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EFFECT OF CURCUMIN AND P53 SIGNALING PATHWAY IN RAT THYMOCYTES TOXICITY INDUCED BY MANCOZEB

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Mancozeb, as a dithiocarbamate fungicide, is widely used in agriculture due to its low acute toxicity and short environmental persistence. We examined the protective role of curcumin on Mancozeb-induced toxicity in rat thymocytes and potential mechanisms involved. Rat thymocytes were exposed to Mancozeb (0.01 μg/ml) and/or curcumin (0.3, 1, 3 μM) and levels of cell viability, caspase-3, caspase-9 activity, cytochrome C oxidase, catalase activity, reactive oxygen species (ROS) production and p53 signaling involvement were evaluated after 24 h of incubation. Cells treated with Mancozeb showed increased toxicity, caspase-3, 9 activity and ROS production with decreased cytochrome C oxidase and catalase activity. Inhibition of caspase-3 and 9 activity resulted with reduced rat thymocytes toxicity while inhibition of p53 signaling pathway suppressed caspase-3 activity in cells. Co-treatment with curcumin (1, 3 μM) displayed significantly reduced toxicity, caspase-3, 9 activity and ROS production, together with increased cytochrome C and catalase activity in cells. These findings propose that Mancozeb-induced apoptosis in rat thymocytes is caspase dependent and is partially attributed to p53 signaling pathway. Certain curcumin concentrations may modulate Mancozeb-induced rat thymocytes toxicity, due to its anti-oxidative effect, and may be useful for providing potential therapeutic strategy in immunomodulation induced by Mancozeb.

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Key words: *Mancozeb, curcumin, p53, thymocytes, toxic*

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Introduction

The application of pesticides represents the most effective means of protecting plants without causing much damage to non-target species. However, humans are often exposed to pesticides through persistent bio-accumulative residues in the environment (1) which may lead to increased risk of adverse health effects, including genotoxicity and cancer (2). Mancozeb is a broad-spectrum fungicide of the ethylene-bis-dithiocarbamate (EBDC) family. Despite various studies which reported toxic effects of Mancozeb in different immune cells (1, 3, 4, 5), this fungicide has been widely used globally due to its low acute toxicity and short environmental persistence (6). Continuous exposure to pesticides raises the risk of immunomodulation (7). Earlier studies showed that occupational exposure to Mancozeb resulted with modulated T cell functional response and alterations in Th1 and Th2 cytokines profiles (6, 8). *In vitro* experiments suggested that Mancozeb mainly targets mitochondrial enzymes (9) and induces reactive oxygen formation with resulted cytotoxicity (3).

Curcumin is a polyphenol derived from the rhizome of the plant Curcuma longa and has been a commonly used seasoning spice and medical plant in Asia for thousands of years. Due to its anti-inflammatory and anti-oxidant properties, curcumin has been proposed as a potential candidate for the prevention and treatment of different diseases (10, 11). Moreover, several reports demonstrated that curcumin, under in vitro conditions, stimulated apoptosis and inhibited proliferation in different cancer cells (12, 13). It has been considered that mechanism for anti-cancer effect of curcumin includes its inhibition of multiple signaling pathways and anti-oxidant property (14). On the other hand, some studies demonstrated cytotoxic effects of curcumin (15), suggesting that the specific mechanisms of curcumin induced cytotoxicity remains controversial due to the variable anti and pro-apoptotic signaling pathways in

different cell types (16). Therefore, in the present study we evaluated the effect of Mancozeb in rat thymocytes and tested whether there is any preventive role of curcumin, along with underlying mechanisms involved.

Materials and methods

Animals

Experiments were performed on adult male Wistar rats (190-220 g), 9-11 weeks old, bread at the Vivarium of the Institute of Biomedical Research, Medical Faculty, Niš, under conventional laboratory conditions and in accordance with national animal protection guidelines. All procedures were performed in line with the recommendations for the proper use and care of laboratory animal and confirmed to the European Communities Council Directive of November 1986 (86/609/EEC).

Materials

Culture medium (CM) was prepared using RPMI 1640 (Sigma-Aldrich, St. Louis, 16 Mo., USA) according to the manufacturer's instructions. CM contained 25 mM HEPES, 2 mM glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml) and 10 % fetal calf serum (FCS).

Cell Counting Kit (CCK-8), Cytochrome C Oxidase Assay kit, Catalase Assay, Pifithrin-α hydrobromide (PFT-α Kit), Z-VAD-FMK, Z-LEHD-FMK and Curcumin were purchased from Sigma-Aldrich (St. Louis, Mo., USA). Caspase-3 colorimetric assay and caspase-9 colorimetric assay were obtained from R&D Systems (Minneapolis, USA). Mancozeb was purchased from Galenika-Fitofarmacija a.d., Belgrade, Serbia.

Preparation of thymocytes

Rat thymocytes were isolated as described previously (17). The viability of the isolated cells, as determined by trypan blue dye exclusion test, was always over 94 %. Isolated thymocytes were counted and adjusted to a density of 1x106 cells/ml.

Cell culture

Isolated rat thymocytes were cultivated in 96-well round-bottom plates (NUNC, Aarhus, Denmark), containing a 100 μl of cell suspension (1x105 cells) in each well. Cells were cultured with Mancozeb (0.01 μg/ml) without or with increasing concentrations (0.3, 1, 3 μM) of curcumin. Control cells were treated with appropriate amounts of vehicle alone, diluted in CM. All cell cultured are done in triplicates and cultivated for 24 h in an incubator (Galaxy, Wolf Laboratories, USA) with 5 % CO_2 at 37°C. When indicated, rat thymocytes were cultured in either the presence or absence of Cyclic Pifithrin-α hydrobromide (PFT-α), an inhibitor of p53 protein, at a final concentration of 20 μM (18), Z-VAD-FMK, a pan-inhibitor of caspases, at final concentration of 10 μM (19) or Z-LEHD-FMK, caspase-9 inhibitor, at final concentration of 20 μM (20). Mancozeb solutions were prepared immediately before use in dimethyl sulfoxide (DMSO) and diluted in CM. Control cells were treated with the same amount of vehicle alone. The final DMSO concentration never exceeded 0.5 % (v/v). Based on the results in our revious study (4), regarding the dose dependent toxicity in rat thymocytes induced by Mancozeb, as well as on proposed acceptable daily intake (0.05 mg/kg body weight) of Mancozeb in humans (21), in our experiment we used 0.01 μg/ml of Mancozeb which corresponds to an in vivo exposure 0.1 mg/kg body weight (3, 22).

