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Therapeutic activity of curcumin in natural and nano forms on copper oxide nanoparticles induced renal toxicity, oxidative stress, and DNA damage in rat

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Abstract

Metal nanoparticles (NPs) that are considered to be copper oxide nanoparticles (CuONPs) have received more attention in recent years and have a significant impact on everyday life due to their large area of influence. Therefore, the current study sought to explore the therapeutic effects of curcumin (Cur) and/or curcumin nanoparticles (CurNPS) concerning DNA damage, oxidative stress, apoptosis, and inflammation in the kidneys of rats. 60 male rats in the maturity stage were divided into 6 groups (G1, control; G2, Cur; G3, Cur NPS; G4, CuONPs; G5, CuONPs+Cur; G6, CuONPs+CurNPS). Current results revealed a significant elevation in serum levels of creatinine, urea, potassium ions, chloride ions, kidney MDA, DNA damage, kidney injury, PCNA, and P53 expressions, and a significant decline in sodium ions, and calcium ions when compared to control. Interestingly, treatment of CuONPs with Cur or CurNPS revealed modulation in these parameters compared to the CuONPs group with the most favorable results observed in CuONPs+CurNPs.

Keywords: Copper oxide nanoparticles, Curcumin nanoparticles, kidney, oxidative stress, PCNA& P53.

Introduction

CuONPs are metal particles that are relatively low-cost-photocatalytic and stable in terms of their physical and chemical properties. They play a significant role in everyday life due to their wide-reaching impact area [1-3]. Nanoparticles can enhance cellular toxicity by constructing ROS which leads to apoptosis via activation of intracellular signals plus DNA damage and subsequently results in autophagic death [4-8]. Although CuNPs have proven beneficial within biomedical applications, the potential adverse effects are still the primary drawback hindering their use in medicine despite reasons for favoring it [9-11].

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CuONPs exposure has been shown to cause DNA damage, genotoxicity, and oxidative stress. Many in vivo and in vitro studies demonstrate this finding [12-15]. On the other hand, numerous studies have reported: that some plant-derived chemical compounds are safe and yet have documented therapeutic effects [16-18].

A polyphenolic compound of significant yellowish hue, curcumin originates from the fundamental principles of turmeric (Curcuma longa). It has high antioxidant properties. The use of this component is used in treating many diseases related to inflammation such as atherosclerosis, Alzheimer's, diabetes, arthritis, and intestinal disease [19-21]. Some limitations associated with the clinical use of curcumin include poor watery solubility, lack of stability, and fast digestion plus a short half-life which causes photodegradation that results in a lack of bioavailability when administered [22].

In addition: there are some plants or chemicals that can be transformed into NPs free from any toxic effects and thus applied for the treatment of a wide range of other diseases [23-25]. To improve the efficacy of curcumin, it can be converted into CurNPs— nanoparticles that have low pharmacokinetic availability and solubility. An approach demonstrated by Nawar et al. [26] and other studies [27-29]. The use of nanotechnology has enabled the development of CurNPs from curcumin. In this research, we studied the protecting effects of Cur as well as its analogs (including CurNPs) against toxicity induced by CuONPs in male rats through enhancement oxidative stress plus DNA damage leading to decreased renal function.

Materials and methods

Materials

CuONPs with a size of 25 ± 5 nm were approved by Nano-Fab-Technology in Cairo, Egypt. We bought curcumin (Cur) from ADVENT CHEMBIO in Mumbai, India. The Egyptian nanotech company was the source of Cur NPs.

Ethics approved

The rats were acquired from the NRC in Giza, Egypt and the research design was approved by the Institutional Ethics Committee for Animal Care and Use (IACUC-SCI-TU-0314).

Animals and Experimental Design

60 male rats (Rattus norvigicus; weighing 180 g and aged 12 weeks) were divided randomly into six groups: G1, which had no treatment at all; G2, the control group that received (50 mg/kg body weight/day) orally via a stomach tube for two weeks; G3, the control NP group that received (50 mg/kg body weight/day) orally via a stomach tube for two weeks; and G4, the CuO NPs group that received (200 mg/kg body weight/day) intraperitoneally for four weeks.

