difference in hematological parameters (RBCs, WBCs & platelets), serum enzymes (AST & ALT), hepatic & bone marrow oxidative stress markers (MDA & GSH levels) and COMET assay in curcumin and CONPs groups compared to control group.

- **Hematological parameters:**

Carboplatin intoxicated group showed significant ($p<0.05$) decrease of RBCs, WBCs & platelets levels when compared to control groups, indicating hematotoxicity. Carboplatin intoxicated groups treated with either curcumin or CONPs showed improvement in hematological parameters when compared with those in the carboplatin intoxicated group.

Co-administration of curcumin and CONPs with carboplatin significantly ($p<0.05$) improved the lowered levels of WBCs & platelets, while non-significantly improved RBCs count compared with those in the carboplatin intoxicated group (Table 1).

- **Serum level of Liver enzymes:**

Carboplatin caused significant ($p<0.05$) increase in serum AST and ALT levels in carboplatin intoxicated group when compared to Control groups. Carboplatin intoxicated groups treated with either curcumin or CONPs showed significant reduction in serum level of these enzymes when compared with those in the carboplatin intoxicated untreated group. Co-administration of both curcumin and CONPs with carboplatin significantly ($p<0.05$) decreased the high levels of AST & ALT compared with the carboplatin group with better improvement than each one administered alone with carboplatin (Table 2).

- **Hepatic MDA and GSH levels:**

Carboplatin treatment significantly increased hepatic MDA content in the group intoxicated with carboplatin when compared to control group indicating lipid peroxidation. However, treatment of carboplatin intoxicated groups with either curcumin or CONPs showed significant reduction in MDA level when compared with those in the carboplatin intoxicated untreated group. Co-administration of both curcumin and CONPs with carboplatin significantly decreased the high levels of MDA when compared to each of them administered alone with carboplatin (Table 3).

Injection of carboplatin caused a significant ($p<0.05$) decrease in hepatic GSH level as compared to control group indicating oxidative stress. Carboplatin intoxicated groups treated with either curcumin or CONPs showed significant ($p<0.05$) increase in GSH level when compared with those in the carboplatin intoxicated untreated group but not returned to control group levels. Co-administration of curcumin and CONPs with carboplatin significantly ($p<0.05$) increased GSH level, compared to the carboplatin intoxicated group, better than administration of each one alone (Table 3).

- **Bone marrow levels of MDA and GSH:**

Carboplatin caused significant ($p<0.05$) increase bone marrow MDA level in group treated with carboplatin when compared to Control group indicating lipid peroxidation. carboplatin intoxicated groups treated with either curcumin or CONPs showed significant ($p<0.05$) reduction in MDA level when compared with those in the carboplatin group but not

returned to control group levels. Co-administration of curcumin and CONPs with carboplatin significantly ($p<0.05$) decreased the high levels of MDA compared with the carboplatin group. The decrease in the combined administration of curcumin and CONPs was more than each one administered alone (Table 3).

Injection of carboplatin caused a significant ($p<0.05$) decrease in bone marrow GSH level when compared to Control group indicating oxidative stress. Carboplatin intoxicated groups treated with either curcumin or CONPs showed significant ($p<0.05$) increase in GSH level when compared with those in the carboplatin group but not returned to Control groups level. Co-administration of curcumin and CONPs with carboplatin significantly ($p<0.05$) increased GSH when compared with the carboplatin group better than each one alone (Table 3).

III.3 Comet assay:

To evaluate the effects of exposure to carboplatin, bone marrow cells were tested for DNA damage using (Comet assay). Comet assay detects the percentage of DNA damage. DNA damage was significantly ($p<0.05$) higher in carboplatin-intoxicated group compared with the Control group indicating DNA damage. However, treatment of carboplatin intoxicated groups with either curcumin or CONPs showed significant ($p<0.05$) decrease in comet assay parameters when compared with those in the carboplatin intoxicated group but not returned to control group levels. Co-administration of both Curcumin and CONPs with carboplatin significantly improved the DNA damage when compared with the carboplatin group with better improvement than each one administered alone with carboplatin (Table 4) (Figure 2).

III.4 Histopathological results:

- **Bone marrow histopathological results**

Bone marrow biopsy from carboplatin intoxicated group showed marked hypocellularity which is largely lacking hematopoietic cells and contains mainly fat cells, scattered lymphocytes, and degenerated marrow cells. Co-administration of curcumin and CONPs with carboplatin revealed some improvement in bone marrow biopsy (Figure 3).

- **Bone marrow 8-OHdG immunoreactivity**

Carboplatin injection for four weeks caused a high significant ($P < 0.001$) increase in 8-OHdG immunoexpression in bone marrow

cells when compared with the control, curcumin and CONPs groups. Carboplatin intoxicated groups with either curcumin or CONPs showed significant reduction in 8-OHdG immunoreactivity when compared with those in the carboplatin intoxicated group. Co-administration of both curcumin and CONPs with carboplatin significantly decreased the 8-OHdG immunoreactivity compared with the carboplatin group better than each one alone with carboplatin (Table 5, Figure 4).

- **Liver histopathological results**

Liver tissue histopathological examination of Control, curcumin and CONPs groups revealed a normal preserved architecture with no fibrosis. Polygonal hepatocytes and hepatocyte cords exhibiting radial pattern around the central vein and liver sinusoids between cords were in a normal histological appearance.

Carboplatin intoxicated group showed that carboplatin induced septal fibrosis with architectural distortion but no obvious cirrhosis (Stage 3). Treatment of carboplatin intoxicated groups with either Curcumin or CONPs showed some improvement in liver pathology when compared with those in the carboplatin intoxicated untreated group in the form of slightly distorted architecture due to portal fibrosis and the presence of some septa (Stage 2). Also, some sections from these groups showed fibrous portal expansion (Stage 1) with inflammation. However, Co-administration of curcumin and CONPs with carboplatin revealed more improvement of hepatic histopathology, showing preserved architecture with mild inflammation in some cases but no fibrosis (Stage 0) (Figure 5).

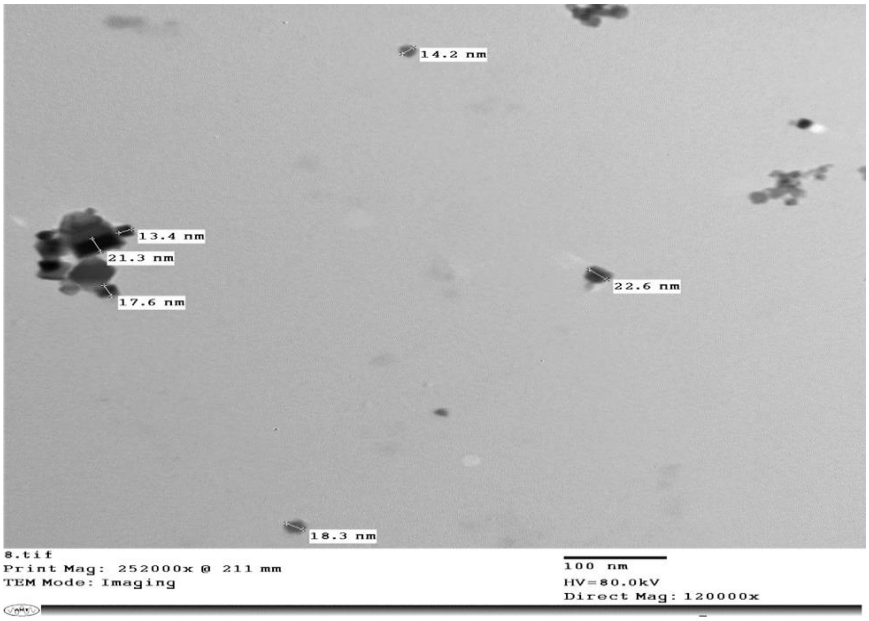

Figure (1): Characterization of CONPs: CONPs as a mixture of cubes and octahedrons. Size observed from TEM was approximately in the range < 25 nm. Some agglomeration was also present.

