Toxicology Reports 4 (2017) 399–407

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

# Anti-inflammatory and antioxidant role of resveratrol on nicotine-induced lung changes in male rats

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ARTICLE INFO

*Keywords:*
Nicotine
Resveratrol
8-hydroxydeoxyguanosine
Nitric oxide
Enzymatic/non-enzymatic antioxidant

ABSTRACT

Male albino rats of Wistar strain were injected intraperitoneally with nicotine or/and resveratrol for 4 weeks. Serum Interleukin-2, Interleukin-6, alpha-fetoprotein and tumor necrosis-alpha, as well as plasma 8-hydroxydeoxyguanosine of nicotine-treated rats were increased significantly. Myeloperoxidase, xanthine oxidase, nitric oxide, lipid peroxidation and total oxidative status of the lung in nicotine-treated rats were increased significantly, which were brought down to normal in resveratrol co-treated group. Endogenous antioxidant status as the activity of superoxide dismutase, catalase, glutathione peroxidase, and glucose-6-phosphate dehydrogenases were found to be decreased significantly in the lung of the nicotine-treated group, which were significantly raised in resveratrol-administered groups. The non-enzymatic antioxidants as total antioxidant and thiol levels were decreased significantly as the effect of nicotine that was effectively enhanced by resveratrol treatment. The lung of nicotine-treated rats showed severe congestion of the alveolar lung tissues with scattered congestion per bronchiolar and perivascular cells, as well as, inflammatory cells were observed. The data suggested that resveratrol exerts its protective effect by modulating the extent of oxidative status and improving the enzymatic/non-enzymatic antioxidant defense system, moreover, decreases the pathological changes in animals against the lung damage caused by nicotine.

## 1. Introduction

Nicotine is the chemical responsible for addiction to tobacco. It can be absorbed through the epithelium of the lung and the skin in alkaline media Sanner and Grimsrud, 2015. Carpagnano et al. (2003) reported that nicotine causes lesion and cancer of the lungs as well as obstructive pulmonary diseases Hackett et al., 2003 and histopathological changes [15]. It also induced oxidative stress by increasing the level of lipid peroxidation (LPO) as the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [5] in animal and different types of systems.

Furthermore, it decreased the antioxidant defense mechanisms by reducing the activity of glutathione peroxidase (GPx) in different organs including the lung (Ozukutat et al., 2005; Muthukumaran et al., 2008). Chronic nicotine administration for 18 weeks caused a significant increase in the activates of superoxide dismutase (SOD) and catalase (CAT) in lung tissue as compared to control rats suggesting an oxidative damage [13]. They also found alterations in the pulmonary structures such as pulmonary congestion, hemorrhage into alveoli and interstitial areas and edema.

Nicotine increases the cytokines production that could be involved in liver injury El-Zayadi, 2006. The inflammatory process in the lungs is characterized by the production of a variety of cytokines. Among the pro-inflammatory cytokines, interleukin-6 (IL-6), which is considered to contribute to the initiation and extension of the inflammatory process Jirik et al., 1989 and it has the ability to inhibit the production of alpha-tumor necrosis factor (α-TNF).

8-hydroxy-2′-deoxyguanosine and 8-hydroxyguanosine are combined with degenerative diseases [53]. ROS can interact with DNA causing DNA breaks and changes the nucleotide that is a significant contributor to different diseases including cancer. 8-hydroxydeoxyguanosine (8-OHdG), the oxidized form of the nucleoside 2′-deoxyguanosine present in DNA, is one of the most reliable and abundant markers of DNA damage because it reflects extremely low levels of oxidative damage [18]. The association of ROS and the use of 8-OHdG as a biomarker of oxidative stress have been investigated in many diseases [49].