In the end, rats were anesthetized using sodium pentobarbital and killed. Blood samples were collected in sterile tubes that allowed clotting for ten minutes at room temperature; then centrifuged for fifteen minutes at 3000 r.p.m.

Chemical analysis:

Before that, the serum was put into Eppendorf tubes and stored at -60 ˚C. Animals were beheaded after the preparation of their dissection, and then kidneys were taken out. Two halves are made from each kidney part, one to be used in histopathological studies after washing with physiological saline (0.9%) and fixation in neutral formalin solution (10%). The other half was lumped together for different analyses after balancing each of the paired organs.

The assessment of renal function and electrolyte balance

The serum levels of urea and creatinine were determined based on the method outlined by Patton and Crouch [30] while the study by Mutar et al. [31] used Indian Sensa-core electrolyte bottles that are commercially available to measure potassium, calcium, sodium, and chloride ions.

Kidney homogenate biochemical assays

After the tissues from the kidneys were individually weighed, they were homogenized with a Potter Elvenhjem tissue homogenizer. After collecting the

crude tissue homogenate by centrifuging for 15 minutes at 11.739 rcf in a chilled centrifuge, the supernatant was saved for multiple estimations.

Enzymatic and non-enzymatic antioxidant assays

Mesbah et al. [32] and Beutler et al. [33], reported MDA content in kidney homogenate and GSH concentration both in the same tissue. CAT was introduced by Saggu et al. [34] while TAC was introduced by Lim and Lim [35].

Detection of total genomic DNA fragmentation via agarose gel electrophoresis

Elgharabawy et al. [36] reported that the salting-out extraction procedure revealed DNA fragmentation detection.

Histological processing

A procedure was done in fixing the kidneys by immersing them into 10% formalin, which is later neutralized by a nontoxic solution after 24-48 hours of dehydration, clearing, and paraffin impregnation were carried out on sections of the tissues then stained with hematoxylin and eosin [37].

Detection of proliferating cell nucleus antigen (PCNA), and apoptotic P53

PCNA was identified as well as the p53 expression in kidney samples by the Avidin-Biotin complex method — according to Tousson et al. [38,39] respectively. The brown cells were quantitated after being excised from those that were stained using the thresholding function of the Image J program, which isolated the brown color from the rest of the pixels for cell count purposes.

Statistical analysis

ANOVA was used as the statistical procedure to compare the means of different treatment groups. The data were summarized using mean ± standard error. The results were considered significant if $p < 0.01$, and the software used in this study was Graph Pad Prism developed by Inc., La Jolla, CA, USA.

Results

Impacts of Cur NPs on kidney functions and electrolytes

Table 1 indicated that CuONPs altered kidney function parameters by increasing creatinine, urea, potassium, and chloride ions while decreasing sodium and calcium ion levels as compared to the control group. Conversely, interactions between CuONPs and Cur or CurNPs (CuONPs+Cur or CuONPs+CurNPs) changed profiles of renal functions and electrolytes in comparison to CuONPs alone; the most favorable effects were observed in CuONPs+CurNPs.

Impact of Cur NPs on Oxidative Stress

Table 2 showed that CuONPs increased the level of MDA in the kidneys, and decreased the amount of GSH, CAT, and total antioxidant capacity (TAC), in comparison to controls. Conversely, the treatment of CuONPs with Cur or Cur NPs (CuONPs+Cur or CuONPs+CurNPs) demonstrated a difference in these parameters compared to the CuONPs group, the greatest benefit was observed in CuONPs+CurNPs.

Impact of CurNPs in kidney DNA fragmentation

An analysis in Figure 1 revealed that CuONPs escalated DNA harm within the kidney tissue compared to the control sample. However, the administration of Cur or CurNPs extract with CuONPs led to an increment in the count of DNA damaged molecules in kidneys, when compared to CuONPs, and the highest rise in DNA damage was observed for CuONPs+ CurNPs.