Table (1): Effect of curcumin, CONPs and the combination on carboplatin-induced toxic effect on WBCs, RBCs & platelets counts in in different groups

Parameter / Groups	WBCs ($X10^3$/ml)	RBCs ($X10^6$/ml)	Platelets ($X10^3$/ml)
Group IA: Negative Control	20.43±4.399^{a}	0.668±0.0652^{a}	1833±333.6^{a}
Group II: Curcumin-treated	19.28±3.274^{ac}	0.700±0.0863^{a}	1911±249.0^{a}
Group III: CONPs-treated	21.24±3.174^{a}	0.680±0.114^{a}	1870±234.2^{a}
Group IV: Carboplatin intoxicated	8.193±1.067^{b}	0.368±0.0747^{b}	1163±141.5^{b}
Group V: Curcumin-treated carboplatin intoxicated	15.65±3.592^{ac}	0.457±0.0565^{b}	1614±134.2^{ab}
Group VI: CONPs-treated carboplatin intoxicated	13.69±3.193^{bc}	0.442±0.0535^{b}	1596±326.8^{ab}
Group VII: Curcumin & CONPs-treated carboplatin intoxicated	15.85±4.189^{ac}	0.487±0.0878^{b}	1717±257.1^{a}

WBCs: White blood cells, RBCs: Red blood cells, CONPs: Cerium oxide nanoparticles

Values represent Mean±SD. Within the same column, values without common superscript small letters are significantly different ($p<0.05$). Number of sacrificed rats in each group=6 rats. Test used is ANOVA and Post Hoc test.

Table (2): Effect of curcumin, CONPs and the combination on carboplatin-induced effect on serum ALT and AST levels in different groups

Parameter / Groups	ALT (U/L)	AST (U/L)
Group IA: Negative Control	74.85±8.221[a]	50.79±7.907[a]
Group II: Curcumin-treated	76.62±8.860[a]	51.08±4.350[a]
Group III: CONPs-treated	80.81±6.854[a]	52.66±8.717[a]
Group IV: Carboplatin intoxicated	158.6±11.68[b]	276.6±16.96[b]
Group V: Curcumin-treated carboplatin intoxicated	113.6±15.64[c]	171.1±19.73[c]
Group VI: CONPs-treated carboplatin intoxicated	105.8±11.29[c]	164.2±15.81[c]
Group VII: Curcumin & CONPs-treated carboplatin intoxicated	86.65±6.363[a]	135.6±10.67[d]

ALT: Alanine transferase enzymes, AST: Aspartate transaminase, CONPs: Cerium oxide nanoparticles

Values represent Mean±SD. Within the same column, values without common superscript small letters are significantly different ($p<0.05$), Number of sacrificed rats in each group=6 rats, Test used is ANOA and Post Hoc test.

Table (3): Effect of curcumin, CONPs and their combination on carboplatin-induced effect on hepatic and bone marrow levels of MDA and GSH in different groups

Parameter / Groups	Hepatic MDA *(nmol/g tissue)*	Hepatic GSH *(mg/g tissue)*	Bone marrow MDA *(nmol/g tissue)*	Bone marrow GSH *(mg/g tissue)*
Group IA: Negative Control	22.84±2.292[a]	44.42±7.450[a]	16.22±2.838[a]	6.228±0.4536 [a]
Group II: Curcumin-treated	25.06±3.229[a]	45.46±6.991[a]	17.04±2.767[a]	6.282±0.4171[a]
Group III: CONPs-treated	25.86±2.906[a]	45.49±5.560[a]	16.99±2.661[a]	6.383±0.5370[a]
Group IV: Carboplatin intoxicated	52.64±9.069[b]	21.14±2.012[b]	36.23±4.882[b]	2.653±0.4381[b]
Group V: Curcumin-treated carboplatin intoxicated	36.28±5.253[c]	30.84±3.375[c]	25.32±2.546[c]	4.132±0.4693[c]
Group VI: CONPs-treated carboplatin intoxicated	37.32±6.343[c]	30.49±1.982[c]	26.15±2.553[c]	4.320±0.4897[c]
Group VII: Curcumin & CONPs-treated carboplatin intoxicated	25.51±3.404[a]	39.23±4.971[ac]	18.93±2.941[a]	5.600±0.5616[a]

MDA: malondialdehyde, GSH: reduced glutathione, CONPs: Cerium oxide nanoparticles

Values represent Mean±SD. Within the same column, values without common superscript small letters are significantly different ($p<0.05$), Number of sacrificed rats in each group=6 rats, Test used is ANOVA and Post Hoc test.

Table (4): Effect of curcumin, CONPs and the combination on carboplatin-induced toxic effect on bone marrow Comet assay parameters in different groups

Parameter / Groups	Tailed *(%)*	Tail length *(µm)*	Tail DNA *(%)*	Tail moment *(Units)*
Group IA: Negative Control	3.000±0.8944[a]	1.325±0.0745[a]	1.167±0.1147[a]	1.838±0.3812[a]
Group II: Curcumin-treated	2.667±0.8165[a]	1.322±0.0877[a]	1.162±0.1061[a]	2.010±0.3839[a]
Group III: CONPs-treated	2.833±0.9832[a]	1.325±0.0862[a]	1.193±0.0948[a]	1.873±0.1911[a]
Group IV: Carboplatin intoxicated	14.50±1.049[b]	3.982±0.5168[b]	4.548±0.3823[b]	17.84±2.265[b]
Group V: Curcumin-treated carboplatin intoxicated	10.33±0.8165[c]	3.215±0.2599[c]	3.408±0.2617[c]	12.75±1.352[c]
Group VI: CONPs-treated carboplatin intoxicated	11.33±0.8165[c]	3.267±0.2406[c]	3.322±0.1888[c]	11.90±1.089[c]
Group VII: Curcumin & CONPs-treated, carboplatin intoxicated	7.667±0.8165[d]	2.633±0.2974[d]	2.765±0.1843[d]	7.572±0.6047[d]

CONPs: Cerium oxide nanoparticles, Values represent Mean±SD. Within the same column, values without common superscript small letters are significantly different ($p<0.05$), Number of sacrificed rats in each group=6 rats, Test used is ANOVA and Post Hoc test.

Table (5): Effect of curcumin, CONPs and the combination on carboplatin-induced toxic effect on 8-OHdG immunoreactivity in the bone marrow.