Curcumin was dissolved in DMSO as a stock solution. The stock solution was stored at -20°C and diluted in CM before use. The final concentration of DMSO, applied to the cells, was less than 0.5 %. Incubation of increasing concentrations of curcumin (0.3, 1, 3 μM) was chosen due to our recently published findings (5) and previous study results in rat thymocytes (23), which showed that 3 μM was the lowest concentration which was not able to induce any cytotoxic actions in rat thymocytes.

Analysis of cell viability

Cell viability of rat thymocytes, after cultivation period, was evaluated by CCK-8 assay as it was previously described (24). Ten microliter of reaction mixture was added in each well. After 2h of incubation, the solubilized formazan product was quantified spectrophotometrically. Absorbance was measured at 450 nm. For each sample, basal intensity values were subtracted from those obtained after different treatments. Absorbances were presented as a ratio of control for further comparison.

Caspase-3 and caspase-9 activity assay

The enzymatic activity of the caspases were determined by a colorimetric assay (by using the chromogenic substrate DEVD-pNA and LEHD-pNA), according to the manufacturer's protocol. The reaction was measured by determining the change in absorbance at 405 nm. The activity was expressed as fold change of treated cell over the non-treated cells. The background values were subtracted from the experimental results before calculation the fold induction.

Cytochrome C oxidase activity

The change in cytochrome c oxidase activity was assayed by using colorimetric kit following the manufacturer's protocol and as previously reported (25). The method is based on observing the decrease in absorbance at 550 nm of ferrocytochrome c, which is caused by its oxidation to ferricytochrome c by cytochrome c oxidase. The activity was expressed as fold change of treated cell over the non-treated cells (25). The background values were subtracted from the experimental results before calculation of the fold induction.

Catalase assay

Catalase (CAT) enzyme activity was analyzed with Catalase Assay Kit. The assay was performed following the manufacturer's instructions. The CAT degrades H_2O_2 to water and molecular oxygen and the amount of degraded H_2O_2 is proportional to the enzymatic activity. The color change of reaction mixture was evaluated spectrophotometrically at 240 nm and activity was expressed as fold change of treated cell over the non-treated cells.

Measurement of intracellular reactive oxygen species (ROS) production

A redox-sensitive probe (H2DCF-DA) was used to determine changes in overall cellular ROS levels, as described previously (4). The change in fluorescence was measured using a Epics XL flow cytometer (Coulter, Krefeld, Germany). Basal inten-sity values were subtracted from the experimental results before calculation the fold induction.

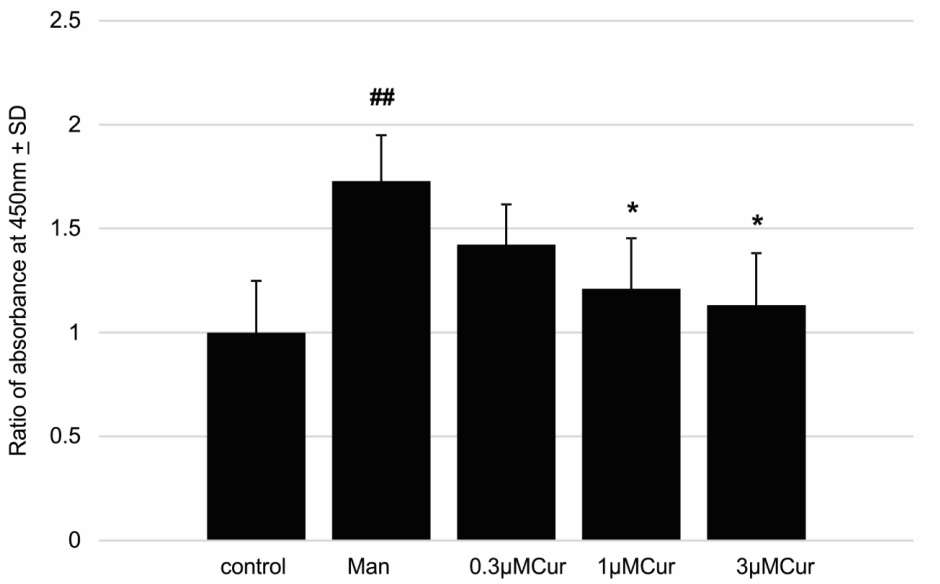
Statistical analysis

Results are presented as mean ± SD. The comparisons among groups were carried out using the analysis of variance (ANOVA) coupled to the Dunnett's post hoc test and student's t test. A p value < 0.05 was considered significant.

Results

Based on the results of our recently published study (5) where we optimized curcumin dose and previous study results in rat thymocytes (23), we found that 3 μM was the lowest concentration which was not able to induce any cytotoxic actions in rat thymocytes. Therefore, we used 0.3, 1 and 3 μM curcumin in all of the experiments in this study.