Table 1: Changes in kidney functions and electrolytes parameter in different groups.

	Control	**Cur**	**CurNPs**	**CuONPs**	**CuONPs+Cur**	**CuONPs+CurNPs**
Creatinine (mg/dl)	$0.44^{\#} \pm 0.051$	$0.40^{\#} \pm 0.051$	$0.38^{\#} \pm 0.044$	$0.94^{*} \pm 0.093$	0.82 ± 0.037	0.68 ± 0.037
Urea (mg/dl)	$33.5^{\#} \pm 2.39$	$30.8^{\#} \pm 1.37$	$31.0^{\#} \pm 3.76$	$87.0^{*} \pm 6.17$	$54.7^{*\#} \pm 4.19$	$40.5^{\#} \pm 1.85$
Na^{+} (mmol/l)	$135.5^{\#} \pm 8.29$	$136.8^{\#} \pm 7.44$	$136.8^{\#} \pm 6.24$	$129.5^{*} \pm 9.43$	$132.2^{*\#} \pm 8.11$	$138.9^{\#} \pm 6.82$
K^{+} (mmol/)	$4.01^{\#} \pm 0.30$	$4.19^{\#} \pm 0.48$	$4.14^{\#} \pm 0.45$	$4.89^{*} \pm 0.29$	$4.51^{*\#} \pm 0.26$	$4.27^{*\#} \pm 0.34$
Cl^{-} (mmol/l)	$100.6^{\#} \pm 5.52$	$103.0^{\#} \pm 7.39$	$102.9^{\#} \pm 5.99$	$113.9^{*} \pm 7.33$	$111.2^{*\#} \pm 6.77$	$104.1^{\#} \pm 8.39$
Ca^{2+} (mmol/l)	$1.098^{\#} \pm 0.03$	$1.164^{\#} \pm 0.05$	$1.110^{\#} \pm 0.04$	$0.868^{*} \pm 0.04$	$1.102^{*\#} \pm 0.04$	$1.122^{\#} \pm 0.02$

Data were expressed as mean ± SE of 10 observations. Significant difference from the control at $^{*}p < 0.01$ and from the CuNPs group at $^{\#}p < 0.01$.

Table 2: Changes in the levels of MDA (nmol/mg), GSH (nmole/g tissue), CAT (µmole/min/g tissue), and total antioxidant capacity (nmole/g tissue) activities in rats kidney tissues in different groups.

kidney	**Control**	**Cur**	**CurNPs**	**CuONPs**	**CuONPs+Cur**	**CuONPs+CurNPs**
MDA (nmol/mg)	$0.82^{\#} \pm 0.017$	$0.88^{\#} \pm 0.052$	$0.67^{\#} \pm 0.069$	$1.51^{*} \pm 0.052$	$1.30^{*\#} \pm 0.012$	$1.14^{*\#} \pm 0.058$
CAT (U/g)	$3.0^{\#} \pm 0.108$	$3.13^{\#} \pm 0.145$	$3.08^{\#} \pm 0.176$	$0.99^{*} \pm 0.084$	$1.35^{*\#} \pm 0.880$	$1.97^{*\#} \pm 0.145$
GSH (nmol/mg)	$1.84^{\#} \pm 0.041$	$2.09^{\#} \pm 0.043$	$2.12^{\#} \pm 0.096$	$0.51^{*} \pm 0.032$	$0.98^{*\#} \pm 0.049$	$1.71^{\#} \pm 0.031$
TAC (g/dl)	$43.1^{\#} \pm 0.67$	$38.7^{\#} \pm 0.81$	$40.5^{\#} \pm 0.40$	$20.5^{*} \pm 0.44$	$35.4^{*\#} \pm 0.69$	$39.2^{\#} \pm 0.85$

Data were expressed as mean ± SE of 10 observations. Significant difference from the control at $^{*}p < 0.01$ and from CuNPs group at $^{\#}p < 0.01$.