Period		4 weeks			
8-OHdG Immunoreactivity		Negative (-)	Weak (+)	Moderate (++)	Strong (+++)
Groups (n= 6	Group IA: Negative Control	6	0	0	0
	Group II: Curcumin-treated *n=6*	5	1	0	0
	Group III: CONPs-treated	4	2	0	0
	Group IV: Carboplatin intoxicated	0	0	1	5
	Group V: Curcumin-treated carboplatin intoxicated	0	2	3	1
	Group VI: CONPs-treated carboplatin-intoxicated	0	4	2	0
	Group VII: Curcumin & CONPs-treated carboplatin-intoxicated	0	5	1	0
^ P		0.000**			

CONPs: Cerium oxide nanoparticles; 8-OHdG: 8-hydroxy-2-deoxy guanosine (8-OHdG)
** Statistically highly significant difference (P ≤ 0.001), n: number (number of sacrificed rats in each group=6 rats), ^ = Chi-square test.

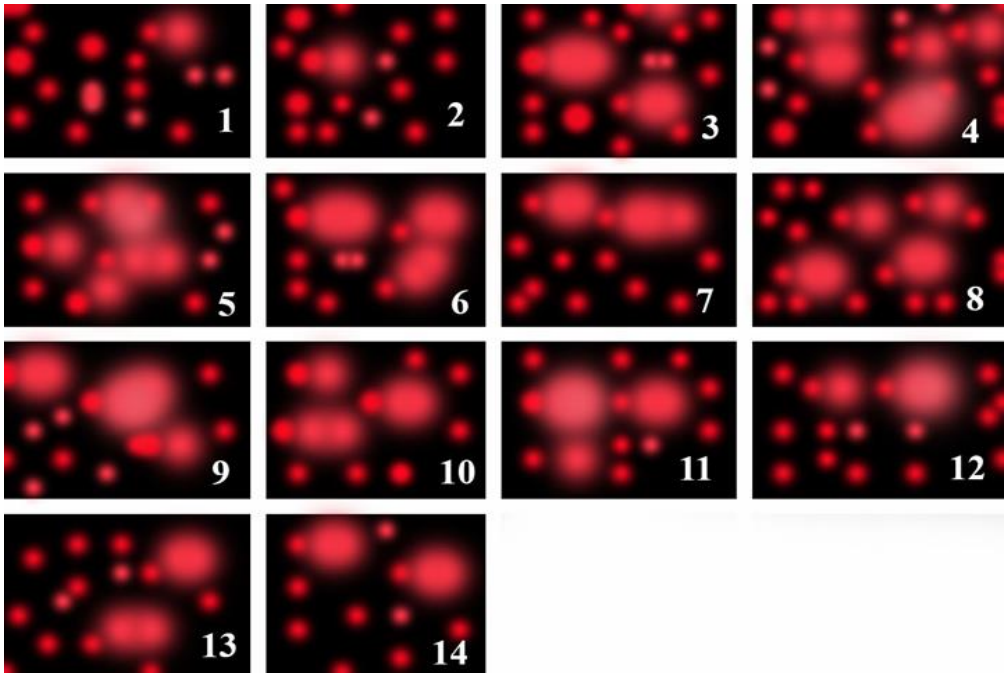

Figure (2): Bone marrow Comet assay (1,2: control groups; 3, 4, 5: carboplatin intoxicated group; 6, 7, 8: carboplatin+curcumin group; 9, 10, 11: carboplatin+CONPs group and 12, 13, 14: carboplatin+curcumin+CONPs group).

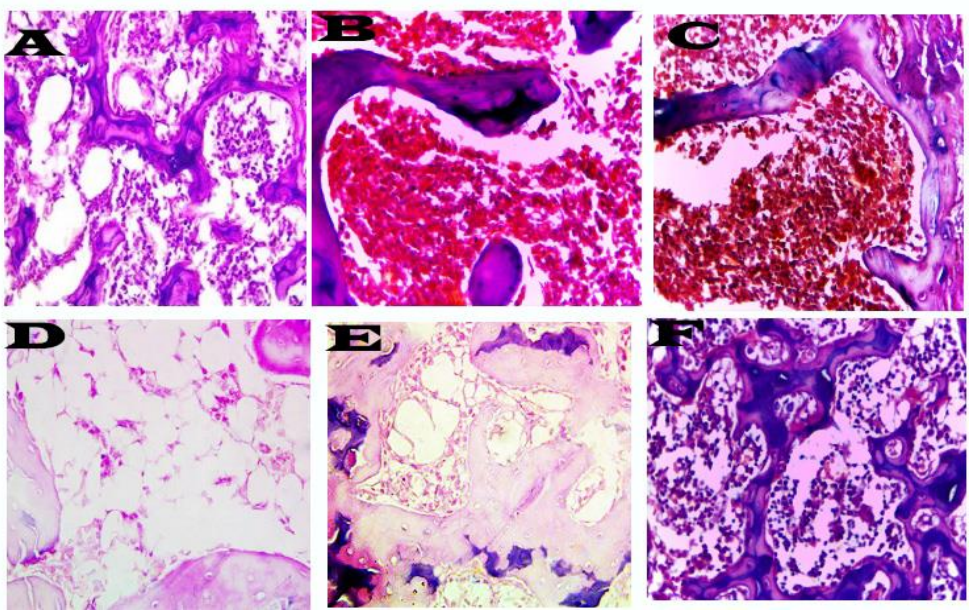

Figure (3): Photomicrograph of bone marrow sections of different groups (hematoxylin and eosin X400 except (F) X200): (A) Control groups showing normal bone marrow biopsy, (B) Curcumin group showing normal bone marrow cellularity, (C) CONPs group with normal cellularity, (D) Carboplatin intoxicated group showing marked hypocellularity which is largely lacking hematopoietic cells and contains mainly fat cells and scattered lymphocytes (E) Carboplatin+Curcumin and Carboplatin+ CONPs groups showing mild hypocellularity with decreased hematopoietic cells in relation to stromal components, (F) Carpoplatin+Curcumin+CNOPs showing improvement in bone marrow cellularity

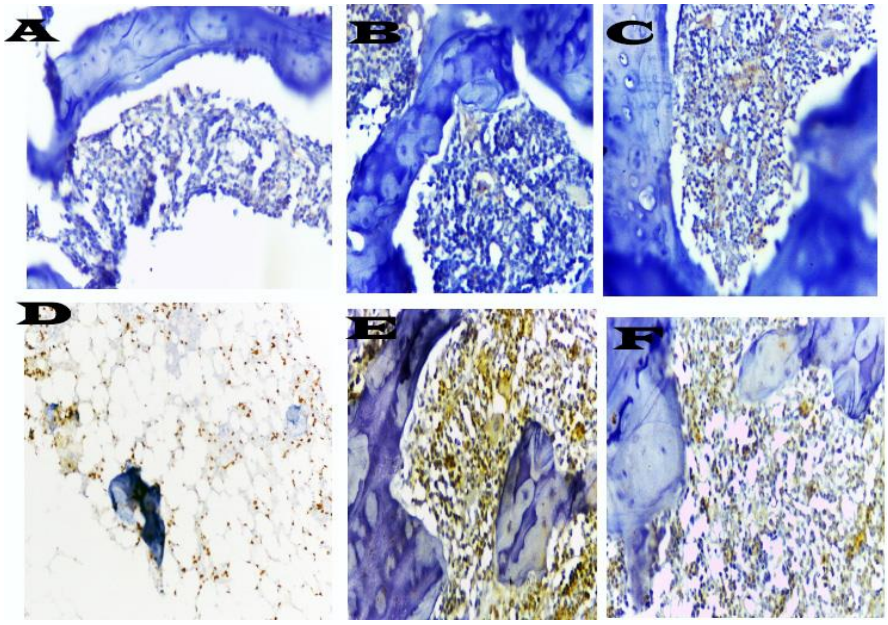

Figure (4): 8OHdG immunostaining of bone marrow sections of different groups (IHCX400): (A) Control groups showing negative 8OHdG immunostaining, (B) Curcumin group with negative 8OHdG immunostaining, (C) CONPs group with negative 8OHdG immunostaining, (D) Carboplatin intoxicated group showing strong nuclear immunostaining in a sever hypocellular bone marrow specimen, (E) Carboplatin + curcumin and Carboplatin+CONPs groups revealing moderate nuclear and cytoplasmic 8OHdG immunostaining.(F) (carboplatin +curcumin+ CNOP) showing mild nuclear and cytoplasmic 8OHdG immunostaining.

