DOI: https://doi.org/10.53350/pjmhs22163671

ORIGINAL ARTICLE

Synergistic Effect of Mesenchymal Stem Cells and L-2-Oxothiazolidine-4-Carboxylate against P53/Caspase-3-Mediated Apoptosis in Lung Tissue of Rats Exposed to Chlorpyrifos

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ABSTRACT

Chlorpyrifos [O, O-diethyl O-(3, 5, 6-trichloro-2-pyridinyl) phosphorothioate), which causes lung dysfunction, was used in this investigation to see if mesenchymal stem cells (MSCs) or l-2-oxothiazolidine-4-carboxylate (OTC) could alleviate the symptoms (CPF). Six sets of male albino rats (12010g) were formed. H2O was administered as a control, whereas OTC was administered orally at a dose of 100mg/kg b.wt./day and CPF was administered orally at a dose of 17.5mg/kg. It is possible to combine the use of one intravenous injection of MSCs (a single dose of 2106 cells) with the use of CPF+OTC, CPF+MSCS, or even CPF+MSCS+MSCS (CPF co-treated with OTC and MSCs). Results after a month demonstrated that treatment with OTC or/and MSCs improved GPx, MDA, and TAC in glutathione peroxidase (TAC). Lung tissue organisation was recovered by H&E after treatment with OTC and/or MSCs. As a result, it's possible that OTC and MSCs work in concert to help protect lung tissue against the apoptotic effects of CPF.

Keywords: MSCs; p53/Caspase-3-mediated apoptosis; OTC; Lung.

INTRODUCTION

Lung dysfunction is a critical cause of mortality (Caley et al., 2021) even in non-smokers, with low lung function being a concern of reduced growth in utero, childhood and failure in adult life (Kung et al., 2021). Lung dysfunctions in adults may be caused by anatomical, physiological or immunological age-related changes as well as lung was influenced by genetics or/and environmental exposures as smoking and other air pollutions (Kling & William. 2021).

Pesticide use has increased recently, causing major problems such as reproductive dysfunction in lab animals or people, endocrine disorders, immunological alarms, neurologic syndromes, and kidney or liver impairments. Acute pesticide exposure causes bronchospasm, respiratory failure, and even death (Tan, 2021; Peiris et al., 2017; Darwiche et al., 2018, and Gadah et al., 2019).

Numerous studies have shown that supplementing stem cells with antioxidants improves their ability to resist oxidative stress and thus the overall therapeutic result of their implantation (Ashfaq et al., 2020). The experimental evidence also supports the role of antioxidants in regulating stem cells and increasing their proliferative capability (Stavely and Nurgali, 2020). Plant extracts and known antioxidants like ascorbic acid and resveratrol have been studied for their ability to stimulate stem cell proliferation (Kwon and Park, 2020). The ongoing investigation may emphasise the role of redox balance in stem cell regulation.

Adult stem cells can self-renew and differentiate into many lineages (Li, Y et al., 2020 and Bhatti et al., 2018). In specific experimental and physiological circumstances, MSCs develop into ligaments, tendon, bone, cartilage, muscle, and adipose tissue (Young et al., 2020 and Karamini et al., 2020). MSCs also release extracellular vesicles (EVs), including exosomes, which promote regeneration processes in several disease scenarios. Exosomes from MSCs have therapeutic characteristics similar to parent MSCs. The substantial therapeutic effects of MSCs on COVID-19 patients are mediated through regulation of the immune response (Florindo et al., 2020; Gupta et al., 2020).

Thus, the current work attempted to assess the effect of MSCs or OTC on p53 and then restore histological characteristics in a CPF-induced lung toxicity rat model.

MATERIALS & METHODS

OTC and CPF were acquired from Sigma Chemical Company (St. Louis, U.S.A). The National Research Center in Cairo contributed 48 male albino rats (bwt 12010g). The rat caged All rats were fed and watered two weeks before the test. The National Research Center's animal facilities followed all ethical guidelines. Its animal welfare committee approved all study animals (13/165).

Induction of pulmonary toxicity: The pulmonary toxicity model was induced by Chlorpyrifos administered orally to male rats 17.5mg/kg one month (Peiris and Dhanushka 2017).

