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Curcumin ameliorates mancozeb-induced neurotoxicity in rats

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Abstract

The study investigated the ameliorative effect of curcumin (CUR) against mancozeb (MZ)-induced neurotoxicity in rats. MZ-treated rats (750 mg/kg b.wt) showed a significant increase in lipid peroxidation, protein carbonyl content, nitric oxide, glutamate and gamma-aminobutyric acid levels and a significant decrease in reduced glutathione level and activity of glutathione peroxidase, glutathione reductase and glutathione-S-transferase in brain. Furthermore, MZ increased immunohistochemical expression of inducible nitric oxide synthase in cerebral cortex. Concurrent treatment with CUR mitigated all these toxic effects. This study concluded that CUR ameliorated MZ-induced neurotoxicity in rats.

Keywords: Curcumin, Mancozeb, Neurotoxicity, Oxidative Stress.

Introduction

Mancozeb (MZ) is a manganese/zinc ethylene-bis-dithiocarbamate (Mn/Zn-EBDC) fungicide that is commonly used in agriculture for controlling a broad variety of fungal infections of vegetables and ornamental plants[21)]. However MZ has low acute toxicity, it possesses neurotoxic and hepatotoxic effects in experimental animals[21,24)]. Curcumin (CUR) diferuloylmethane) is a yellow pigment isolated from the rhizome of *Curcuma longa*. CUR has a variety of therapeutic applications owing to its antioxidant and free radical scavenging properties[16)]. Moreover, CUR possesses neuroprotective effects against various toxic agents such as arsenic[23)]. This study aimed to investigate the ameliorative effect of CUR against MZ-induced neurotoxicity in rats through estimation of oxidative stress markers, amino acid neurotransmitters in brain and histopathological and immunohistochemical examinations of cerebral cortex.

Materials and Methods

Chemicals and reagents:

Dithane M-45 (Mancozeb 80 % wettable powder) was provided from Dow Agro Sciences Co., France while CUR was obtained from Sigma–Aldrich (St. Louis, MO, USA).

Experimental design:

Twenty-four healthy adult male Sprague-Dawley rats, weighting 200 ± 10 g were divided into four groups (six per each). Group I (control group) received oral daily doses of 0.5% carboxymethyl cellulose (1ml/rat) as a vehicle for 10 weeks. Group II (CUR-treated group) was daily gavaged with 100 mg/kg b.wt CUR dissolved in 0.5% carboxymethyl cellulose for 10 weeks[23)]. Group III (MZ-treated

group) was orally administered MZ dissolved in distilled water at a dose of 750mg/kg b.wt (1/20 LD_{50}) daily for10 weeks. The LD50 of MZ in rats was 15000 mg/kg b.wt[13]. Group IV (MZ + CUR-treated group) was co-treated with MZ (750 mg/kg b.wt) and CUR (100 mg/kg b.wt) as previously mentioned.

Sample collection:

Animals were anesthetized using diethyl ether and then sacrificed. The brain was immediately excised and perfused with ice cold saline. Some brain specimens were preserved at -20°C while cerebral cortex was dissected and preserved in 10% neutral-buffered formalin for histopathological and immunohistochemical examinations.

Estimation of oxidative stress markers and amino acid neurotransmitters:

Brain specimens were homogenized in potassium phosphate buffer solution (10% w/v, pH 7.4), then centrifuged at 3000 rpm for 15 min. Protein carbonyl (PC) content, Malondialdehyde (MDA), Reduced glutathione (GSH), Nitric oxide (NO) concentrations and Glutathione peroxidase (GPx), Glutathione reductase (GR) and Glutathione-S-transferase (GST) activities were estimated according to the methods of Levine *et al.* [15], Ohkawa *et al.* [18], Beutler *et al.* [2], Cattell *et al.* [5], Gross *et al.*, [10], David and Richard[6] and Harada *et al.* [11] respectively. The glutamate and gamma-aminobutyric acid (GABA) levels were estimated in brain homogenate using Enzyme linked immunosorbent assay (ELISA) kits (MyBioSource, Inc., California, USA).

Histopathological and immunohistochemical studies:

For histopathological investigation, the cerebral cortex specimens were processed and stained with hematoxylin and eosin (H&E) [1]. The immunohistochemical detection of inducible nitric oxide synthase

(iNOS) positive cells using primary rabbit polyclonal anti-rat iNOS antibody (Thermo Fisher Scientific Inc., Fremont, CA, USA) was performed by avidin–biotin complex method[20].

Statistical analysis:

The data was analyzed using one way analysis of variance (ANOVA) followed by the post hoc Duncan's test for comparison between different experimental groups (IBM SPSS Statistics, version 22).