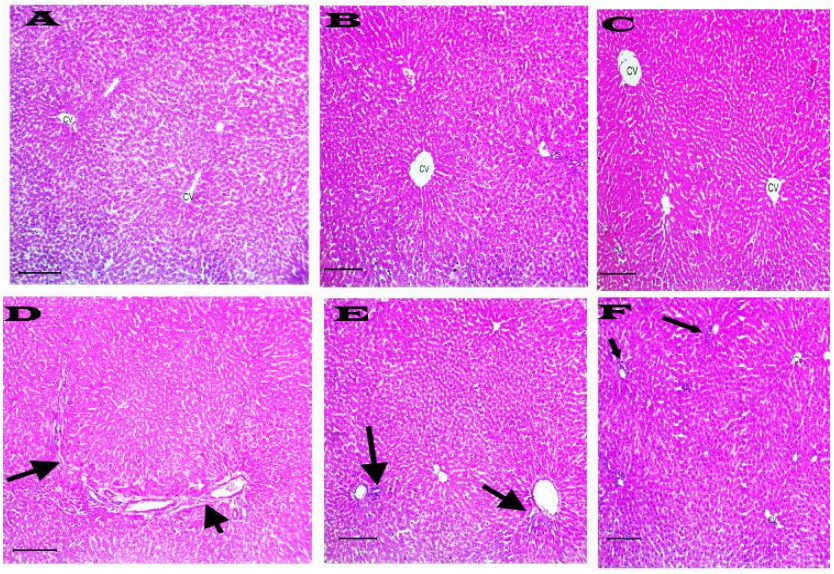

Figure (5): Photomicrographs of liver sections (hematoxylin and eosin X100) of different groups: **(A)** control groups, **(B)** Curcumin group and **(C)** CONPs group showed preserved architecture with no fibrosis (Stage 0), Central Vein: CV. **(D)** Carboplatin intoxicated group showed septal fibrosis(arrows) with architectural distortion but no obvious cirrhosis (Stage 3). **(E)** Carboplatin+curcumin and carboplatin+CONPs treated groups showed some improvement in liver pathology, slightly distorted architecture due to portal fibrosis and portal expansion with inflammation (arrows) (Stage 1). **(F)** Co-administration of curcumin and CONPs with Carboplatin showed preserved architecture with mild inflammation (arrows)but no fibrosis (Stage 0) (scale bar =30 µm.

IV. DISCUSSION

Carboplatin, a platinum compound, is used for the management of many types of cancers in oncology clinics. The clinical use of carboplatin has been linked with various organ toxicities (Pujari and Bandawanea 2019).

Myelosuppression is the major toxic adverse effect of this compound, alongside the noticeable release of free radicals that cause cytotoxicity (Forlani et al., 2018; Erisgin et al. 2019).

In the current study, carboplatin administration induced hematotoxicity, which was confirmed by a significant decrease in hematological parameters (RBCs, WBCs & platelets count). This occurred in association with a significant increase in bone marrow MDA level and a significant decrease in GSH level, indicating oxidative stress. Bone marrow biopsy from carboplatin intoxicated group showed marked hypocellularity which is largely lacking hematopoietic cells and contains mainly fat cells, scattered lymphocytes, and degenerated marrow cells.

Bone marrow cells were tested for DNA damage (comet assay), which revealed significantly higher

percentage of tailed DNA, tail length, tail DNA, and tail moment in carboplatin intoxicated group indicating DNA damage (Unger et al. 2009).

ROS is produced either endogenously or exogenously. In living cells, ROS can attack proteins, lipids, and nucleic acid instantaneously. Free radicals can damage mitochondrial & nuclear DNA leading to severe lesions. 8-hydroxydeoxyguanosine (8-OHdG) is one of these DNA lesions. It is the end-product of the hydroxylation of guanine base. So, it is the most frequently detected and studied DNA lesion (Wu et al. 2004).

DNA lesions in normal conditions are successively excised through a normal base excision repair (BER) pathway. This pathway inhibits the replication of these lesions in DNA. But when the production of free radicles exceeds the capability of repair mechanism of the cell, this will increase DNA injury & mutagenesis (Feng et al. 2006). Therefore, 8-OHdG is an essential marker for assessing the effect of endogenous oxidative damage to DNA by reactive oxygen and nitrogen species (Valavanidis et al. 2009).

In our study, there is a significant increase in 8-OHdG immunostaining in the bone marrow cells of rats intoxicated with carboplatin than control rats which revealed –ve immunostaining.

In our study, carboplatin induced hepatotoxicity was confirmed by the biochemical results which revealed significantly increased levels of serum ALT & AST in carboplatin-intoxicated group compared with the control group in association with the changed pattern of liver cells in histopathological examination in the form of architectural distortion and septal fibrosis. Also, carboplatin produced an increase in the levels of MDA in liver indicating lipid peroxidation, beside a significant decrease in hepatic GSH level indicating oxidative stress.

Several antioxidants have been used in many previous studies to evaluate their efficiency in prevention of carboplatin cytotoxicity (Moon et al. 2011; Erisgin et al. 2019; Hassan et al. 2019). Curcumin as a natural product and nanoparticles of cerium oxide produce good results as antioxidants in several studies (Tomeh et al. 2019; Abdelhamid et al., 2020).

In this work, carboplatin induced hematotoxicity and

hepatotoxicity was improved significantly with combined curcumin and CONPs administration better than each of them alone with carboplatin.

About curcumin, previous studies demonstrated its effectiveness as hematoprotective and hepatoprotective agent. Yılmaz Savcun et al. (2013) reported that curcumin has strong anti-inflammatory and antioxidant effects against the hepatorenal damage caused by free oxygen radicals and lipid peroxidation in experimental model of sepsis induced in rats. Zhang et al. (2015), demonstrated that carboplatin leads to severe myelosuppression and curcumin, as an anti-inflammatory attenuates the effect to some extent.

Chen et al. (2017) concluded that curcumin attenuates carboplatin-induced myelosuppression by activating the DNA repair pathway in bone marrow cells. In their study curcumin significantly improved the survival rate of carboplatin-intoxicated mice. Histologic analysis of bone marrow revealed that curcumin improved carboplatin-induced myelosuppression.

Mohapatra et al. (2019) study on Wistar albino rats with acetylsalicylic acid induced hepatotoxicity reported that curcumin significantly decreased hepatic MDA levels, increased hepatic SOD and GSH levels and improved the histopathology of the livers.