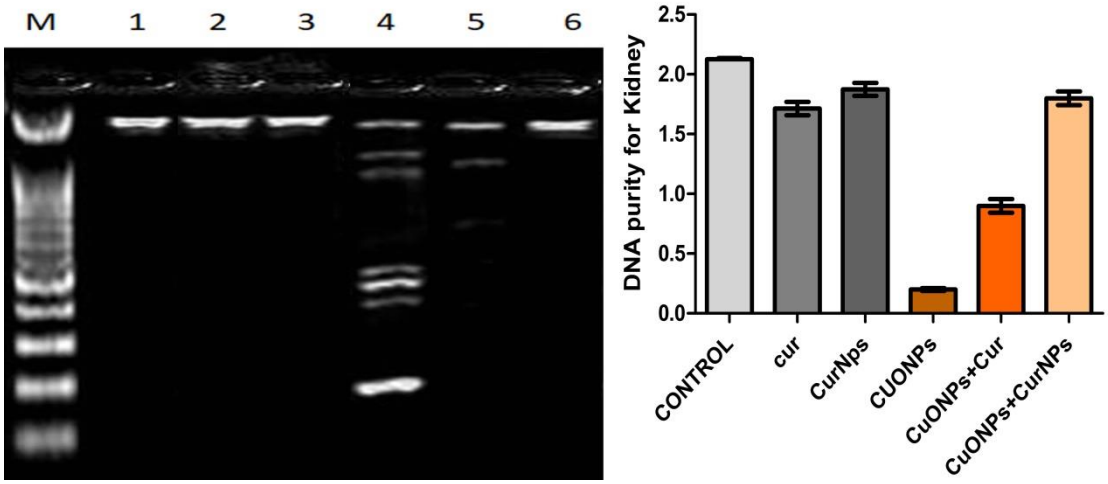

Figure 1: Changes in kidney DNA fragmentation in different groups. Where, G1, control; G2, Cur; G3, CurNPs; G4, CuONPs; G5, CuONPs+Cur; G6, CuONPs+Cur NPs.

3.4. Impact of Cur and CurNPs on the Kidney Structure

Control and treated groups' kidney samples were observed for glomeruli, renal tubules, cortex, and medulla (Figures 2A–2C). The control group had a normal distribution. However, in the treated rats there was a decrease in the inability to open Bowman's capsules and increase in size; inflammation and necrosis were also present (Fig.2D). CuONPs+Cur group showed mild changes in renal corpuscle size with damage to Bowman's capsules and mild necrosis (Fig.2E); while CuONPs and CurNps induced abnormal changes with presence of mild necrosis only (Fig.2F).

3.5. Impact of Cur and Cur NPs on PCNA expression

Regarding the PCNA expressions in rat kidneys, control rats and treated rats with Cur or its NPs depicted positive reactions which were mild (Fig. 3A–3C); nonetheless, an escalation was noted in the expression of PCNA with the use of CuO NPs (Fig.3D). Conversely, moderate to mild levels of PCNA were observed when CuO NPs+Cur and CuO Nps+Cur Nps were present, respectively (Fig. 3E, 3F).

3.5. Impact of Cur and Cur NPs on P53 expression

In control rats, Cur or its NPs did not show any positive reactions at all but only negative or faint positive reactions (Fig. 4A-4C) concerning P53 expression in kidney sections. However, there was a moderate to strong positive relationship between P53 and behaviors of treated rats observed for CuO NPs (Fig.3D). On the contrary, when it came to the presence of CuO NPs+Cur and CuO Nps+Cur Nps, the reactions were strongly positive for the P53 gene (Fig. 4E, 4F).