Results and Discussion

Our results revealed that MZ induced brain oxidative damage that was evident by increased lipid peroxidation (MDA), PC content (index of protein oxidation) and NO level. Moreover, a significant ($p<0.05$) decrease in GSH concentration and antioxidant enzyme activities (GPx, GR and GST) was observed in brain of MZ-treated rats when compared with control group (Table 1). In a similar study, MZ induced oxidative stress in liver of rats[24]. The MZ-induced oxidative stress may be attributed to its chemical structure containing transitional metals which catalyze the production of ROS through the Fenton reaction[4] and subsequently causing oxidative damage to macromolecules such as lipids, protein and DNA[8].

In Table 1, a significant ($p<0.05$) increase in brain GABA and glutamate concentrations was recorded in MZ-treated rats when compared with control group. These findings were concordant with a previous study revealing that MZ induced damage to GABAergic cells[7].The increase in brain glutamate level may be attributed to the inhibition of glutamate uptake by MZ exposure[22]. The role of Mn in the neurotoxic effect of MZ had been reported[7]. Additionally, Mn exposure caused alterations in glutamatergic and GABAergic neurotransmitter systems[19].

Our results also indicated that co-administration of CUR with MZ mitigated oxidative and nitrosative stress in rat brain caused by MZ through a significant ($p<0.05$) reduction of lipid peroxidation, protein oxidation and NO level. Besides, a significant increase in GSH concentration and antioxidant enzyme activities (GPx, GR and

GST) was also observed in brain of MZ + CUR-treated group when compared with MZ-treated one (Table 1). This effect may be attributable to its antioxidant properties and scavenging activity

Table 1. Effect of CUR on oxidative stress markers and amino acid neurotransmitters in the brain of MZ-treated rats (Mean ± SE) (n = 6).

	Groups			
Parameters	Control	CUR	MZ	MZ + CUR
PC (nmol/mg protein)	4.03 ± 0.09 [c]	4.01 ± 0.08 [c]	5.77 ± 0.05 [a]	4.56 ± 0.06 [b]
MDA (nmol/g tissue)	46.80 ± 3.20 [c]	47.51 ± 3.72 [c]	164.76 ± 3.63 [a]	72.84 ± 2.99 [b]
NO (μmol/g. tissue)	37.23 ± 1.00 [c]	38.64 ± 2.58 [c]	98.05 ± 2.16 [a]	67.66 ± 1.41 [b]
GSH (μg/g tissue)	6.45 ± 0.23 [a]	6.70 ± 0.19 [a]	3.24 ± 0.13 [c]	4.75 ± 0.15 [b]
GPx (ng/g tissue)	31.40 ± 0.73 [a]	31.66 ± 0.57 [a]	15.68 ±1.69 [b]	25.61 ± 0.35 [a]
GR(ng/g tissue)	4.00 ± 0.13 [a]	3.52 ± 0.08 [a]	1.17 ± 0. 23 [c]	2.39 ± 0. 28 [b]
GST (ng/g tissue)	0.73 ± 0.07 [a]	0.72 ±0.04 [a]	0.23 ± 0.02 [c]	0.45 ± 0.02 [b]
GABA (pg/ml tissue)	52.27 ± 1.91 [c]	50.85 ± 2.12 [c]	120.96 ± 3.47 [a]	67.72 ± 2.94 [b]
Glutamate (nmol /ml tissue)	0.45 ± 0.06 [c]	0.48 ± 0.08 [c]	1.25 ± 0.11 [a]	0.78 ± 0.06 [b]

