Curcumin and Nano-Curcumin Mitigate Neurotoxicity

Subjects: Biochemistry & Molecular Biology

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Curcumin (CUR) is a hydrophobic polyphenolic compound found natively in turmeric. It exhibits antioxidant, antimicrobial, anti-inflammatory, pulmoprotective, anti-diabetic, hepatoprotective, nephroprotective, and antitumor actions. In addition to these pharmacological effects, CUR possesses neuroprotective activity where it protected the brain against oxidative injury induced by heavy metals.

Keywords: curcumin ; GSK-3β ; inflammation ; DNA damage ; oxidative stress

1. Introduction

Copper (Cu) is a redox-active metal found in many organs and tissues. It is essential for a plethora of biochemical processes such as blood clotting, iron absorption, protein homeostasis, energy production, and cellular metabolism [1]. It acts as a cofactor necessary for many redox-regulating proteins[2]. Cu homeostasis is maintained within the normal level by precise regulatory mechanisms that regulate its absorption, excretion, and blood level[3]. Genetic alteration in Cu-regulating ATPases, ATP7A, and ATP7B can cause Menkes disease (MD) and Wilson disease (WD), respectively [2][4][5]. MD is associated with a defect in Cu absorption and severe Cu deficiency, while WD results in Cu toxicity and affects several organs, including the liver, brain, and eye. Chronic exposure to Cu has been implicated in the pathogenesis of neurodegenerative diseases such as Alzheimer's disease [6]. Parkinson's disease[7], and familial amyotrophic lateral sclerosis (ALS) [2][8].

Copper sulphate (CuSO4) is a well-known pesticide used for repelling pests that decreases the crop yield in agriculture. It is commonly used in tissue culture incubators to minimise the contamination risk as it has bactericidal and fungicidal properties. Accidental or intentional CuSO4 intoxication can induce multiorgan dysfunctions that could be fatal. The systemic absorption of Cu occurs through the gastrointestinal tract, lungs, and skin[9]. The clinical manifestations of Cu toxicity are erosive gastropathy, acute liver and kidney injuries, intravascular hemolysis, arrhythmia, rhabdomyolysis, and seizures [10]. Although the mechanisms of CuSO4 toxicity are not fully addressed, they represent a combination of significant oxidative stress and endocrine perturbation in the vulnerable organs of the body[11]. Animal studies showed that the chronic oral administration of CuSO4 causes liver and kidney functional impairment due to increased Cu levels in the respective organs [12]. The toxic effects of Cu on the liver and kidney have been studied extensively, while the toxicities of other vital organs of the body are less documented. Similar to other metals, the management of Cu toxicity includes the use of chelating agents such as D-penicillamine, tetrathiomolybdate, and trientine[13]. Other chelators such as deferoxamine (DFO) have an affinity for Cu binding[14]. Despite the effectiveness of these chelators, they often associated with some serious adverse effects on cardiovascular, gastrointestinal, respiratory, and nervous systems, which necessitates the use of safer alternatives. In addition, the limited or moderate effectiveness of these chelators has been found in some cases.

2. N-CUR and CUR Attenuate Cu-Induced Cerebral Oxidative Stress

The ameliorative effect of CUR and N-CUR on oxidative stress in the brain of Cu-exposed rats was evaluated through the assessment of malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT). Cerebral MDA was significantly elevated in Cu-administered rats when compared with the control group ($p < 0.001$; **Figure 1**A). In contrast, cerebral GSH content (**Figure 1**B), SOD activity (**Figure 1**C), and CAT activity (**Figure 1**D) were decreased in Cu-administered rats ($p < 0.001$). Treatment with DFO, CUR, and N-CUR decreased MDA and increased GSH, SOD, and CAT in the brain of Cu-administered rats. The effect of both CUR and N-CUR on cerebral MDA was significant compared to DFO ($p < 0.01$).