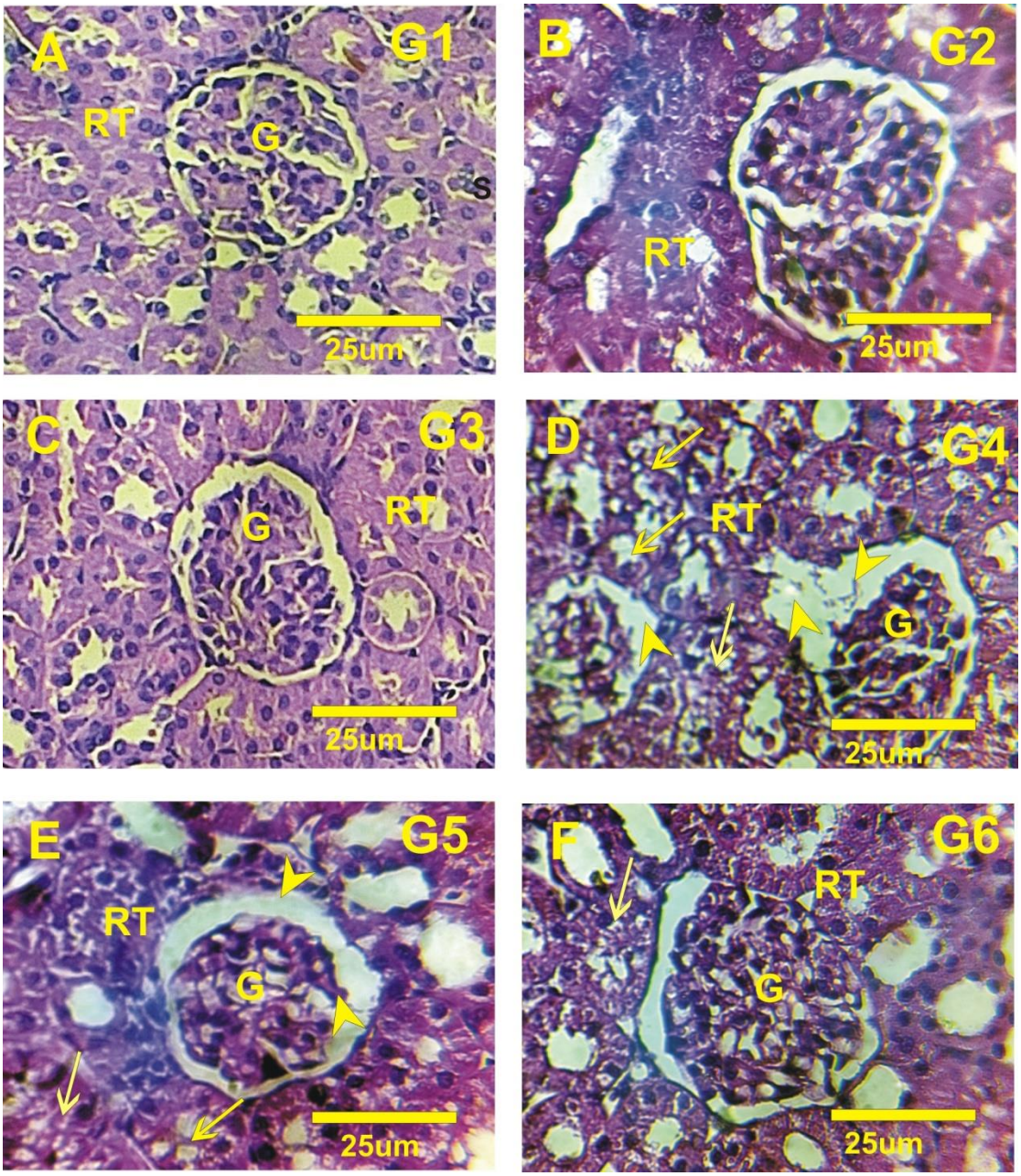

Figure 1: Kidney sections of different groups stained with H&E. A-C: Normal structure of glomeruli and renal tubules in control, Cur and Cur NPs groups. D: Marked hypertrophy in glomeruli (arrowheads) and renal tubules and marked necrosis (arrows) in tubular cells. E: Moderate hypertrophy of the renal corpuscles(arrows) and moderate necrosis (arrows) in tubular cells in kidney sections in CuONPs+Cur. F: Normal structure except only mild necrosis (arrows) in tubular cells in kidney sections in the CuONPs+CurNps group.