Alpha-fetoprotein (α-FP) was undertaken to correlate the various

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http://dx.doi.org/10.1016/j.toxrep.2017.07.003
Received 17 March 2017; Received in revised form 3 July 2017; Accepted 14 July 2017
Available online 17 July 2017

types of neoplasms with the presence of these tumor markers in the tissue and serum [32]. Myeloperoxidase (MPO) is also proposed to contribute to tumor proliferation, angiogenesis, and metastasis Amy et al., 2014. Determination of lung MPO has been used to detect pulmonary leukostasis in rabbits [22]. It is a heme peroxidase enzyme that neutrophils express and secrete. It generates ROS/RNS as a mechanism for pathogen removal. Upon neutrophil activation, primary granules containing MPO can fuse with the plasma membrane to secrete contents into the extracellular.

Nitric oxide (NO) is formed from L-arginine by NO synthase (NOS) that synthesis?? to the effects of nicotine in the vascular system. Therefore, nicotine induces relaxation in peripheral [61] and cerebral arteries [2] by the effect of NO.

Glucose-6-phosphate dehydrogenase (G6PD) plays an important role in the oxidative pentose-phosphate pathway. It provides NADPH that helps in the protection of cells against oxidative damage Singh et al., 2012. G6PD reduced nicotinamide adenine dinucleotide phosphate produced from pentose-phosphate pathway might reflect, in part, the capacity of the lung to tolerate oxygen exposure.

Resveratrol (trans- 3,5,40 trihydroxystilbene) is a phenolic phytoalexin [55] that occurs naturally in various foods, including grapes, plums, cranberries, and peanuts. Özcan et al. [38] found that the antioxidant properties of resveratrol are dependent upon the up-regulation of endogenous cellular antioxidant systems but, its mode of action is still unclear Fantinelli et al., 2005. Resveratrol had found to prevent the hyperoxic lung injury through its anti-inflammatory and antioxidant properties [39]. Dihydro-resveratrol improved acute pancreatitis-associated lung injury by inhibiting pro-inflammatory response that was associated with a suppression of the nuclear factor-kappaB [35].

There has been a general consensus concerning the positive effects of resveratrol in prevention and treatment of various disease conditions due to its potent anti-inflammatory and antioxidant potentials [57]. Eleawa et al. [14] found that resveratrol might be a promising agent in the treatment of cadmium chloride induced testicular damage by enhancing endogenous antioxidant potential, reducing inflammation and its ability to act as antiapoptotic agent.

A lot of works are necessary before the proposal of resveratrol in the treatment of nicotine. Therefore, we are currently investigating the mode of action of resveratrol with detoxifying enzymes, such as SOD, GPx, CAT, MPO, G6PD and xanthine oxidase (XO), which could be implicated in lung disorders as the effect of nicotine. The association between oxidative DNA damage and nicotine exposure have not been studied before, so, the plasma level of 8-OHdG was determined as a marker for DNA oxidation. The goals of this study also were to examine the effects nicotine without/with resveratrol exposure on lung inflammation by evaluating the role of IL-6 and IL-2. Tumor markers were evaluated as α-FP and α-TNF. Moreover, the pathological changes were investigated.

## 2. Materials and methods

### 2.1. Chemicals

Nicotine hydrogen tartrate was obtained from Sigma Chemical Company, St. Louis, Missouri, USA. All the other chemicals and reagents used were of analytical grade and were purchased from Sigma Chemical Company, St. Louis, MO, USA. Trans-Resveratrol ( > 98% purity) was obtained from Cayman Chemical Company (Ann Arbor, MI, USA).

### 2.2. Animals and experimental design

Male albino Wistar rats of 8 weeks and weighing 150–180 g were obtained from the animal house of Faculty of Pharmacy, Zagazig University, Zagazig, Egypt. They were maintained in a polypropylene cage and provided with standard pellet diet (Valley Group Co., Egypt) and water *ad libitum* in an air-conditioned room with regular alternate cycles of 12 h light and darkness. The animals were acclimatized for 14 days prior to their use in experiments and were handled in accordance with the Ethical Committee of Faculty of Pharmacy, Zagazig University (ECAHZU) (Number P11-3-2013 and P12-3-2013). Every effort was made to minimize the number of animals used and their suffering.