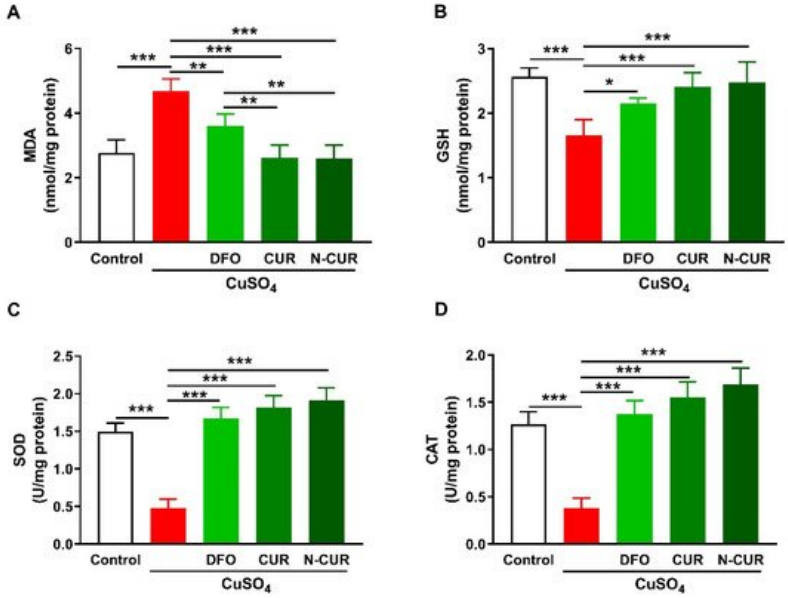

Figure 1. N-CUR and CUR attenuate Cu-induced cerebral oxidative stress. Treatment with N-CUR, CUR, and DFO decreased MDA (**A**) and increased GSH (**B**), SOD (**C**), and CAT (**D**) in the brain of Cu-administered rats. Data are mean ± SEM, (n = 8). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

3. N-CUR and CUR Suppress Cerebral Inflammation in Cu-Administered Rats

Cerebral levels of NF-κB p65, TNF-α, and IL-6 were assayed to determine the ameliorative effect of CUR and N-CUR on inflammation induced by Cu ingestion (**Figure 2**). Cu administration increased NF-κB p65 (**Figure 2**A), TNF-α (**Figure 2**B), and IL-6 (**Figure 2**C) in the cerebrum of rats ($p < 0.001$). All treatments (DFO, CUR, and N-CUR) decreased the levels of cerebral NF-κB p65, TNF-α, and IL-6 significantly ($p < 0.001$). N-CUR was more effective in decreasing cerebral NF-κB p65 ($p < 0.05$) than DFO, and TNF-α, and IL-6 as compared to either DFO or CUR.

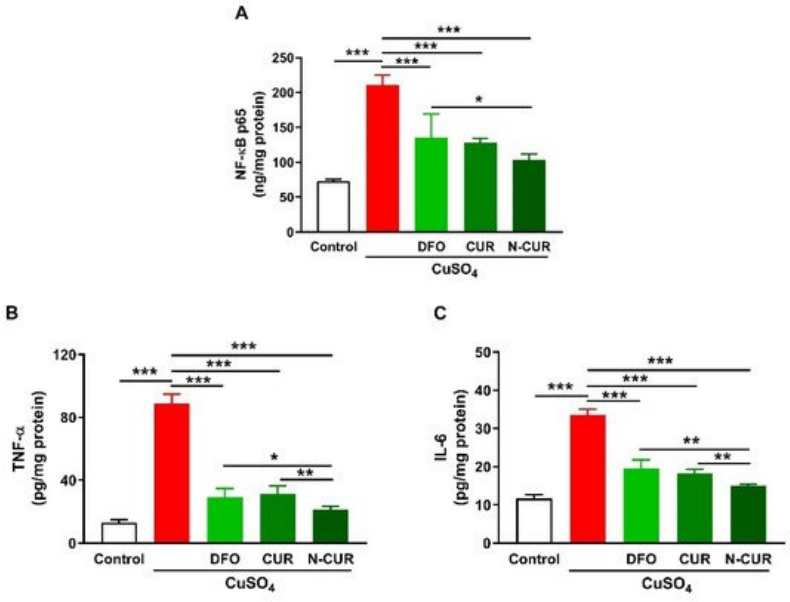

Figure 2. N-CUR and CUR suppress inflammation in Cu-administered rats. Treatment with N-CUR, CUR, and DFO decreased cerebral (**A**) NFκB p65, (**B**) TNF-α, and (**C**) IL-6. Data are mean ± SEM, (n = 8). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

4. N-CUR and CUR Prevent Apoptosis in Cu-Administered Rats

The expression levels of BAX, caspase-3, and p53 were significantly increased in the cerebrum of rats exposed to Cu as compared to the control group, as depicted in **Figure 3**. In contrast, rats administered with Cu exhibited a remarkable downregulation of cerebral BCL-2 expression. DFO, CUR, and N-CUR significantly downregulated BAX, p53, and caspase-3 and upregulated BCL-2 in the cerebrum of Cu-administered rats. The effect of N-CUR on BAX and BCL-2 was

significant when compared with CUR, whereas its effect was more potent on BAX, caspase-3, and p53 than the effect of DFO.