Preparation of bone marrow-derived MSCs: The bone marrow of 6-week-old male albino rats was extracted after flushing with DMEM (GIBCO/BRL) and adding 10% foetal bovine serum to the tibiae and femurs. It was utilised to isolate nucleated cells, which were subsequently placed in complete culture media with 1% penicillin–streptomycin (GIBCO/BRL).

Flow cytometry: The researchers used a Fluorescence Activated Cell Sorter (FACS) flow cytometer (Coulter Epics Elite, Miami, FL, USA). PBS washed twice with MSC. Each run utilised 1105 MSCs. The cells were maintained in 100 l PBS with 3 l for 20 minutes. Each litre of blood had 0.1 mg mL-1 antibody. After resuspension, they were washed twice with PBS.

Fluorescence Labeling of MSCs: It was employed in the Sigma technique to mark MSCs with PKH26 fluorescent dye (Saint Louis, Missouri USA). Serum free media was used to perform a double wash of the cells. The cells were then pelleted and dissolved in a dye solution and injected into the tail veins. (Marina et al., 2008). After 10 days, fluorescence microscopes were used to look for migrating labelled cells in lung sections (Mokbel et al., 2011).

Experimental design: All rats were divided into two main groups

GROUP A. 16 rats were divided into 2 groups (each of 8 rats):

Group I: control group received distilled water.

Group II: OTC group received oral dose of l-2-oxothiazolidine-4-carboxylate (100mg/kg for one month) at the beginning of MSCs administration.

Group B. 32 rats were received oral doses of Chlorpyrifos; CPF (17.5mg/kg for one month) and then divided into 4 equal CPF groups as following:

Group III: CPF group left with no further treatment.

Group IV: CPF group treated with OTC (100mg/kg for one month) **(Choi et al., 2013).**

Group V: CPF group treated with MSCs (a single intravenous injection (2×10^6 cell) for one month).

Group VI: CPF group treated with both MSCs companied with OTC. Lung tissue was dissected and placed on formalin for histological investigation 1 month after MSC injection. 1 gramme lung tissue homogenised Before biochemical tests, the homogenates were stored at -80°C..

Biochemical analysis: GPx activity assay was determined according to the method of Sies et al., (1979) and Almeida and Bainy (2006). MDA and TAC, and were assessed by commercial kits (Biodiagnostic Co., Egypt). p53 and Caspase-3 contents were

determined by ELISA technique using rat ELISA kit (Glory Science Co., Ltd, USA) according to the manufacturer's instruction.

Histopathological examinations: Ten percent formalin solution (FFBE) blocks were made from lungs preserved in formalin solution. Histopathological sections of 3-micron thickness were stained with haematoxylin and eosin to examine changes (H&E).

RESULTS

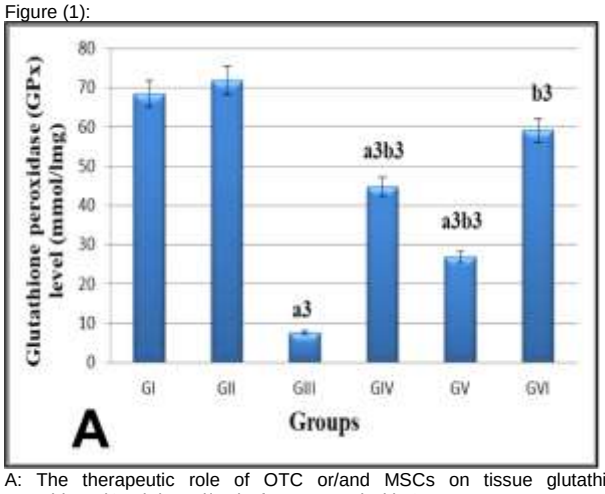
Recognition and Characterization of MSCs: MSCs in culture were recognized morphologically as shown in. S1 (a,b,c, and d). Also, MSCs were characterized by surface markers expression of CD90 (+ve) and CD34 (-ve) detected by flow cytometry. (S 2).

Homing of migrated MSCs: Labeled MSCs that had been migrating for 12 days had been found in lung tissue, and this was confirmed in lung sections treated with MSCs and OTC at the same time. S3.