In a study of Guo, et al. (2020), the antioxidant, anti-inflammatory and the anti-apoptotic properties of curcumin were assessed on Acrylamide-induced neurotoxicity in rats. They reported that curcumin at the dose of 100 mg/kg/day significantly decreased the levels of MDA, IL-1β and TNF-α, increased levels of GSH and SOD in cerebral homogenates. In addition, Miao et al. (2021) reported that curcumin improves diabetes induced damage through regulating oxidative stress and inflammation in brain of diabetic rats.

Curcumin has been used in the treatment of inflammatory disorders and cancer for many years. Curcumin can inhibit tumor growth through different mechanisms: antitumor angiogenesis, suppression of proliferation, induction of apoptosis, and prevention of metastasis. Several studies reported curcumin's antitumor activity on breast cancer, lung cancer, head and neck squamous cell carcinoma, prostate cancer, and brain tumors showing its capability to target multiple cancer cell lines (Tomeh et al., 2019).

There is some evidence suggesting that curcumin is an ideal chemosensitizer for chemotherapy and that it helps to protect patients from the side effects of treatment (Song, et al., 2017).

Curcumin is reported to have anti- inflammatory activity by suppressing the NF- kB signaling pathway. Pretreatment of neuroblastoma with curcumin induced an anti- inflammatory effect in colistin- induced toxicity, as it decreases the expression of the pro- inflammatory cyclooxygenase- 2 with subsequent reduction in the NF- kB level (Xu et al., 2018).

In El Khateeb et al. (2020) study Pretreatment with curcumin improved most of the adverse effects in rats treated with CuO NPs regarding oxidative stress and inflammatory indices in kidney and showed that curcumin administration attenuates the toxicity in the kidney of CuO NPs- treated rats through its antioxidant، anti- inflammatory, and antiapoptotic effects.

Regarding CONPs, Abdelhamid et al. (2020) study showed the antioxidant and anti-inflammatory properties of CONPs against oxaliplatin & cisplatin induced neurotoxicity and nephrotoxicity in adult male albino rats. As CONPs ameliorated neurotoxicity of oxaliplatin and nephrotoxicity of cisplatin induced experimentally in male albino rats. Also, Adebayo et al. (2020) demonstrated that pretreatment with CONPs attenuate hepatotoxicity in diethyl nitrosamine-intoxicated mice by downregulating the expression of pro-inflammatory cytokines as well as increasing the activities of antioxidant enzymes.

Amiri et al. (2018) reported the hepatoprotective effect of CONPs on cyclophosphamide- induced hepatotoxicity through antioxidant and anti-apoptotic properties. CONPs inhibited the degeneration in the liver tissue.

Previous studies by Pourkhalili et al. (2011), Navaei-Nigjeh et al. (2012) and Najafi et al. (2017) demonstrated that CONPs administration reduced MDA & reactive oxygen molecules concentration in diabetic rats and increased total antioxidant capacity in plasma and studied tissues.

Also, a study of Xu et al. (2016) demonstrated that administration of nanoparticles of CONPs to male mice model of inflammation and oxidative stress induced by air pollution for 24 weeks

have reduced MDA levels, superoxide radical and hydrogen peroxide. In addition, these nanoparticles diminished the levels of (TNF-α, IL-1β and IL-6) pro-inflammatory cytokines in tissues & serum.

Furthermore, the results of Hirst et al. (2013) are in accordance with our findings, they demonstrated that IP administration of CONPs decreased the markers of oxidative damage including MDA and 8-OHdG in carbon tetrachloride-intoxicated mice. Characterization of nanoparticles is vital for any study. Asati et al. (2010) stated that the size, shape, surface reactivity, degree of aggregation & solubility of nanoparticles determines their action in biological systems.

Therefore, in our study the average size, the primary particle morphology and agglomeration status of CONPs were examined by TEM. It was observed that the size of the nanoparticles was less than 25 nm. Their shape is in the form of octahedrons and cubes, beside some agglomeration was detected. These results are like that of the study of Mittal and Pendy (2014). They used the same product of CONPs powder (purity 99.95% and <25nm particle size purchased from Sigma Aldrich Company of chemicals, USA). Their results about characterization by TEM revealed that the particles were cuboidal in shape and their size from 8 nm to 20 nm with some agglomeration. CONPs have effective antioxidant properties. They have the capability to alternate between two states: (Ce^{3+}) or (Ce^{4+}) by either donation or reception of electrons due to the existence of highly mobile lattice oxygen at their surface.

This is correlated with reduction of ROS levels in cells (Heckman et al. 2013). Alternation from Ce^{4+} to Ce^{3+} induces defects & oxygen vacancies on the nanoparticles surface, producing a cage for oxidation-reduction reactions to happen. CONPs can scavenge hydrogen peroxide and superoxide radicles (Pirmohamed et al. 2010). Therefore, CONPs play as antioxidant enzymes, like superoxide dismutase& catalase and have the capability to counteract peroxynitrite radicle (Dowding et al. 2012).

Many previous studies discussed the use of Curcumin and CONPs in cancer treatment. Previous studies proved that CONPs are toxic to cancer cells by inhibition of their invasion and increasing their response to radiation therapy and chemotherapy

(Gao et al., 2014). CONPs are toxic only to cancer cells. This is caused by generation of ROS and the induction of oxidative stress, at least in part by the inherent oxidase activity of the nanoparticle core at acidic pH like that of cancer cells (Lin et al., 2006). On the other hand, they protect normal tissues from various forms of ROS generation as the physiological pH in normal cells, to which CONPs are not toxic, enables canonical radical scavenging by CONPs (Hirst et al., 2009; Singh et al., 2010). This differential cytotoxicity is important death in normal tissues in line with the protection from other methods of inducing oxidative stress (Madero-Visbal et al., 2012).

V. CONCLUSION

From these results, it can be concluded that carboplatin-induced myelotoxicity and hepatotoxicity are related to the generation of oxidative stress markers and DNA damage. Combined Curcumin and CONPs offer more protection to bone marrow and liver cells against carboplatin related toxicities than does each of them when used alone.

VI. RECOMMENDATIONS

Identification of the exact mechanism by which carboplatin induced toxicities for anticancer drugs to distinguish effectively between tumor cells and normal cells (Gao et al., 2014).

Wang et al. (2013) demonstrated the CONPs induced apoptosis of tumor cells by initiating a mitochondrion-mediated apoptosis signaling pathway. The results also indicated that CONPs were rapidly cleared from the organs and that these particles exhibited little systemic toxicity.

Treatment with CONPs prior to radiotherapy exposure decreases the radiotherapy -induced cell damage and might be helpful in improving the therapeutic strategies. Further studies including cancer models are needed to know more about the effect of CONPs and curcumin on carboplatin treatment on both normal and cancer cells. Future studies are needed to know which dose and route of administration of CONPs are suitable for humans. It is important also to evaluate the long-term effect of CONPs treatment.

VII. Conflict of interest

There is no conflict of interest.

VIII. FUND

The researchers were not assisted financially by any organization.