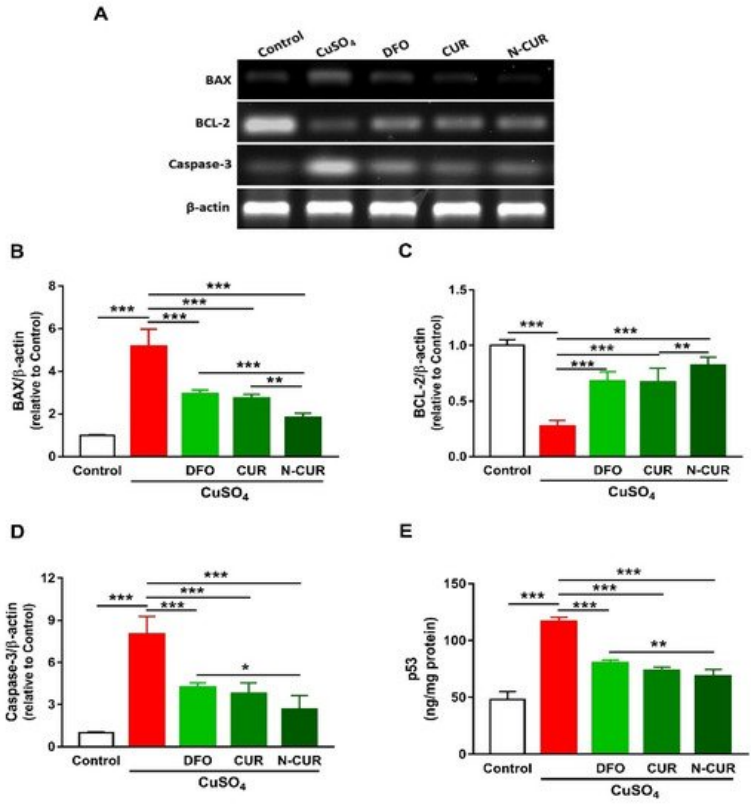

Figure 3. N-CUR and CUR prevent apoptosis in Cu-administered rats. (**A**) Representative blots showing changes in the expression of BAX, BCL-2, and caspase-3. (**B**–**E**) N-CUR, CUR, and DFO decreased (**B**) BAX, increased (**C**) BCL-2, and downregulated (**D**) caspase-3, and (**E**) p53 expression in the brain of Cu-administered rats. Data are mean ± SEM, (n = 8). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

The beneficial effects of CUR and N-CUR against Cu-induced cerebral cell death were further confirmed via assessment of DNA fragmentation (**Figure 4**). Cu-administered rats showed an increase in DNA fragmentation levels as compared to the control group ($p < 0.001$). All treatments (DFO, CUR and N-CUR) prevented the deleterious effect of Cu on DNA integrity.

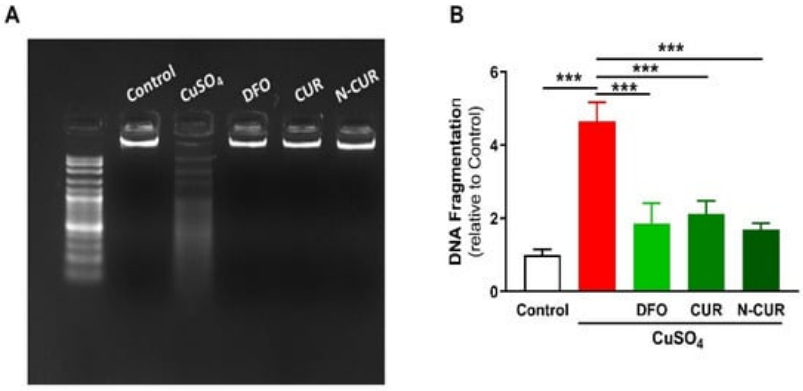

Figure 4. N-CUR, CUR, and DFO prevent DNA fragmentation in the brain of Cu-administered rats. DNA fragmentation was assessed by (**A**) agarose gel electrophoresis and (**B**) colorimetric methods. Data are mean ± SEM, (n = 8). *** $p <$ 0.001.

5. N-CUR and CUR Upregulate AKT/GSK-3β Signaling in Cu-Administered Rats

To investigate the effect of Cu and the ameliorative effect of DFO, CUR, and N-CUR on cerebral AKT/GSK3β signaling, the phosphorylation levels of AKT and GSK3β were determined using Western blotting (**Figure 5**). Cu-treated rats exhibited a significant decrease in pAKT Ser473 and pGSK-3β Ser9 as compared to the normal rats ($p < 0.001$).

Treatment with DFO, CUR, or N-CUR increased cerebral AKT and GSK-3β phosphorylation levels. N-CUR exerted a stronger effect on AKT/GSK-3β signaling than DFO and CUR.