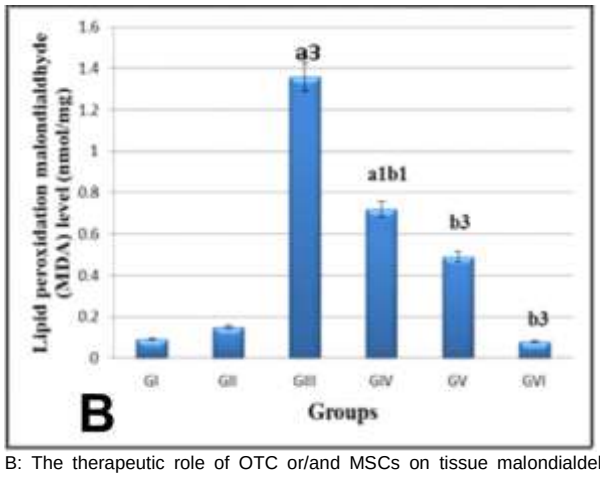
Biochemical analysis: Figure 1 shows data for tissue glutathione peroxidase (GPx), malondialdehyde (MDA), and total antioxidant capacity (TAC) concentration (4, 5 &6).

Throughout the trial, rats treated with OTC (GII) showed no significant alterations (p>0.05). However, the CPF group (GIII) had a substantial (p0.001) decrease in tissue GPx content (7.711.41mmol/mg) compared to the control group (68.373.17mmol/mg) (-88.72 percent change). Compared to CPF rats, the mean value (44.802.58mmol/mg) of the treatment group with OTC (GIV) increased significantly (p0.001) (481.06 percent change).

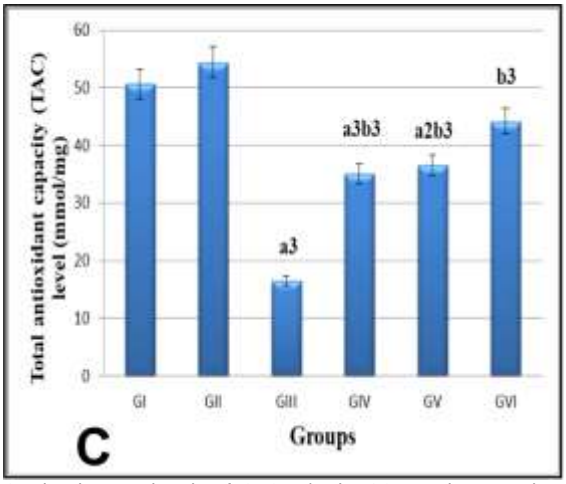
Figure (1):



A: The therapeutic role of OTC or/and MSCs on tissue glutathione peroxidase (GPx) (mmol/mg) of rats treated with CPF.

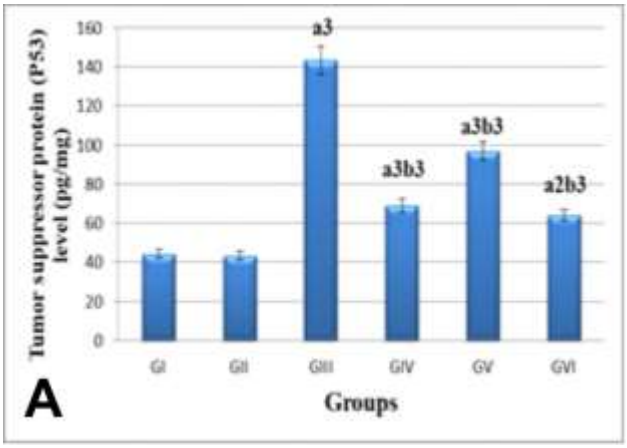


B: The therapeutic role of OTC or/and MSCs on tissue malondialdehyde (MDA) (nmol/mg) of rats treated with CPF.