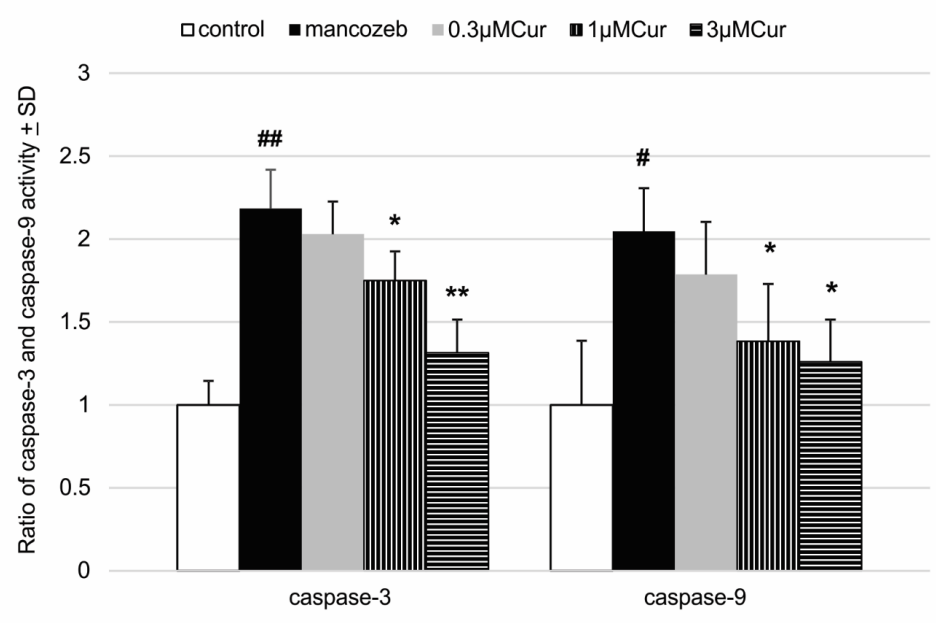
In an attempt to determine the effect of curcumin on Mancozeb treated rat thymocytes, cells were exposed to the increasing curcumin concentrations (0.3, 1, 3 μM) and/or Mancozeb (0.01 μg/ml) for 24 h and assayed for cell viability. The obtained results showed that cells treatment with Mancozeb resulted with significantly reduced cell viability ($p < 0.01$), compared to the control cells (Graph 1). Co-treatment with curcumin, at concentrations of 1 and 3 μM significantly ($p < 0.05$) inhibited cell toxicity induced by Mancozeb. On the other hand, lowest curcumin concentrations (0.3 μM), used in our study, failed to restore rat thymocytes viability after treatment with Mancozeb (Graph 1).



Graph 1. Effect of Mancozeb (Man) and curcumin (Cur) on rat thymocytes toxicity

Cells were treated with Man (0.01 μg/ml) without or with increasing Cur (0.3, 1 and 3 μM), for 24 hours. Data were expressed (mean ± SD) as the absorbance ratio of control for further comparison. Man-cells treated only with Man;
0.3 μMCur-cells treated with Man and Cur (0.3 μM);
1 μMCur-cells treated with Man and Cur (1 μM); 3 μMCur-cells treated with Man and Cur (3 μM); ##-$p < 0.01$ vs. control cells; *-$p < 0.05$ vs. Man treated cells.

It has been shown that caspase is a key executioner of apoptosis (26). Since our previous results (4, 5) demonstrated that Mancozeb-induced toxicity in rat thymocytes involves apoptotic cell death, we next investigated whether caspases play an important role in Mancozeb-induced toxicity. As shown in Graph 2, caspase-3 ($p < 0.01$) and caspase-9 ($p < 0.05$) activity were markedly increased by a 24h Mancozeb treatment, suggesting that caspases are intimately involved in this model of cytotoxicity. Also, co-treatment cells with curcumin (1, 3 μM) significantly downregulatedcaspase 3 ($p < 0.05$; $p < 0.01$) and caspase-9 ($p < 0.05$) activity, indicating the protective role of curcumin in Mancozeb-induced toxicity in rat thymocytes. Application 0.3 μM of curcumin in rat thymocytes culture resulted in no significant reduction of caspase-3 and caspase-9 activity (Graph 2).