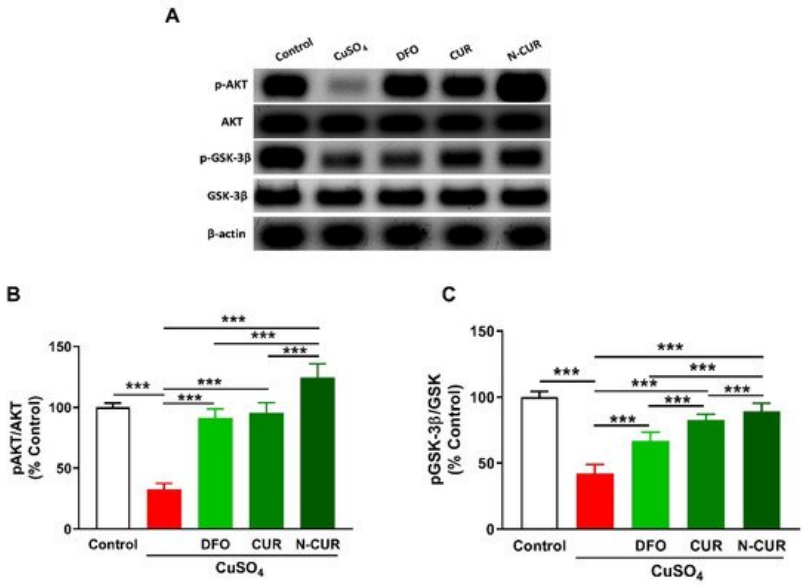

Figure 5. N-CUR and CUR upregulate AKT/GSK-3β signaling in Cu-administered rats. (**A**) Representative blots of pAKT, AKT, pGSK-3β, and GSK-3β. (**B**,**C**) N-CUR, CUR, and DFO increased AKT Ser473 (**B**) and GSK-3β Ser9 (**C**) phosphorylation in the brain of Cu-administered rats. Data are mean ± SEM, (n = 8). *** $p < 0.001$.

6. N-CUR and CUR Upregulate Brain-Derived Neurotrophic Factor (BDNF) in Cu-Administered Rats

The administration of Cu resulted in a significant downregulation of BDNF expression in the cerebrum of rats, as shown in **Figure 6**. Treatment of the Cu-administered rats with DFO, CUR, or N-CUR increased the levels of cerebral BDNF. While the effect of CUR on BDNF was significant as compared to DFO, the effect of N-CUR was more potent when compared to both treatments.

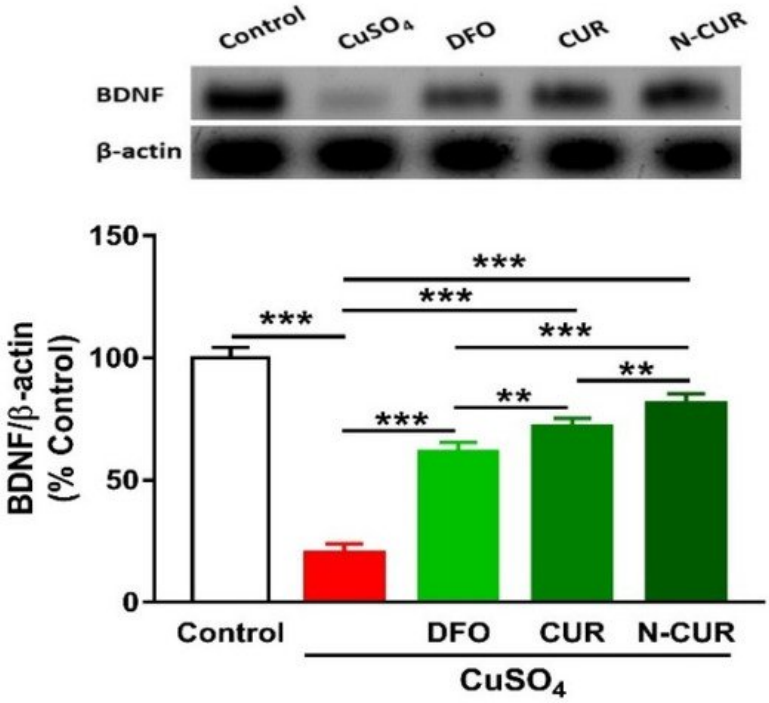

Figure 6. N-CUR and CUR upregulate BDNF in Cu-administered rats. Data are mean ± SEM, (n = 8). ** $p < 0.01$ and *** $p < 0.001$.

7. Conclusions

These results confer new information on the protective effect of N-CUR on Cu neurotoxicity. N-CUR and CUR attenuated oxidative stress, inflammation, cell death, and oxidative DNA damage in the brain of Cu-administered rats. The modulatory effect of N-CUR and CUR on AKT/GSK-3β signaling was involved, at least in part, in their protective activity against Cu neurotoxicity. The neuroprotective effect of N-CUR was stronger when compared to the native form, which is an effect that could be attributed to the improved properties of CUR.

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