Animals were randomized into four groups of 10 rats each and were treated intraperitoneally (i.p) as below for 4 weeks: control group received physiological saline (NaCl 9%; 1 mL/kg b.w.), nicotine group received nicotine (2.5 mg/kg/b.w.) in physiological saline Kalpana and Menon, 2004 successively. This dose presented 0.05 $LD_{50}$ of nicotine to the rats [46]. Resveratrol group was treated with 20 mg/Kg/b.w. of resveratrol [37] at 9.00 A.M every day. Trans-resveratrol was gaven orally to male rats for 28 d at a dose of 20 mg/(kg x d), 1000 times the amount taken by a 70-kg (1.4 g/d./B. W., and food and water consumption did not differ between rats treated with trans-resveratrol and the control group. Thes results indicated that consumption of trans-resveratrol at 20 mg/(kg x d) does not adversely affect in hematology, biochemical and histopathological examination of the organs tested in rats [28]. Finally, nicotine + resveratrol group received the same dose of nicotine and followed by resveratrol at the same doses.

### 2.3. Blood collection

Blood samples of the fasted rats were collected from the retro-orbital venous plexus immediately with capillary tubes (Micro Hematocrit Capillaries, Mucaps, Jiangsu, China, Mainland) under the ether anesthesia Boussarie, 1999. Then, the blood was centrifuged at 3000 rpm for 15 min and serum was collected for different biochemical analyses.

### 2.4. Cytokines and tumor biomarkers

Serum IL-2 and IL-6 were determined using Abcam's IL-2 and Abcam's IL6 rat enzyme-linked immunosorbent (ELISA) kits, respectively. The data were presented as ng/mL.

The levels of α-TNF in were quantitated using an ELISA assay (Komabiotech INC, rat α −TNF ELISA kit, USA) according to the manufacturer's instructions. Serum α-FP was determined using Enzyme Linked Fluorescent Assay (ELFA) technique (VIDAS® AFP kits, BioMerieux, France). α-FP and α–TNF were presented as IU/mL and pg/mL, respectively.

### 2.5. Assay of DNA damage

The levels of 8-OHDG were determined using ELISA kit according to the manufacturer's protocol (Eastbiopharm, Hangzhou, China). DNA damage was prepared as previously explained by Shi et al. [44]. The amount of 8-OHdG was measured by using a mice specific ELISA kit booklet instructions. In brief, all the reagents were made according to the brochure of kit. Using wells, 50 μL of sample and standard were added to each well followed by 50 μL HRP-conjugate and incubated for 1 h at 37 °C. After washing three times, 50 μL of substrates A and B were added again and incubated for 15 min at 37 °C. Stop solution was added and the absorbance was read at 450 nm. The sensitivity limit of the ELISA system was 0.25 ng/mL of 8-OHDG.

### 2.6. Preparation of tissue homogenates

Prior to dissection, tissue was perfused with a 50 mM (sodium phosphate buffer saline (100 mM $Na_2HPO_4/NaH_2PO_4$) (pH 7.4) and 0.1 m ethylenediaminetetraacetic acid (EDTA) to remove any red blood cells and clots. Then, tissues from the lung were homogenized in 5 mL cold buffer per gram tissue and centrifuged at 5000 rpm for ½ an hour. The resulting supernatant was transferred into Eppendorf tubes and