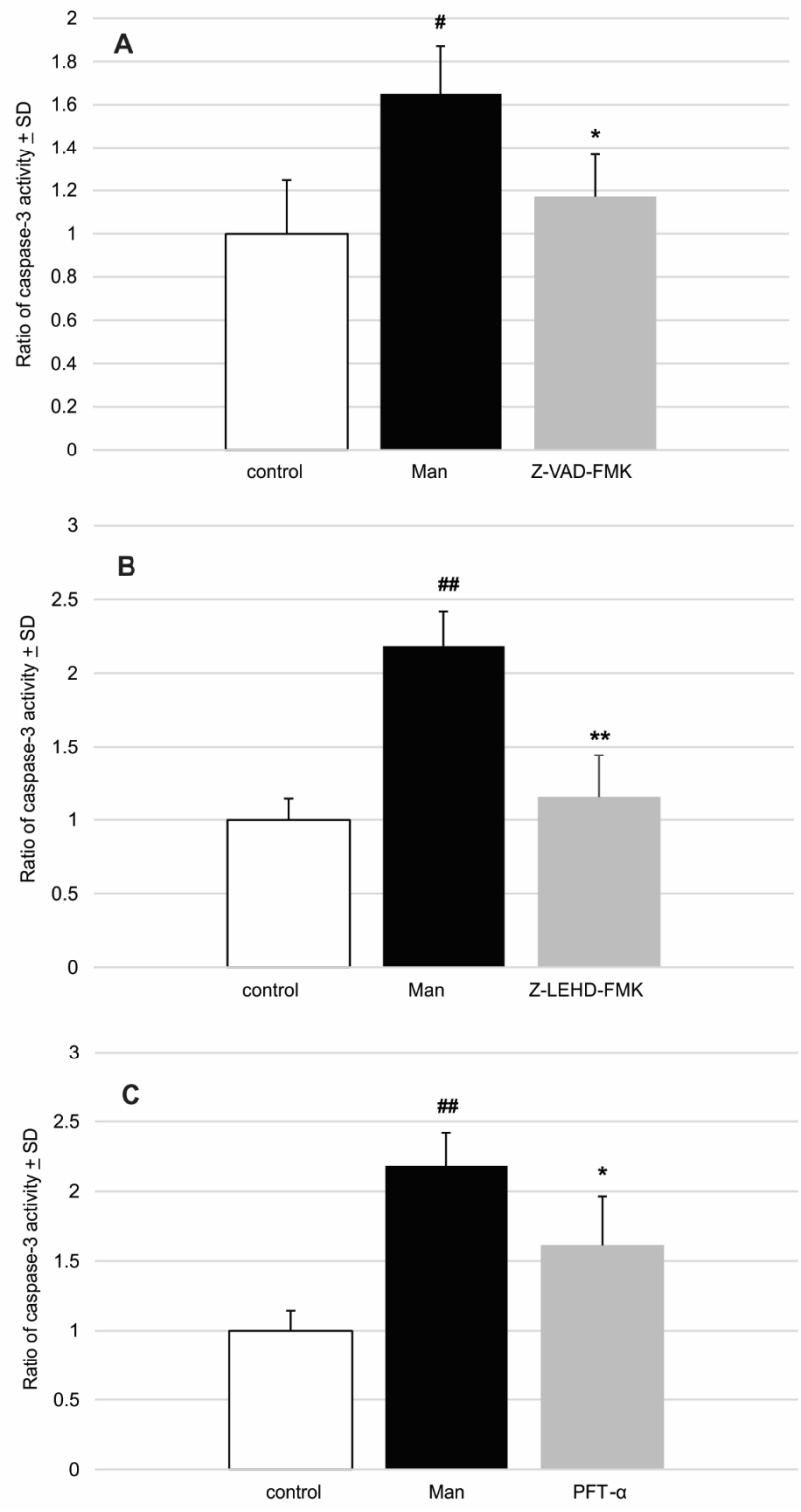


Graph 2. Effect of Mancozeb (Man) and curcumin (Cur) on caspase-3 and caspase-9 activity in rat thymocytes

Cells were treated with Man (0.01 μg/ml) without or with increasing Cur (0.3, 1 and 3 μM), for 24 hours.
Data were expressed (mean ± SD) as the absorbance ratio of control for further comparison.
Man-cells treated only with Man; 0.3 μMCur-cells treated with Man and Cur (0.3 μM); 1 μMCur-cells treated with Man and Cur (1 μM); 3 μMCur-cells treated with Man and Cur (3 μM); #-$p < 0.05$; ##-$p < 0.01$ vs. control cells; *-$p < 0.05$; **-$p < 0.01$ vs. Man treated cells.

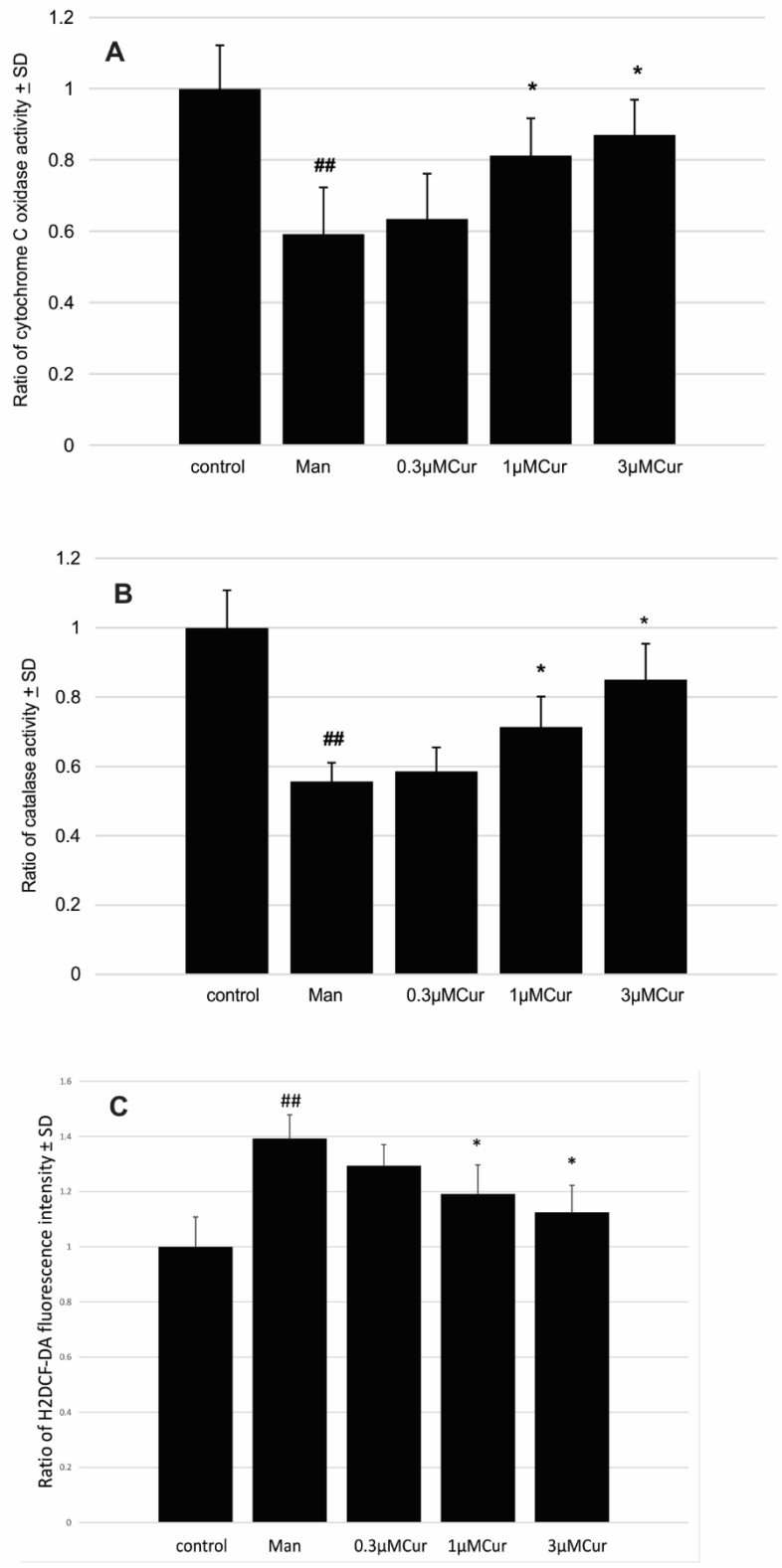
To further elucidate the involvement of caspase-3 and caspase-9 in Mancozeb-induced toxicity in rat thymocytes, cells were simultaneously treated with Mancozeb and Z-VAD-FMK (a pan-inhibitor of caspases) or Z-LEHD-FMK (specific caspase-9 inhibitor). Caspase-3 activity was markedly inhibited in cell treated with Z-VAD-FMK ($p < 0.05$) and Z-LEHD-FMK ($p < 0.01$), proposing that Mancozeb-induced toxicity in ratthymocytes involves caspase dependent toxicity (Graph 3A and 3B). Since caspase inhibitors were not able to completely inhibit caspase-3 activity in rat thymocytes, further analyses are required to evaluate the possibility that Mancozeb-induced toxicity may be caspase independent.