IX. REFERENCES

- Abdelhamid A, Mahmoud S, Abdelrahman A, Said N, Toam M,

Samy w, Amer M (2020) Protective effect of cerium oxide nanoparticles on cisplatin and oxaliplatin primary toxicities in male albino rats. *Naunyn-Schmiedeberg's Archives of Pharmacology* 393. 10.1007/s00210-020-01946-7.

- Adebayo OA, Akinloye O. and Adaramoye, O.A. (2020) Cerium Oxide Nanoparticles Attenuate Oxidative Stress and Inflammation in the Liver of Diethylnitrosamine-Treated Mice. *Biological trace element research* 193(1):214–225. doi: 10.1007/s12011-019-01696-5.
- Amiri FT, Hamzeh M, Beklar SY, Hosseinimehr SJ (2018) Anti-apoptotic and antioxidant effect of cerium oxide nanoparticles on cyclophosphamide-induced hepatotoxicity. *Erciyes Medical Journal* 40(3):148-154. doi: 10.5152/etd.2018.0016.
- Avci H, Epikmen ET, Ipek E, Tunca R, Birincioglu SS, Akşit H, Sekkin S, Akkoç AN, Boyacioglu M (2017) Protective effects of silymarin and curcumin on cyclophosphamide-induced cardiotoxicity. *Experimental and toxicologic pathology, official journal of the Gesellschaft fur Toxikologische Pathologie* 69(5):317–327. doi: 10.1016/j.etp.2017.02.002.
- Asati A, Santra S, Kaittanis C, Perez, JM (2010) Surface-charge-dependent cell localization and cytotoxicity of cerium oxide nanoparticles. *ACS nano*, 4(9): 5321–5331. doi: 10.1021/nn100816s.
- Beutler E, Duron O, Kelly BM (1963) Improved method for the determination of blood glutathione. *The Journal of laboratory and clinical medicine* 61:882-888.
- Chen X, Wang J, Fu Z, Zhu B, Wang J, Guan S, Hua Z (2017) Curcumin activates DNA repair pathway in bone marrow to improve carboplatin-induced myelosuppression. *Scientific reports* 7(1): 17724. doi: 10.1038/s41598-017-16436-9.
- Cheng YJ, Wu R, Cheng ML, Du J, Hu XW, Yu L, Zhao XK, Yao YM, Long QZ, Zhu LL, Zhu JJ, Huang NW, Liu HJ, Hu YX, Wan F (2017) Carboplatin-induced hematotoxicity among patients with non-small cell lung cancer: Analysis on clinical adverse events and drug-gene interactions. *Oncotarget* 8(19):32228-32236. doi: 10.18632/oncotarget.12951.
- Corsi F, Caputo F, Traversa E, Ghibelli L (2018) Not Only Redox: The Multifaceted Activity of Cerium

Oxide Nanoparticles in Cancer Prevention and Therapy. *Frontiers in oncology* 8:309. doi: 10.3389/fonc.2018.00309.

- Dalian D, Haiyan J, Yong F, Salvi R, Someya S, Tanokura M (2012) Ototoxic Effects of Carboplatin in Organotypic Cultures in Chinchillas and Rats. *Journal of otology* 7(2):92-101. doi: 10.1016/S1672-2930(12)50023-1.
- Das S, Singh S, Dowding JM, et al. (2012) The induction of angiogenesis by cerium oxide nanoparticles through the modulation of oxygen in intracellular environments. Biomaterials.;33:7746–7755.
- Das S, Dowding JM, Klump KE, McGinnis JF, Self W, Seal S (2013) Cerium oxide nanoparticles: applications and prospects in nanomedicine. *Nanomedicine (London, England)* 8(9):1483-1508. doi: 10.2217/nnm.13.133.
- Dowding JM, Dosani T, Kumar A, Seal S, Self WT (2012) Cerium oxide nanoparticles scavenge nitric oxide radical (˙NO). *Chemical communications (Cambridge, England)* 48(40):4896–4898. doi: 10.1039/c2cc30485f.
- Du Q, Deng S, Shen K, Hu B (2011) Effects of curcumin on ROS production and apoptosis in murine hepatocarcinoma Hepa 1-6 cells. *Pharmacology and Clinics of Chinese Materia Medica* 27: 28–31.
- Elkhateeb S, IbrahimT, El-Shal A, Ibrahim O (2020). Ameliorative role of curcumin on copper oxide nanoparticles-mediated renal toxicity in rats: An investigation of molecular mechanisms. Journal of Biochemical and Molecular Toxicology. 10.1002/jbt.22593.
- Erisgin Z, Atasever M, Cetinkaya K, Akarca Dizakar SÖ, Omeroglu S, Sahin H (2019) Protective effects of Nigella sativa oil against carboplatin-induced liver damage in rats. *Biomedicine & pharmacotherapy* 110:742–747. doi: 10.1016/j.biopha.2018.12.037.
- Feng Z, Hu W, Marnett LJ, Tang MS (2006) Malondialdehyde, a major endogenous lipid peroxidation product, sensitizes human cells to UV- and BPDE-induced killing and mutagenesis through inhibition of nucleotide excision repair. *Mutation research* 601(1-2):125–136. doi: 10.1016/j.mrfmmm.2006.06.003.
- Forlani GS, Félix SR, Teixeira LV, Michelon L, Bastos RF, Ribeiro

CL, Freitag RA, Felix AD, Nobre MD (2018) Aqueous wheat extract (Triticuma estivum) prevents carboplatin-induced myelosuppression and oxidative stress in Wistar rats. *Ciência Rural, Santa Maris* 48(10), e20170810

- Gao Y, Chen K, Ma JL, Gao F (2014) Cerium oxide nanoparticles in cancer. *Oncology Targets and Therapy* 7:835-840. doi: 10.2147/OTT.S62057.
- Graham MD (2013) The Coulter principle: Imaginary origins. *Cytometry. Part A: the journal of the International Society for Analytical Cytology* 83(12):1057–1061. doi: 10.1002/cyto.a.22398. Epub 2013 Oct 21.
- Guo J, Cao X, Hu X, Li S, Wang J (2020) The anti-apoptotic, antioxidant and anti-inflammatory effects of curcumin on acrylamide-induced neurotoxicity in rats. BMC pharmacology & toxicology 21(1):62.
- Hassan ES, Majeed S, Mohammad AR, Gaen KK (2019) The protective effect of the N-acetylcysteine on acute liver toxicity induced by carboplatin in rat model. *International Journal of Pharmaceutical Research* 11(3):356-364. doi:10.31838/ijpr/2019.11.03.057.
- Heckman KL, DeCoteau W, Estevez A, Reed KJ, Costanzo W, Sanford D, Leiter JC, Clauss J, Knapp K, Gomez C, Mullen P, Rathbun E, Prime K, Marini J, Patchefsky J, Patchefsky AS, Hailstone RK, Erlichman JS (2013) Custom cerium oxide nanoparticles protect against a free radical mediated autoimmune degenerative disease in the brain. *ACS nano* 7(12):10582–10596. doi: 10.1021/nn403743b.
- Hewlings SJ and Kalman DS (2017) Curcumin: A Review of Its Effects on Human Health. *Foods* 6(10):92. doi: 10.3390/foods6100092.
- Hirst SM, Karakoti A, Singh S, Self W, Tyler R, Seal S, Reilly CM (2013) Bio-distribution and in vivo antioxidant effects of cerium oxide nanoparticles in mice. *Environmental toxicology* 28(2):107–118. doi: 10.1002/tox.20704.
- Joe B, Vijaykumar M, Lokesh, BR (2004) Biological properties of curcumin-cellular and molecular mechanisms of action. *Critical reviews in food science and nutrition* 44(2):97-111. doi: 10.1080/10408690490424702.
- Kaplan SV, Limbocker RA, Gehringer RC, Divis JL, Osterhaus GL, Newby MD, Sofis MJ,

Jarmolowicz DP, Newman BD, Mathews TA, Johnson MA (2016) Impaired Brain Dopamine and Serotonin Release and Uptake in Wistar Rats Following Treatment with Carboplatin. *ACS Chemical Neuroscience* 7(6):689-699. doi: 10.1021/acschemneuro.5b00029.