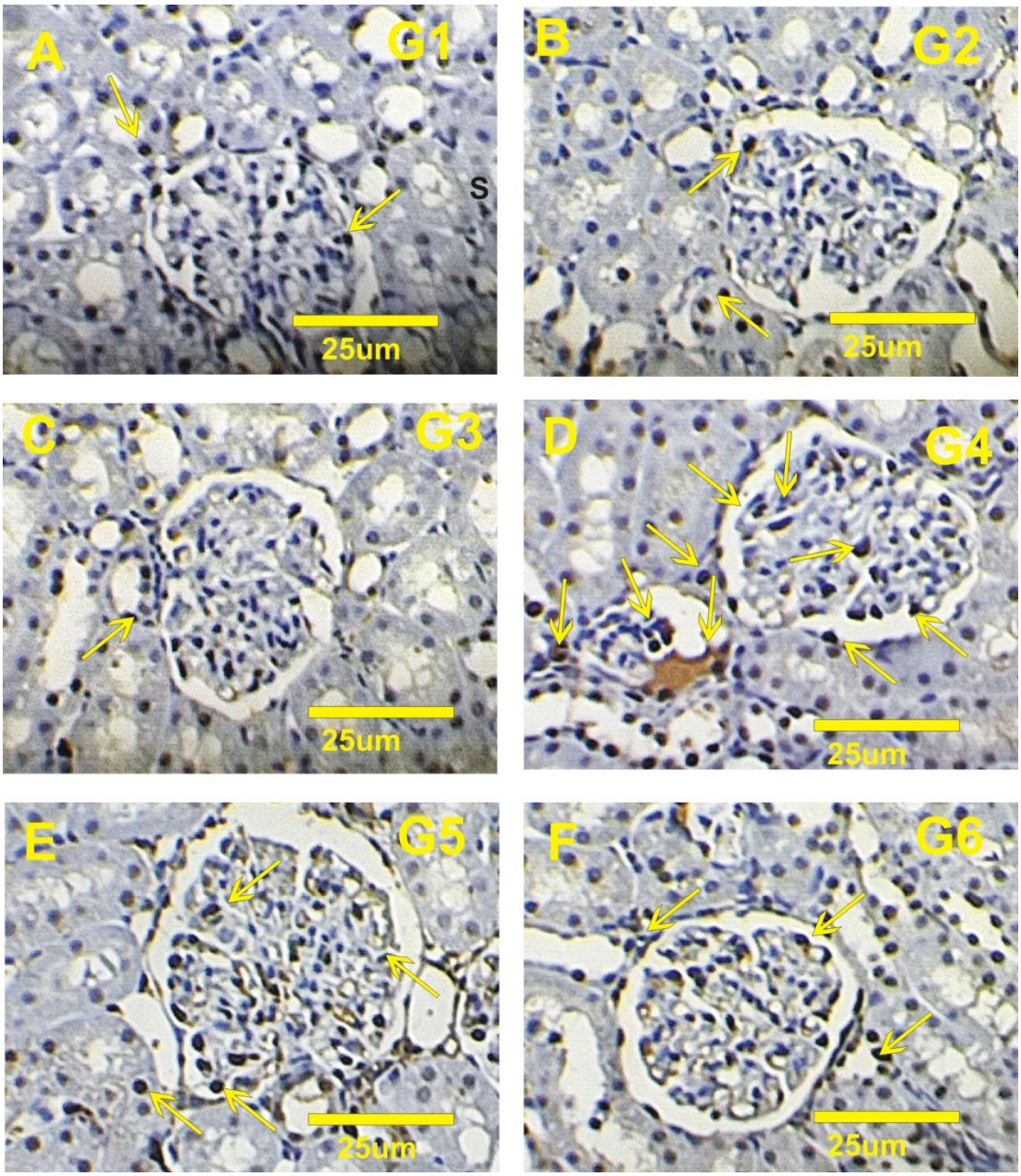

Figure 3: Photomicrographs of kidney sections stained with PCNA in different groups. A-C: Mild positive reaction for PCNA expressions in control (G1), Cur (G2), and Cur NPs (G3) groups. D: Strong positive reaction in CuO NPs group (G4). E, F: Moderate and mild positive reaction in CuO NPs+Cur. And CuO NPs+Cur Nps respectively.