The tumor suppressor p53 has been related to different key cellular processes, including the regulation of apoptotic cell death (27, 28). Different reports propose crosstalk between p53 and caspases in apoptosis induction in various cells (26, 29). Therefore, we next examined the potential role of p53 in Mancozeb-induced cytotoxicity. In response to co-treatment with Mancozeb and PFT-α (an inhibitor of p53 protein) caspase-3 activity was significantly downregulated ($p < 0.05$), indicating the involvement of p53 in Mancozeb-induced toxicity in rat thymocytes (Graph 3C). Taking into account that the rescue was not complete, we are not able to neglect the activation of other multiple signaling pathways.



Graph 3. Effect of Mancozeb (Man) and Z-VAD-FMK (pan-inhibitor of caspases) (A), Z-LEHD-FMK (caspase-9 inhibitor) (B), PFT-α (inhibitor of p53 protein) (C) on caspase-3 activity and toxicity in rat thymocytes

Cells were treated with Man (0.01 μg/ml) without or with Z-VAD-FMK, Z-LEHD-FMK, PFT-α, for 24 hours.
Data were expressed (mean ± SD) as the absorbance ratio of control for further comparison.
Man-cells treated only with Man; 0.3 μMCur-cells treated with Man and Cur (0.3 μM); 1 μMCur-cells treated with Man and Cur (1 μM); 3 μMCur-cells treated with Man and Cur (3 μM); #-$p < 0.05$; ##-$p < 0.01$ vs. control cells; *-$p < 0.05$ vs. Man treated cells.



Graph 4. Effect of Mancozeb (Man) and curcumin (Cur) on cytochrome C oxidase (A) catalase activity (B) and ROS production (C) in rat thymocytes

Graph 4. Effect of Mancozeb (Man) and curcumin (Cur) on cytochrome C oxidase (A) catalase activity (B) and ROS production (C) in rat thymocytes. Cells were treated with Man (0.01 μg/ml) without or with increasing Cur (0.3, 1 and 3 μM), for 24 hours. Data were expressed (mean ± SD) as the absorbance ratio of control for further comparison. Man-cells treated only with Man; 0.3 μMCur-cells treated with Man and Cur (0.3 μM); 1 μMCur-cells treated with Man and Cur (1 μM); 3 μMCur-cells treated with Man and Cur (3 μM); ##-$p < 0.01$ vs. control cells; *-$p < 0.05$ vs. Man treated cells.

Since different studies revealed that activity of cytochrome C oxidase activity is closely related to the cell death (30, 31), we next evaluated whether Mancozeb and curcumin treatment had any effect on cytochrome C oxidase activity in rat thymocytes. The results showed that cells exposure to Mancozeb resulted in significantly decreased ($p < 0.01$) cytochrome C oxidase activity, after 24 of incubation (Graph 4A). Simultaneously, co-treatment with curcumin (1, 3 μM) significantly restored ($p < 0.05$) cytochrome C activity in rat thymocytes, as evaluated by colorimetric assay (Graph 4A).

Based on the previous results and because catalase activity may protect rat thymocytes from oxidative injury and apoptosis (17), in next experiments we examined the effect of Mancozeb and curcumin on catalase (CAT) activity in rat thymocytes. As shown in Graph 4B, Mancozeb application to cell culture markedly reduced ($p < 0.01$) catalase activity in rat thymocytes. Also, colorimetric assay revealed that treatment with curcumin (1, 3 μM) significantly restored ($p < 0.05$) altered catalase activity in rat thymocytes, induced by Mancozeb (Graph 4B). Moreover, the analysis of ROS production showed that Mancozeb treatment induced significantly ($p < 0.01$) increased ROS production while application of curcumin (1, 3 μM) reduced ($p < 0.05$) ROS production in rat thymocytes (Graph 4C).

Discussion

Curcumin has been used in traditional Indian and Chinese medicine for centuries due to its various therapeutic properties. Extensive *in vivo* and *in vitro* studies showed that curcumin has a number of biological activities (32, 33), including the increasing of T cell proliferation and inhibition of T cell apoptosis (34).