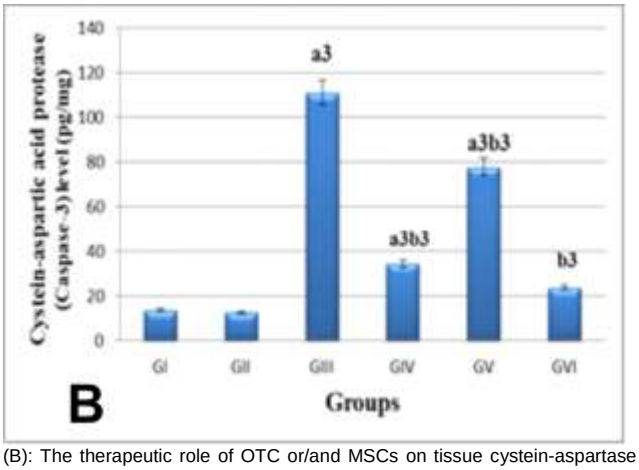


C: The therapeutic role of OTC or/and MSCs on tissue total antioxidant capacity (TAC) content (mmol/mg) of rats treated with CPF

Figure (2)



(A): The therapeutic role of OTC or/and MSCs on tissue tumor suppressor protein p53 content (pg/mg) of rats treated with CPF.



(B): The therapeutic role of OTC or/and MSCs on tissue cystein-aspartase proteases-3 (Caspase-3) content (pg/mg) of rats treated with CPF.

a: When compared to comparable values in control groups (control and OTC), this difference is statistically significant. When compared to the CPF group, this difference is statistically significant. There are three levels of statistical significance: p 0.05, 0.01 and 0.001. Control, OTC, CPF,

CPF+OTC, MSCs, and CPF+OTC+MSCs groups are referred to as GI, GII, GII, GIII, GIV, and GVI.

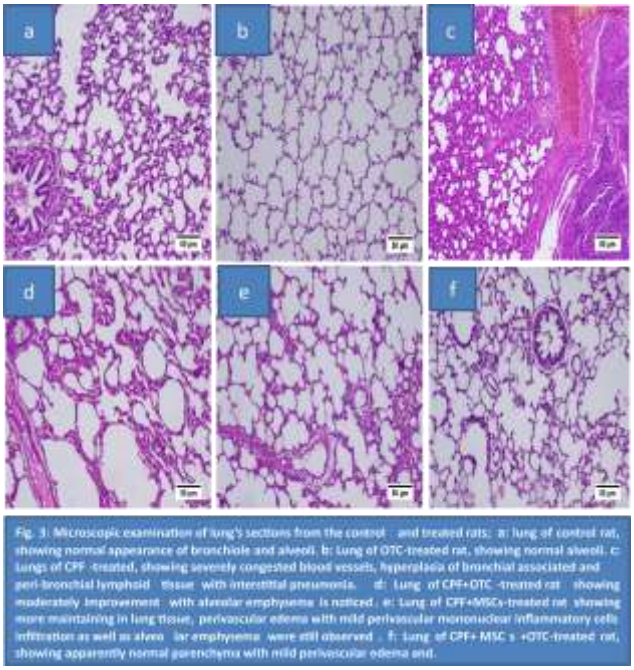


Fig. 3: Microscopic examination of lung's sections from the control and treated rats; a: lung of control rat, showing normal appearance of bronchiole and alveoli. b: Lung of OTC-treated rat, showing normal alveoli. c: Lungs of CPF -treated, showing severely congested blood vessels, hyperplasia of bronchial associated and peri-bronchial lymphoid tissue with interstitial pneumonia. d: Lung of CPF+OTC -treated rat showing moderately improvement with alveolar emphysema is noticed . e: Lung of CPF+MSCs-treated rat showing more maintaining in lung tissue, perivascular edema with mild perivascular mononuclear inflammatory cells infiltration as well as alveolar emphysema were still observed . f: Lung of CPF+ MSC s +OTC-treated rat, showing apparently normal parenchyma with mild perivascular edema and.

Istopathology: Microscopic examination of lung's sections from the control rats showed the normal histological structure; in which the parenchyma is consisted of small air ways; bronchioles and air alveoli (**Fig. 3a**). The group received OTC alone displayed apparently normal lung tissue (**Fig. 3b**) with slight perivascular edema.

Administration of CPF resulted in serious histopathological changes in lung sections; peri-bronchial blood vessels were severely congested with hyperplasia in the bronchial related lymphoid tissue and peribronchial mononuclear cells infiltration (**Fig. 3c**).