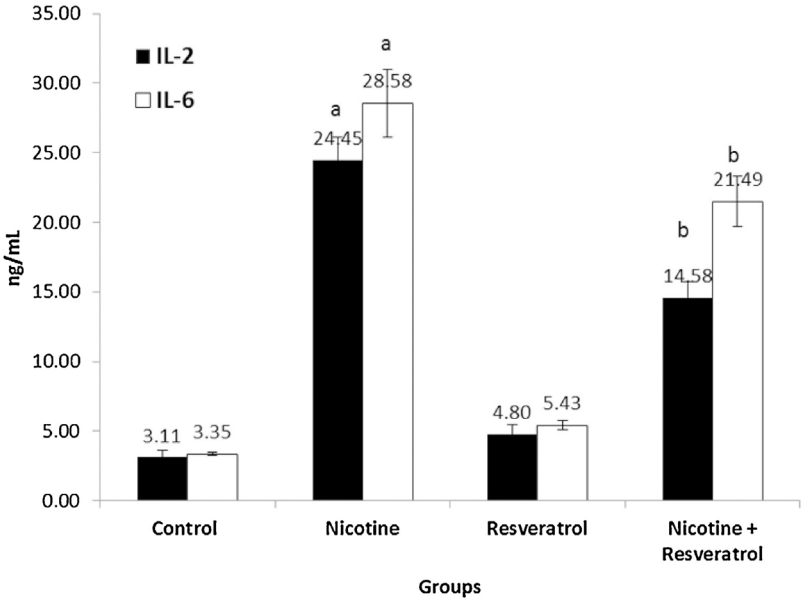


**Fig. 1.** Serum IL2 and IL6 levels of rats that exposed to nicotine (2.5 mg/kg/b.w) or/and resveratrol (20 mg/kg b.w) for 4 weeks.
Values are expressed as means ± SE; n = 10 for each treatment group.
[a] Significant different as compared to control rats and [b] significant different as compared to nicotine group.

preserved in a deep freeze until used for various oxidative/antioxidant assays as following.

*2.6.1. Determination of oxidative stress*

MPO is a peroxidase enzyme that was determined using fluorometric assay kit [50]. The MPO presented as nmol/min/mL.

The LPO was estimated as the concentration of thiobarbituric acid reactive product malondialdehyde (MDA) by using the method of Ohkawa et al., 1979. The amount of MDA was calculated as n mol/g tissue.

The levels of nitrite, a stable end-product of nitric oxide (NO) production, were measured spectrophotometrically based on the Griess reaction [37] and presented as µmole/g.

Total oxidative status (TOS) was measured in the lung using a commercially available kit from Rel Assay Diagnostics [17]. This assay was calibrated with hydrogen peroxide ($H_2O_2$), and the results were expressed as µmol $H_2O_2$ eq./L.

*2.6.2. Determination of antioxidant enzymes*

Xanthine oxidase (XO) activity was assayed spectrophotometrically by the reaction of the enzyme with xanthine, as a substrate, and the absorbance was measured at 650 nm, according to the method described in Litwack et al. [36]. XO activity was determined using the following equations: Concentration of xanthine in control or test = A (sample)/A (standard) × concentration of standard × 48. Xanthine oxidase activity (U/g tissue) = concentration of test concentration of control/0.284.

SOD activity was measured according to Marklund and Marklund (1974) and calculated as the amount of protein that caused 50% pyrogallol auto-oxidation inhibition. SOD activity is expressed as nmol/g tissue. CAT activity was determined according to Aebi [1] and expressed as U/g tissue. GPx activity was determined as described by Hafeman et al. [24] and expressed in mol/g GSH consumed min/g/wet weight tissue. Tissue G6PD activity was measured in lung homogenates using the methods of Aebi [1]. The results were expressed as U/g tissue.

*2.6.3. Evaluation of non-enzymatic antioxidant*

Total antioxidant status (TAS) was measured in the lung using a commercially available kit from Rel Assay Diagnostics (Gaziantep, Turkey) [16]. The method was calibrated using the vitamin E analog Trolox, and data were expressed as mmol Trolox eq./L.

Total thiols level was determined according to Hu (1994) and results are expressed in mM/g. Protein concentration in the homogenate of the lung was determined by the method of Bradford [7] using bovine serum albumin as standard.