The current study results demonstrate that Mancozeb application in cell culture decreased viability and increased caspase-3 and caspase-9 activity in rat thymocytes, as evaluated by colorimetric assay. The obtained results correspond with our inhibition experiments which showed that Z-VAD-FMK (a pan-inhibitor of caspases) almost restored viability of the cells while Z-LEHD-FMK (specific caspase-9 inhibitor) strongly suppressed caspase-3 activity in rat thymocytes, indicating that the caspase cascade is involved in Mancozeb-induced cytotoxicity. These observations are in accordance with our previous results (4, 5) demonstrating the pro-apoptotic potential of Mancozeb in rat thymocytes, as well as with other studies in human immune cells (1, 3). Moreover, our results indicate that rat thymocytes exposure to Mancozeb resulted with decreased cytochrome C oxidase and catalase activity, after 24h of incubation. Cytochrome C oxidase represents the terminal enzyme of mitochondrial respiratory chain. It couples electron transfer from cytochrome c to oxygen to form water with transport of protons from matrix to cytosol thereby maintaining mitochondrial (MMP) membrane potential. Furthermore, since this enzyme induces proton transfer and electron exchange takes place within the enzyme, reactive oxygen species (ROS) generation is inherently prohibited (35). It is well documented that Mancozeb possess ability to reduce MMP and induce ROS generation in immune cells (1, 4, 5), showing that mitochondrial dysfunction and alterations in antioxidant defense systems represent major components of Mancozeb-induced toxicity (36). During mitochondrial dysfunction, several key factors of apoptosis (procaspase, cytochrome C, apoptosis protease-activating factor 1-APAF-1) are released into cytosol. The complex formed of cytochrome C, APAF-1 and caspase-9 leads up to a chain activation of other caspases and results in apoptosis (5, 37). These findings correlate with increased caspase-3 and caspase-9 activity in rat thymocytes, after Mancozeb treatment. On the other hand, our results indicated that CAT activity was markedly decreased in rat thymocytes treated with Mancozeb. Given observations are supported by the earlier reports, which indicated that overexpression of CAT protect thymocytes against oxidative injury and apoptosis (17). The decreased activity of CAT in the rat thymocytes indicated the altered CAT activity to degrade hydrogen peroxide. Increased hydrogen peroxide could be converted to toxic hydroxyl radicals that may contribute to oxidative stress and apoptosis (38). Taken together with our results, it seems that rat thymocytes exposure to Mancozeb resulted in altered activities of antioxidant defense system and mitochondrial dysfunction which may lead to caspase cascade activation and cytotoxicity.

p53, as a tumor suppressor gene, plays a prominent role in the regulation of cell apoptosis (27). Moreover, p53 has been linked to evoking apoptosis by transcriptional activation of pro-apoptotic proteins (Bax) and transcriptional repression of anti-apoptotic (Bcl-2) proteins (39). The present study showed that co-treatment with Mancozeb and PFT-α inhibited caspase-3 activity in cells, suggesting the potential role of p53 in Mancozeb-induced toxicity in rat thymocytes. Activated p53 is able to induce the expression of Bax, MMP damage and activation of caspases that lead to apoptosis (40). These observations are in agreement with our recent report which documented that Mancozeb-induced apoptosis through mitochondrial pathway, by disturbing the Bcl-2/Bax protein ratio in rat thymocytes (5). On the other hand, since the rat thymocytes were not completely rescued after PFT-α treatment, it suggests the involvement of another pathway which can be triggered by Mancozeb. Having in mind our previous results, it appears that Mancozeb-induced toxicity in rat thymocytes may be partially associated with p53 signaling activation, with potential secondary immunological consequences.

Taking into account the obtained results, we next tested the possibility that curcumin may modulate Mancozeb-induced toxicity in rat thymocytes. Present study results showed that curcumin (1, 3 μM) markedly inhibited cytotoxicity, caspase-3 and 9 activity, ROS production and restored cytochrome C oxidase and CAT activity in rat thymocytes after Mancozeb treatment. Curcumin (0.3 μM) failure to suppress Mancozeb-induced cytotoxicity is supported by previous findings which proposed that curcumin protective effect is mainly mediated by micromolar concentrations (23). The obtained findings are

consistent with our recently published results, indicating the protective role of curcumin through mitochondrial pathway in Mancozeb-induced rat thymocytes toxicity (5). The decline in mitochondrial respiratory activity (reduced cytochrome C oxidase activity) results in an increased susceptibility to oxidative stress, indicating the unique crosstalk between cytochrome C oxidase activity and cell death machinery (41). These findings correspond with inhibitory effect of curcumin on rOS production. In line with these observations, we showed that CAT activity was suppressed, suggesting that there may be an imbalance between pro-oxidant and anti-oxidant system after Mancozeb treatment. The preventive effect of curcumin on cytochrome C oxidase activity has been shown earlier (42) and here we demonstrated in rat thymocytes, after Mancozeb treatment. In support of this possibility, the protective effect of curcumin may also involve the promotion of mitochondrial respiratory function due to its anti-oxidative properties (42). Together with our results, we can speculate that protective role of curcumin in Mancozeb induced toxicity in rat thymocytes may be partially attributed to p53 inhibition, but this thesis needs additional studies.

In summary, the current study results demonstrate that Mancozeb exerts toxic effects in rat thymocytes, including caspase activation, cytochrome C oxidase and catalase inhibition. Moreover, Mancozeb-induced cell toxicity may be partially mediated through p53 signaling pathway and restored by certain curcumin concentrations. These findings could potentially provide the basis of curcumin as a potential therapeutic strategy for individuals exposed to pesticides which may suppress immunomodulation and secondary immunological consequence development.