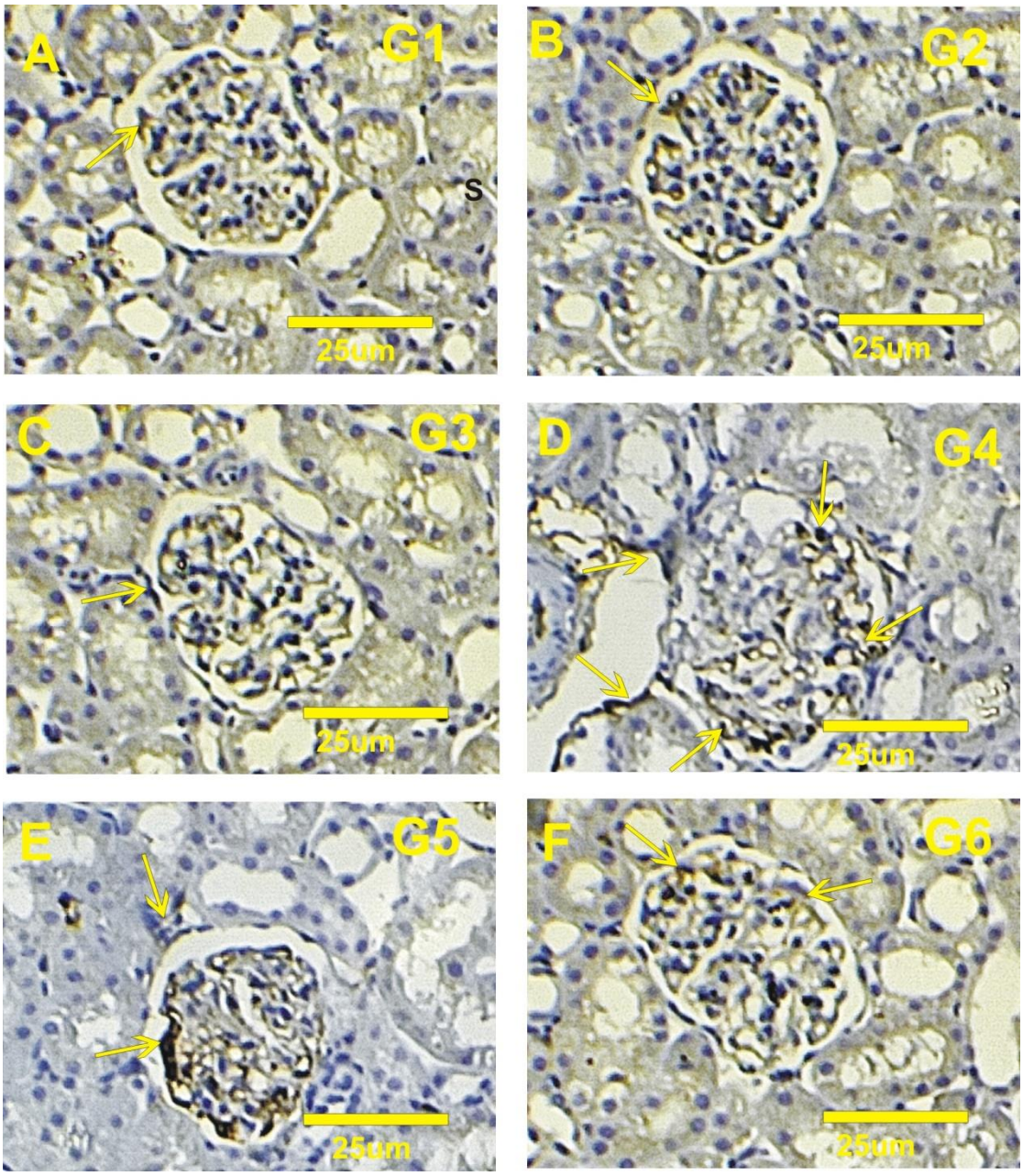

Figure 4: Photomicrographs of kidney sections stained with P53 in different groups. A-C: Faint positive reaction in control (G1), Cur (G2), and Cur NPs (G3) groups. D: Strong positive reaction in CuO NPs group (G4). E, F: Moderate and mild positive reaction in CuO NPs+Cur and CuO NPs+Cur Nps respectively.