OTC moderately improved the adverse effect of CPF on lung tissue, some of the examined sections seemed apparently normal lung tissue with minor scattered foci of interstitial pneumonia (**Fig. 3d**). MSCs exerted the best action among the treated groups in maintaining lungs architecture (**Fig. 3e**). Mild perivascular edema and alveolar emphysema were detected in limited sections.

Co-administration of both OTC + MSCs also restricted the pulmonary destruction induced by CPF to some degree, some of the examined lung sections looked apparently normal with small focal areas of interstitial pneumonia (**Fig. 3f**).

DISCUSSION

Lung is the first organ of the body which comes into contact with toxic substances or chemicals inhaled through the air. Organophosphorus (OPs) insecticides have severe side effects in different organs, plus lung. OPs compounds cause cellular aggregation in the vascular walls or air spaces, immune cells infiltrations, hemorrhage, alveolar congestion, and emphysematous alterations, amongst other lung injuries (Yurumez et al 2007). CPF is a lipophilic OP that easily passes through cell membranes and causes significant damage according to previous research (Deb and Das, 2013 and Hassani et al., 2015).

Given our results, the OTC administration moderately returned the oxido-reductive balance that had been disturbed by CPF-induced irreversible deviations in antioxidant enzymes. This is in accordance with published suggestion that OTC increases GSH levels by providing a cellular cysteine source. Our findings also show that using OTC to advance tissue Gpx and reduce oxidative lung damage may be effective (Ilievska and Hadzi, 2015; Hadzi-Petrushev et al., 2012; Hadzi-Petrushev et al., 2011). OTC was also found to raise cellular GSH and decrease destruction produced by free radicals formed by ionizing radiation when used in vitro. (Angelovski et al 2020).

According to Angelovski et al., 2020; Caspase-3 is an aspartate-specific cysteine protease that has been linked to mitochondrial apoptosis pathway. Also, p53 regulates cell cycle and controls Caspase-3 apoptosis pathway. More to the point, it was known that oxidative stress induce cell death through boosting of p53-Caspase-3 axis (Sritharan and Sivalingam, 2021).

This finding is consistent with (Angelovski et al., 2020), who suggested that the CYP450s/ROS pathway is complicated in atrazine-induced apoptosis. As previously reported, CPF toxicity had an impression on cell cycle and apoptosis, as well as neurotoxicity in SK-N-SH cells Bcl-2, Bax, Caspases. Moreover, CPF can inhibit Bcl-2 and increasing p53, caspase-9, and caspase-3, inducing apoptosis, in carpe gills (Zhang et al., 2019).

As a result, these findings indicated that OTC has an improvement role for lung injuries. After treatment with MSCs, Zhang et al., (2021) Ayala-Cuellar et al., (2019) and Kadry et al., (2018) found that apoptosis was reduced in pancreatic tissues of rats, and they attributed this to MSCs' ability to induce the growth of new islet of cells by using the transcription factor Sox9. MSCs have the ability to transdifferentiate and act as antiapoptotic player (Wu, Y et al., 2021; Holan et al., 2021). Homing of MSCs in lung tissues in these results (fig.3) may boost this expectation.

The concurrent histological findings show significant protective effects of OTC or/and MSCs in CPF-induced lung destruction in rats. Furthermore, OTC+MSCs in the therapeutic group (GVI) were found to be more effective in returning CPF-induced histopathological alterations than in post treatment curative groups (GIV) and (GV).

As mentioned before, the biochemical analysis of lung tissue in this study showed elevation of TAC and GPx. So, the amelioration in histopathological features might be due to accelerated regeneration of lungs parenchyma under the influence of antioxidative effects of OTC, which increase intracellular concentrations of GSH above physiological concentrations. (Promsote et al., 2014; John and Arockiasamy 2021; Boese and Kang, 2021). Therefore, GSH and GSH-px modulation is progressively relevant in the treatment of oxidative stress-related diseases (Terziev et al., 2020).

CONCLUSION

Based on our results, it can be concluded that the treatment with OTC or MSCs alone or in combination may improve lung tissue of rats against CPF-induced disruption via reducing of oxidative stress and hence suppression of p53/caspase-3-mediated apoptosis.

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