References

1. Srivastava AK, Ali W, Singh R, Bhui K, Tyagi S, Al-Khedhairy AA,et al. Mancozeb-induced genotoxicity and apoptosis in cultured human lymphocytes. Life Sci 2012;90 (21-22):815-24.[PubMed][CrossRef]
2. Zahm SH, Blair A. Cancer among migrant and seasonal farmworkers: an epidemiologic review and research agenda. Am J Ind Med 1993;24(6):753-66. [PubMed][CrossRef]
3. Calviello G, Piccioni E, Boninsegna A, Tedesco B, Maggiano N, Serini S,et al. DNA damage and apoptosis induction by the pesticide mancozeb in rat cells: involvement of the oxidative mechanism. ToxicolAppl Pharmacol 2006;211(2):87-96.[PubMed][CrossRef]
4. Pavlovic V, Cekic S, Kamenov B, Ciric M, Krtinic D. The effect of ascorbic acid on Mancozeb-induced toxicity in rat thymocytes. Folia Biol 2015;61(3):116-23. [PubMed]
5. Pavlovic V, Cekic S, Ciric M, Krtinic D, Jovanovic J. Curcumin attenuates Mancozeb-induced toxicity in rat thymocytes through mitochondrial pathway. Food ChemToxicol 2016;88:105-11.[PubMed][CrossRef]
6. Mandarapu R, Ajumeera R, Venkatesan V, Prakhya BM. Proliferation and TH1/TH2 Cytokine Production in Human Peripheral Blood Mononuclear Cells after Treatment with Cypermethrin and MancozebIn Vitro. J Toxicol 2014;2014:308286. [PubMed][CrossRef]
7. Vine MF, Stein L, Weigle K, Schroeder J, Degnan D, Tse CK,et al. Effects on the immune system associated with living near a pesticide dump site. Environ Health Perspect 2000;108 (12):1113-24. [PubMed][CrossRef]
8. Colosio C, Barcellini W, Maroni M, Alcini D, Bersani M, Cavallo D, et al. Immunomodulatory effects of occupational exposure to mancozeb. Arch Environ Health 1996;51(6):445-51. [PubMed][CrossRef]
9. Domico LM, Zeevalk GD, Bernard LP, Cooper KR. Acute neurotoxic effects of mancozeb and Maneb in mesencephalic neuronal cultures are associated with mitochondrial dysfunction. Neurotoxicology 2006;27 (5):816-25. [PubMed][CrossRef]
10. Guan F, Ding Y, Zhang Y, Zhou Y, Li M, Wang C. Curcumin Suppresses Proliferation and Migration of MDA-MB-231 Breast Cancer Cells through Autophagy-Dependent Akt Degradation. PLoS One 2016;11 (1): e0146553. [PubMed][CrossRef]
11. Demir EA, Oz M, Alp MI, Gergerlioglu HS, Nurullahoglu KE, Yerlikaya FH. Co-administration of cisplatin and curcumin does not altermood-associated behaviors. BratislLekListy2016; 117(2):106-11. [PubMed][CrossRef]
12. Liao YF, Hung HC, Hour TC, Hsu PC, Kao MC, Tsay GJ. Curcumin induces apoptosis through an ornithine decarboxylase-dependent pathway in human promyelocytic leukemia HL-60 cells. Life Sci 2008;82(7-8): 367-75. [PubMed][CrossRef]
13. Mackenzie GG, Queisser N, Wolfson ML, Fraga CG, Adamo AM, Oteiza PI. Curcumin induces cell-arrest and apoptosis in association with the inhibition of constitutively active NF-kappaB and STAT3 pathways in Hodgkin's lymphoma cells. Int J Cancer 2008; 123 (1):56-65. [PubMed][CrossRef]
14. Chen J, Xu T, Chen C. The critical roles of miR-21 in anti-cancer effects of curcumin. Ann Transl Med 2015; 3(21):330. [PubMed]
15. Banerjee A, Kunwar A, Mishra B, Priyadarsini KI. Concentration dependent antioxidant/prooxidant activity of curcumin studies from AAPH induced hemolysis of RBCs. ChemBiol Interact 2008;174(2):134-9. [PubMed][CrossRef]