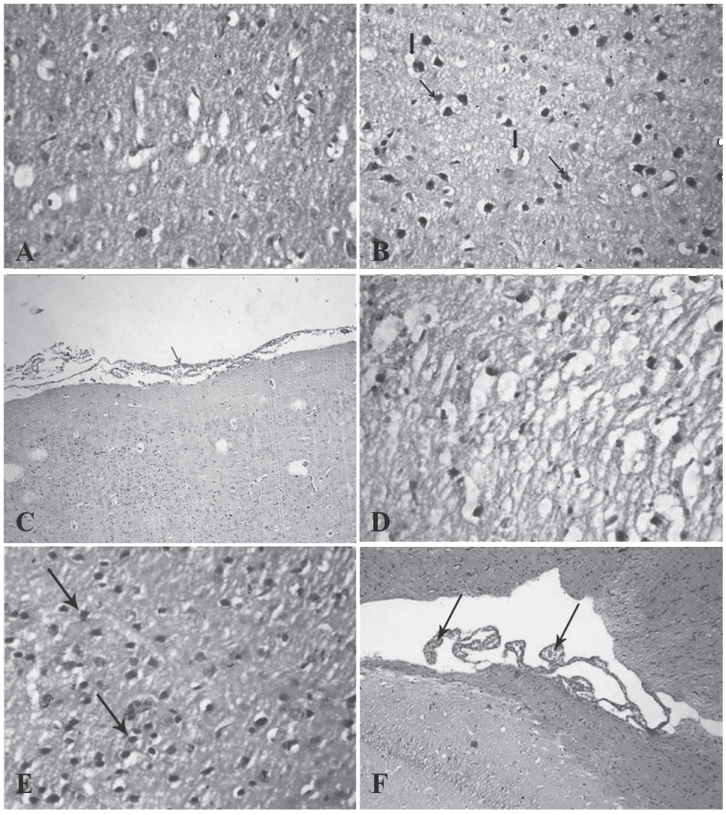

Fig. 1. Photomicrographs of cerebral cortex sections of control and experimentally treated rats stained with H&E under light microscopy. A; Control and CUR-treated groups showing normal histological structure of cerebral cortex (1200x). (B-D); MZ-treated group showing: B; Numerous necrotic neurons (thin arrow) beside perinuronal and perivascular edema in cerebral cortex (thick arrow) (1200x). C; Sub meningeal edema and congested meningeal blood vessels (arrow) (300x). D; Demyelination of the nerve axons (1200x). (E-F); MZ + CUR-treated group showing: E; Mild degeneration and congestion in meningeal blood vessels in few neurons (arrow) (1200x). F; Slightly dilated choroid plexus and focal gliosis (arrows) (300x).

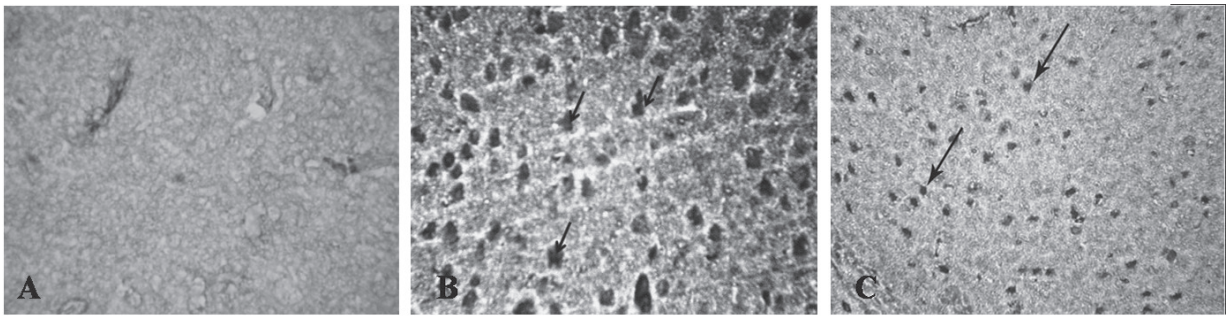

Fig. 2. Immunohistochemical staining of iNOS in rat cerebral cortex. (A); control and CUR-treated groups showing no immunoreactivity in neurons other than a very small number of iNOS positive cells (1000x). (B); MZ-treated group showing intense positive immunohistochemical staining in degenerated neurons (arrows). (C); MZ + CUR-treated group showing moderate immunohistochemical staining in some neurons (arrows) (1200x) .

of reactive oxygen and nitrogen species owing to the presence of methoxy and phenolic groups on the phenyl ring and 1, 3-diketone group in its structure[3]. In addition, CUR may act as a chelator and directly binds to iron, which catalyzes the formation of free radicals through the Fenton reaction[12]. Similarly, CUR attenuated brain oxidative damage induced by arsenic[23].

A significant reduction in GABA and glutamate concentrations ($p<0.05$) was observed in brain of MZ + CUR-treated group when compared with MZ-treated one (Table 1). Similarly, a remarked reduction of glutamate concentrations in CUR-treated rats after subarachnoid hemorrhage was noticed as a result of preservation of glutamate transporter-1 expression which is essential for maintaining a low extracellular glutamate level[14].

Our results were supported with the histopathological findings of cerebral cortex. The cerebral cortex revealed extensive damage and structural alterations in neurons of MZ-treated rats (Fig. 1B-D). These lesions were remarkably reduced by co-administration of CUR (Fig. 1E-F). Our results coincided with other report showing that CUR provided histological protection against neurotoxicity induced by lipopolysaccharide in rats[25].

iNOS is a high-output isoform of NOS, calcium-independent, which is usually undetectable in central nervous system under normal conditions but is expressed after exposure of cells to several deleterious agents such as cytokines or lipopolysaccharide[17]. The present results depicted nearly negative immunohistochemical expression of iNOS in cerebral cortex of control and CUR-treated rats except a very small number of iNOS positive cells (Fig. 2A). However, over expression of iNOS was detected in cerebral cortex of MZ-treated group (Fig. 2B). The up-regulation of iNOS provides an abundant amount of NO, which can cause neurotoxicity[17]. Interestingly, moderate iNOS immunoreactivity was observed in cerebral cortex of MZ + CUR-treated group (Fig. 2C). These results coincided with a similar study demonstrating that CUR reduced brain iNOS expression in lipopolysaccharide-induced neurotoxicity in rats[25].

In conclusion, this study confirmed that CUR exhibited ameliorative efficacy against MZ-induced neurotoxicity in rats by modulating oxidative stress and amino acid neurotransmitter levels due to its antioxidant and free radical scavenging properties.

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