Discussion

Recently, CuONPs have been used in the production of various pharmaceuticals antibiotics primarily developed to help fight or prevent infection caused by resistant Staphylococcus aureus or E. coli as well as sensors, catalysts, pigments, rotating semiconductors, and other devices. Therefore, this study tries to probe the impact of curcumin naturally and in micro size on CuONPs-induced toxicity via rats' damage to DNA and oxidative stress. The current findings include an increase in creatinine plus urea level, potassium ions which are due to kidney damage with decreasing levels of sodium ions noted, and calcium ions from the rat samples when treated with CuONPs. This effect can be seen as causing an increase in potassium ions while decreasing those of sodium ions which is a clear indication of kidney damage hence our findings support Shotop [40] while Ghonimi et al. [41] supported Mohamed et al. [42] that documented; toxicity caused by CuONPs in male rats' liver and kidneys.

The findings from the ongoing investigation showed that administering CuONPs as well as either Cur or CurNPs significantly affected kidney function and electrolytes compared to when only CuONPs were administered. The most pronounced changes were recorded when both Cur and CuONPs were used together (CuONPs+CurNPs). Our results are in agreement with those of Elkhateeb [43] who looked into the therapeutic effects of curcumin on CuONPs-induced nephropathy among rats.

The latest data indicates that CuONPs have brought about an elevation of MDA levels in kidneys and a decrease in GSH, catalase, and TAC compared to controls. This study is supported by Yahya et al. [44] who found that copper oxide nanoparticles increased oxidative damage in male albino rats. Our results are consistent with Mohamed et al. [42] who found that CuONPs reduced antioxidant defense in male Westar rats. Copper oxide nanoparticles have been shown to lower antioxidant defenses in rat kidneys. Husain et al. [45] documented this finding, hence endorsing our results as well. Similarly, Yahya et al. [44] found that copper oxide nanoparticles induce oxidative damage in male rats which contradicts the development of an effective defense system thus affirming our findings also.

We arrived at the same findings as Chen et al. [46], who showed that CuONPs had a significant toxicological effect and acute kidney injury in experimental mice these results were also supported by Tousson and El-Gharbawy [15] who documented that CuO NPs increased stressors in cardiac tissues of rats. In our current results, we observed that treatment of CuONPs with Cur or CurNPs changed these parameters compared to the CuONPs group; the most beneficial results were found in CuONPs+CurNPs.

The current study showed that CuO NPs have a significant effect on the kidneys, this effect is associated with apoptosis, DNA damage, and an increase in PCNA expression. These findings are additionally supported by Ahmed et al. [47], who reported that; CuONPs lead to genotoxicity in human lung cells of epithelial origin. These findings are supported by Husain et al. [45] documented that; the administration of CuONPs to rats leads to DNA damage in the kidneys. Other pertinent information is also endorsed by Mosa et al. [48], which studies the protective effects of CurNPs towards the DNA damage and stress associated with hydroxyapatite particles in male rats. Our results are in line with those of Naz et al. [49], who documented the damage to DNA in different parts of the Japanese quail's anatomy. Our results are in line with the findings of Ghonim et al. [41] who documented the damage to the liver and kidneys in adult males from the Westar breed. Our results are in agreement with Ahmed et al. [50] described CuONPs as causing glomerular thickening along with the addition of mononuclear cells that are associated with inflammation, as well as an increase in the number of renal interstitial spaces. The current results demonstrated that; treatment of CuONPs with Cur or CurNPs decreased

the number of DNA damage, PCNA, and P53 in comparison to CuONPs. These findings are also supported by Mosa et al. [48], who study the healing role of CurNPs in preventing the development of pathological changes in hydroxyapatite nanoparticles.

Conflict of interest: None

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