16. Guo LD, Chen XJ, Hu YH, Yu ZJ, Wang D, Liu JZ. Curcumin inhibits proliferation and induces apoptosis of human colorectal cancer cells by activating the mitochondria apoptotic pathway. Phytother Res 2013; 27(3):422-30. [PubMed][CrossRef]
17. Pavlovic V, Cekic S, Sokolovic D, Djindjic B. Modulatory effect of monosodium glutamate on rat thymocuyes proliferation and apoptosis. BratislLekListy 2006: 107(5):185-91. [PubMed]
18. Meslin F, Thiery J, Richon C, Jalil A, Chouaib S. Granzyme B-induced Cell Death Involves Induction of p53 Tumor Suppressor Gene and Its Activation in Tumor Target Cells. J BiolChem 2007;282(45):32991-9. [PubMed][CrossRef]
19. Nishimura Y, Oyama TB, SakanashiY, Oyama TM, Matsui H, Okano Y. Some characteristics of quercetin-induced cytotoxicity on rat thymocytes under in vitro condition. ToxicolIn Vitro 2008;22(4):1002-7. [PubMed][CrossRef]
20. Park GB, Choi Y, Kim YS, Lee HK, Kim D, Hur DY. ROS and ERK1/2-mediated caspase-9 activation increases XAF1 expression in dexamethasone-induced apoptosis of EBV-transformed B cells. Int J Oncol 2013;43 (1): 29-38.[PubMed][CrossRef]
21. Rohm. Dithane fungicide. Material Safety Data Sheetpp. Philadelphia: X; 1995. p. 1–11.
22. Vaccari A, Saba P, Mocci I, Ruiu S. Dithiocarbamate pesticides affect glutamate transport in brain synaptic vesicles. J PharmacolExpTher 1999;288(1):1-5. [PubMed]
23. Koizumi K, Kawanai T, Hashimoto E, Kanbara Y, Masuda T, Kanemaru K, et al. Cytometric analysis on cytotoxicity of curcumin on rat thymocytes: Proapoptotic and antiapoptotic actions of curcumin. ToxicolIn Vitro 2011;25(4):985-90.[PubMed][CrossRef]
24. Hori R, Kashiba M, Toma T, Yachie A, Goda N, Makino N, et al. Gene transfection of H25A mutant heme oxygenase-1 protects cells against hydroperoxide-induced cytotoxicity. J BiolChem 2002;277(12): 10712-8. [PubMed][CrossRef]
25. Al-Gayyar MM, Al Youssef A, Sherif IO, Shams ME, Abbas A. Protective effects of arjunolic acid against cardiac toxicity induced by oral sodium nitrite: Effects on cytokine balance and apoptosis. Life Sci 2014;111 (1-2):18-26. [PubMed][CrossRef]
26. Choi EY, Shin KC, Lee J, Kwon TK, Kim S, Park JW. Treatment with a Small Synthetic Compound, KMU-193, induces Apoptosis in A549 Human Lung Carcinoma Cells through p53 Up-Regulation. Asian Pac J Cancer Prev 2015;16(14):5883-7. [PubMed][CrossRef]
27. Slovackova J, Smarda J, Smardova J. Roscovitine-induced apoptosis of h1299 cells depends on functional status of p53. Neoplasma 2012;59(6):606-12. [PubMed][CrossRef]
28. Dastjerdi MN, Valiana A, Mardani M, Ra MZ. Adenosine A1 receptor modifies P53 expression and apoptosis in breast cancer cell line Mcf-7. BratislLekListy 2016; 117(4):242-6. [PubMed][CrossRef]
29. Amaral JD, Xavier JM, Steer CJ, Rodrigues CM. The role of p53 in apoptosis. Discov Med 2010;9(45):145-52. [PubMed]
30. Srinivasan S, Avadhani NG. Cytochrome c oxidase dysfunction in oxidative stress. Free RadicBiol Med 2012;53(6):1252-63. [PubMed][CrossRef]
31. Leadsham JE, Sanders G, Giannaki S, Bastow EL, Hutton R, Naeimi WR, et al. Loss of cytochrome c oxidase promotes RAS-dependent ROS production from the ER resident NADPH oxidase, Yno1p, in yeast. Cell Metab 2013;18(2):279-86. [PubMed][CrossRef]
32. Srinivasan A, Menon VP, Periaswamy V, Rajasekaran KN. Protection of pancreatic beta-cell by the potential antioxidant bis-o-hydroxycinnamoyl methane, analogue of natural curcuminoid in experimental diabetes. J Pharm PharmSci 2003;6(3):327-33. [PubMed]
33. Tu CT, Han B, Liu HC, Zhang SC. Curcumin protects mice against concanavalin A induced hepatitis by inhibiting intrahepatic intercellular adhesion molecule-1 (ICAM-1) and CXCL10 expression. Mol Cell Biochem 2011;358(1-2):53-60. [PubMed][CrossRef]
34. Li X, Liu X. Effect of curcumin on immune function of mice. J HuazhongUnivSciTechnolog Med Sci 2005;25 (2):137-40. [PubMed][CrossRef]
35. Tripathy MK, Mitra D. Differential modulation of mitochondrial OXPHOS system during HIV-1 induced T-cell apoptosis: up regulation of Complex-IV subunit COX-II and its possible implications. Apoptosis 2010;15 (1):28-40. [PubMed][CrossRef]
36. Medjdoub A, Merzouk S, Merzouk H, Chiali F, Narce F. Effects of mancozeb and metribuzin on in vitro proliferative responses and oxidative stress of human and rat spleen lymphocytes stimulated by mitogens. PesticBiochemPhysiol 2011;101(1):27-33. [PubMed][CrossRef]
37. Badr H, Hashemi M, Javadi G, Movafagh A, Mahdian R. Assessment of global ischemic/reperfusion and Tacrolimus administration on CA1 region of hippocampus: gene expression profi les of BAX and BCL2 genes. BratislLekListy 2016; 117(6):358-62. [PubMed][CrossRef]
38. Kaushik S, Kaur J. Chronic cold exposure affects the antioxidant defense system in various rat tissues. ClinChimActa 2003;333(1):69-77.[PubMed][CrossRef]
39. Brady CA, Attardi LD. p53 at a glance. J Cell Sci 2010;123:2527-32. [PubMed][CrossRef]
40. Kotipatruni RP, Ren X, Thotala D, Jaboin JJ. NDRG4 is a novel oncogenic protein and p53 associated regulator of apoptosis in malignant meningioma cells. Oncotarget 2015;6(19):17594-604. [PubMed][CrossRef]
41. Schüll S, Günther SD, Brodesser S, Seeger JM, Tosetti B, Wiegmann K, et al. Cytochrome c oxidase deficiency accelerates mitochondrial apoptosis by activating ceramide synthase 6. Cell Death Dis 2015;6: e1691. [PubMed][CrossRef]
42. Kaur H, Bal A, Sandhir R. Curcumin supplementation improves mitochondrial and behavioral deficits in experimental model of chronic epilepsy. Pharmacol BiochemBehav 2014;125:55-64. [PubMed][CrossRef]

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EFEKAT KURKUMINA I P53 SIGNALNOG PUTA U TOKSIČNOSTI PACOVSKIH TIMOCITA IZAZVANE MANKOZEBOM

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Mankozeb, kao ditiokarbamatni fungicid, nalazi se u širokoj upotrebi u poljoprivredi, prvenstveno zbog svoje male akutne toksičnosti i kratkog poluživota u spoljašnjoj sredini. U našem radu ispitivali smo preventivnu upotrebu kurkumina na mankozebom indukovanu toksičnost pacovskih timocita, kao i potencijalne mehanizme uključene u ovaj proces. Timociti pacova bili su izloženi delovanju mankozeba (0,01 μg/ml) i/ili kurkuminu u rastućim koncentracijama (0.3, 1 3 μM). Varijabilnost ćelija, aktivnost kaspaze 3, aktivnost kaspaze 9, aktivnost citohrom C oksidaze, katalazna aktivnost, produkcija reaktivnih kiseoničkih radikala (ROS) i aktivnost p53 signalnog puta ispitivani su nakon inkubacije od 24 sata. Ćelije tretirane mankozebom pokazale su povećanu toksičnost, aktivnost kaspaze 3 i kaspaze 9 i produkciju ROS-a, zajedno sa sniženom aktivnošću citohroma C oksidaze i sniženom katalaznom aktivnošću. Inhibicija aktivnosti kaspaze 3 i kaspaze 9 dovela je do smanjene toksičnosti timocita pacova, dok je inhibicija p53 signalnog puta suprimirala aktivnost kaspaze 3 u ćelijama. Kotretman kurkuminom (1, 3 μM) pokazao je značajnu redukciju toksičnosti, aktivnosti kaspaze 3 i 9 i produkcije ROS-a, zajedno sa povećanom aktivnošću citohroma C i povećanom katalaznom aktivnošću u ćelijama. Dobijeni rezultati pokazuju da je mankozebom indukovana apoptoza u timocitima pacova zavisna od kaspaza, kao i da se parcijalno odigrava preko p53 signalnog puta. Odgovarajuće koncentracije kurkumina mogu modelirati mankozebom indukovanu toksičnost pacovskih timocita, prvenstveno preko svog antioksidativnog efekta, što može predstavljati potencijalno mesto terapijske strategije u imunomodulaciji koja je indukovana mankozebom.

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Ključne reči: *mankozeb, kurkumin, p53, timociti toksičnost*