- Karihtala P, Soini Y (2007) Reactive oxygen species and antioxidant mechanisms in human tissues and their relation to malignancies. *APMIS: acta pathologica, microbiologica, et immunologica Scandinavica* 115(2):81–103. doi: 10.1111/j.1600-0463.2007.apm_514.x.
- Kuhad A and Chopra K (2007) Curcumin attenuates diabetic encephalopathy in rats: behavioral and biochemical evidences. *European journal of pharmacology 576*(1-3): 34–42. doi: 10.1016/j.ejphar.2007.08.001. Epub 2007 Aug 14.
- Lin W, Huang YW, Zhou XD, Ma Y. (2006) Toxicity of cerium oxide nanoparticles in human lung cancer cells. International Journal of Toxicology; 25:451–457.
- Madero-Visbal RA, Alvarado BE, Colon JF, et al. (2012) Harnessing nanoparticles to improve toxicity after head and neck radiation. Nanomedicine.;8:1223–1231.
- Miao C, Chen H, Li Y, Guo Y, Xu F, Chen Q, Zhang Y, Hu M, Chen G (2021) Curcumin and its analog alleviate diabetes-induced damages by regulating inflammation and oxidative stress in brain of diabetic rats. *Diabetology & metabolic syndrome* 13(1):21. doi: 10.1186/s13098-021-00638-3.
- Mittal S and Pandey AK (2014) Cerium oxide nanoparticles induced toxicity in human lung cells: role of ROS mediated DNA damage and apoptosis. *BioMed research international* 2014:891934. doi:10.1155/2014/891934.
- Mohapatra TK, Nayak RR, Subudhi BB, Garrido, G. (2019) Exploration of anti-inflammatory and hepatoprotective effect of curcumin on co-administration with acetylsalicylic acid. *Journal of Pharmacy & Pharmacognosy Research* 7:310-322.
- Moon IJ, Kim KR, Chu HS, Kim SH, Chung WH, Cho YS, Hong SH (2011) N-acetylcysteine and N-nitroarginine methyl ester attenuate Carboplatin-induced ototoxicity in dissociated spiral ganglion neuron cultures. *Clinical and experimental*

otorhinolaryngology 4(1):11–17. doi: 10.3342/ceo.2011.4.1.11.

- Moss DW (1982) Alkaline phosphatase isoenzymes. *Clinical chemistry;* 28(10): 2007–2016.
- Najafi R, Hosseini A, Ghaznavi H, Mehrzadi S, Sharifi AM (2017) Neuroprotective effect of cerium oxide nanoparticles in a rat model of experimental diabetic neuropathy. *Brain research bulletin* 131: 117–122. doi: 10.1016/j.brainresbull.2017.03.013.
- Navaei-Nigjeh M, Rahimifard M, Pourkhalil N, Nili-Ahmadabadi A, Pakzad M, Baeeri M, Abdollahi M (2012) Multi-organ Protective Effects of Cerium Oxide Nanoparticle/Selenium in Diabetic Rats: Evidence for More Efficiency of Nanocerium in Comparison to Metal Form of Cerium. *Asian Journal of Animal and Veterinary Advances* 7(7): 605-612. doi:10.3923/AJAVA.2012.605.612.
- Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry* 95(2):351-358. doi: 10.1016/0003-2697(79)90738-3..
- Pirmohamed T, Dowding M, Singh S, Wasserman B, Heckert E, Karakoti AS, King JE, Seal S, Self WT (2010) Nanoceria exhibit redox state-dependent catalase mimetic activity. *Chemical communications (Cambridge, England)* 46(16):2736–2738. doi: 10.1039/b922024k. Epub 2010 Mar 11.
- Pourkhalili N, Hosseini A, Nili-Ahmadabadi A, Hassani S, Pakzad M, Baeeri M, Mohammadirad A, Abdollahi M (2011) Biochemical and cellular evidence of the benefit of a combination of cerium oxide nanoparticles and selenium to diabetic rats. *World journal of diabetes* 2(11):204–210. doi: 10.4239/wjd.v2.i11.204.
- Pujari RR, Bandawane, DD (2019) Ameliorative effects of Gentisic acid on carboplatin induced hematological toxicities in Wistar Rats. *International Journal of PharmTech Research* 12(3): 22-30. doi: 10.20902/ijptr.2019.120303
- Shiha, G. and Zalata, K. (2001) Ishak versus METAVIR: Terminology, Convertibility and Correlation with Laboratory Changes in Chronic Hepatitis C. In: Takahashi, H., Ed., Liver Biopsy, InTech, Rijeka, 155-170.
- Singh S, Kumar A, Karakoti A, Seal S, Self WT. (2010) Unveiling the mechanism of uptake and sub-cellular

distribution of cerium oxide nanoparticles. Molecular Biosystem; 6:1813–1820.

- Singh NP, McCoy MT, Tice RR, Schneider EL (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental cell research* 175(1):184-191. doi: 10.1016/0014-4827(88)90265-0.
- Song W, Muthana M, Mukherjee J, Falconer RJ, Biggs CA, Zhao X (2017) Magnetic-Silk Core–Shell Nanoparticles as Potential Carriers for Targeted Delivery of Curcumin into Human Breast Cancer Cells. ACS Biomaterials Science and Engineering; 3, 1027–1038.
- Stevens SM, McClelland CM, Trusheim JE, Lee MS (2018) Carboplatin-associated Cranial Neuropathy. *Neuroophthalmology* 42(5):302-305. doi: 10.1080/01658107.2017.1419367.
- Stojanovska V, Sakkal S, Nurgali K (2015) Platinum-based chemotherapy: gastrointestinal immunomodulation and enteric nervous system toxicity. *American Journal of Physiology. Gastrointestinal and Liver Physiology* 308(4): G223-G232. doi: 10.1152/ajpgi.00212.2014.
- Sundar Dhilip Kumar S, Houreld NN, Abrahamse H (2018) Therapeutic Potential and Recent Advances of Curcumin in the Treatment of Aging-Associated Diseases. Molecules 23(4):835. doi: 10.3390/molecules23040835.
- Tomeh MA, Hadianamrei R, Zhao X (2019) A Review of Curcumin and Its Derivatives as Anticancer Agents. *International journal of molecular sciences* 20(5): 1033. doi: 10.3390/ijms20051033.
- Toyokuni S, Tanaka T, Hattori Y, Nishiyama Y, Yoshida A, Uchida K, Hiai H, Ochi H, Osawa T (1997) Quantitative immunohistochemical determination of 8-hydroxy-2'-deoxyguanosine by a monoclonal antibody N45.1: its application to ferric nitrilotriacetate-induced renal carcinogenesis model. *Laboratory investigation. a journal of technical methods and pathology* 76(3):365–374.
- Unger FT, Klasen HA, Tchartchian G, de Wilde RL, Witte I (2009) DNA damage induced by cis- and carboplatin as indicator for in vitro sensitivity of ovarian carcinoma cells. *BMC Cancer* 9:359. doi: 10.1186/1471-2407-9-359.
- Valavanidis A, Vlachogianni T, Fiotakis C (2009) 8-hydroxy-2' -

deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. *Journal of environmental science and health. Part C, Environmental carcinogenesis & ecotoxicology reviews* 27(2):120–139. doi: 10.1080/10590500902885684.

- Wang Y, Yang F, Zhang HX, et al. (2013) Cuprous oxide nanoparticles inhibit the growth and metastasis of melanoma by targeting mitochondria. Cell Death and Disease; 4: e783.
- Wu LL, Chiou CC, Chang PY, Wu JT (2004) Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. *Clinica chimica acta; international journal of clinical chemistry* 339(1-2): 1–9. doi: 10.1016/j.cccn.2003.09.010.
- Xu MX, Zhu YF, Chang HF, Liang Y (2016) Nanoceria restrains PM2.5-induced metabolic disorder and hypothalamus inflammation by inhibition of astrocytes activation related NF-κB pathway in Nrf2 deficient mice. *Free radical biology & medicine 99*: 259–272. doi: 10.1016/j.freeradbiomed.2016.08.021.
- Xu XY, Meng X, Li S, Gan RY, Li Y, Li HB (2018) Bioactivity, Health Benefits, and Related Molecular Mechanisms of Curcumin: Current Progress, Challenges, and Perspectives. Nutrients.;10(10):1553. doi: 10.3390/nu10101553.
- Yılmaz Savcun G, Ozkan E, Dulundu E, Topaloğlu U, Sehirli AO, Tok OE, Ercan F, Sener G (2013) Antioxidant and anti-inflammatory effects of curcumin against hepatorenal oxidative injury in an experimental sepsis model in rats. *Ulusal travma ve acil cerrahi dergisi = Turkish journal of trauma & emergency surgery* 19(6):507–515. doi: 10.5505/tjtes.2013.76390.
- Zhang BY, Wang YM, Gong H, Zhao H, Lv XY, Yuan GH, Han SR (2015) Isorhamnetin flavonoid synergistically enhances the anticancer activity and apoptosis induction by cis-platin and carboplatin in non-small cell lung carcinoma (NSCLC). *International journal of clinical and experimental pathology 8*(1):25–37.
- Zilva JF, Pannall PR (1979) Plasma enzymes in diagnosis in clinical chemistry in diagnosis and treatment. *Lioyd – Luke London* Chapter 17:338.

الملخص العربي

التأثير الوقائي للكركمين و جسيمات أكسيد السيريوم النانوية على السمية النخاعية والسمية الكبدية المستحثة بالكاربوبلاتين في ذكور الجرذان ويستار البالغة

مروة عبد المنعم عامر[1]، دينا محمد نجيب عبد المعوض[1]، ريهام سامح[2]، رشا أحمد عجاجة[3]، نسرين علواني[4]، أميرة محمد عبد الحميد[4]

[1] قسم الطب الشرعي والسموم الإكلينيكية، كلية الطب البشري، جامعة الزقازيق، مصر.
[2] قسم علم الأمراض، كلية الطب البشري، جامعة الزقازيق، مصر.
[3] قسم علم التشريح والأجنة، كلية الطب البشري، جامعة الزقازيق، مصر.
[4] قسم علم الأدوية، كلية الطب البشري، جامعة الزقازيق، مصر.

خلفية البحث: الكاربوبلاتين هو دواء كيميائي يستخدم في علاج العديد من أنواع السرطانات المختلفة. يؤثر الكاربوبلاتين سلبًا على أعضاء متعددة فى الجسم مثل نخاع العظام والكبد والجهاز الهضمي والكلى. **الهدف من البحث:** يهدف العمل الحالي إلى تقييم التأثير الوقائي المحتمل للكركمين و جسيمات أكسيد السيريوم النانوية على السمية النخاعية والسمية الكبدية المستحثة بالكاربوبلاتين في ذكور الجرذان ويستار البالغة.**طريقة البحث:** تم اجراء البحث على ثمانية وأربعون من ذكور الجرذان ويستار البالغة و قد صنفوا إلى سبع مجموعات متساوية. I. المجموعة الضابطة (نقسم الي المجموعة الضابطة السالبة والمجموعة الضابطة الموجبة) ، II. مجموعة الكركمين ، III. مجموعة جسيمات أكسيد السيريوم النانوية ، IV. مجموعة الكاربوبلاتين ، V. مجموعه الكاربوبلاتين و الكركمين، VI. مجموعه الكاربوبلاتين و جسيمات أكسيد السيريوم النانوية، VII. مجموعه الكاربوبلاتين و الكركمين و جسيمات أكسيد السيريوم النانوية. وكانت مدة الدراسة 4 أسابيع متتالية، تم بعدها تخدير الجرذان و جمع عينات الدم و عينات من نخاع العظام و الكبد لإجراء دراسات بيوكيميائية ، ودراسات الهستوباثولوجيا والكيمياء المناعية واختبار المذنب كومت للحمض النووى. **النتائج**: أدى العلاج بالكاربوبلاتين إلى انخفاض عدد خلايا الدم وارتفاع نسبه إنزيمات الكبد وارتفاع نسبه المالونديالديهيد فى الكبد و نخاع العظام، بينما انخفضت مستويات الجلوناثايون فى الكبد و نخاع العظام. وقد أظهر اختبار المذنب كومت تلف الحمض النووي فى خلايا نخاع العظام. تسبب الكاربوبلاتين أيضا في نقص الخلايا في عينات نخاع العظم وأظهر نشاط مناعي قوي لل8-هيدروكسي-2-ديوكسي غوانوزين، كما تسبب في حدوث تليف و تشويه في أنسجة الكبد. وأظهرت النتائج أن تناول الكركمين و جسيمات أكسيد السيريوم النانوية قد خفف من هذه التأثيرات السامة للكاربوبلاتين وأن استخدامهم معا اعطى تحسن أفضل بكثير من أي منهما بمفرده. **الخلاصة**: استخدام الكركمين و جسيمات أكسيد السيريوم النانوية يحسن السمية النخاعية والسمية الكبدية التي يسببها الكاربوبلاتين.

التوصيات: قد يكون تحديد الآلية الدقيقة لكيفية التسمم بالكاربوبلاتين مفيدًا في تحسين الاستراتيجيات العلاجية. هناك حاجة إلى عمل مزيد من الدراسات التى تضم نماذج للسرطان لمعرفة المزيد عن تأثير جسيمات أكسيد السيريوم النانوية والكركمين على العلاج بالكاربوبلاتين على كل من الخلايا الطبيعية والسرطانية. و هناك حاجة أيضا لدراسات مستقبلية لمعرفة الجرعة والطريقة المناسبة لاستخدام جسيمات أكسيد السيريوم النانوية فى الإنسان، كما أنه من المهم أيضًا تقييم التأثير طويل المدى للعلاج بجسيمات أكسيد السيريوم النانوية.