*2.7. Histological evaluation*

For histological examination, a portion of the lung was fixed in 10% neutral buffered formalin embedded in paraffin, sectioned and stained with hematoxylin and eosin as described by Gabe [20]. Then semi-thin sections (0.5-1 µm) were prepared by using LKB ultramicrotome. The sections were stained with toluidine blue, examined with a light microscope and photographed. Semi-quantitative histopathologic assessment of slides evaluated while blinded to exposure status, followed by nonparametric statistical analysis, is an established standard technique for evaluating morphologic changes in tissue sections from toxicology studies [23].

Tissue alterations were scored for severity (0 none, 1 minimal, 2 mild, 3 moderate, 4 marked, and 5 severe) as previously described by Hubbs et al. [26]. Multiple lung sections were taken from each rat to assure representative evaluation of lung pathology. For each treatment group, five sections were evaluated from the left lung lobe, the right diaphragmatic lung lobe, the right cardiac lung lobe and the right apical lung lobe.

*2.8. Statistical analyses*

Values of the biochemical assays were given as mean ± standard error (SE). Statistically significant differences between groups were determined using Analysis of Variance (ANOVA), followed by the Tukey multiple comparison tests. $P < 0.05$ was considered to be significant.

## 3. Results

*3.1. Immunological, tumor and DND biomarkers*

Fig. 1 showed the effect of resveratrol or/and nicotine on IL2 and IL6 levels. Exposed the rats to nicotine increased the IL2 and IL6 levels by 7.9- and 8.5-folds as compared to control group, respectively. However, the treatment the rats with resveratrol before the administration of nicotine decreased the IL2 and IL6 by 40% and 24.8% as compared to nicotine-treated animals, respectively.

The nicotine increased the levels of α-FP and α-TNF in the rat as compared to control animals while resveratrol in combination with

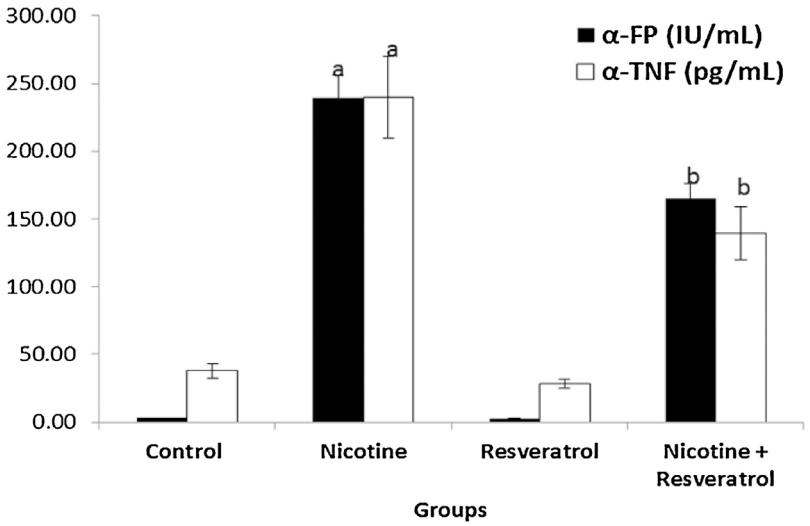


**Fig. 2.** Serum α-FP and TNF levels of rats that exposed to nicotine (2.5 mg/kg/b.w) or/and resveratrol (20 mg/kg b.w) for 4 weeks.
Values are expressed as means ± SE; n = 10 for each treatment group.
[a] Significant different as compared to control rats and [b] significant different as compared to nicotine group.

nicotine decreased their levels as compared to nicotine group (Fig. 2).

The nicotine increased the activities of MPO and XO by 3.4- and 3.2-folds as compared to control group, respectively (Fig. 3). The concurrent administration of resveratrol and nicotine decreased the activity of MPO and XO by 23.9% and 30% as compared to nicotine group, respectively.

The serum 8-OHDG level rose approximately by 2-fold in nicotine group as compared to control rats (Fig. 4). However, the treatment of the rats with resveratrol before nicotine had decreased the level of 8-OHDG by 39.7% as compared to nicotine group.

### 3.2. Oxidative/antioxidant parameters

The level of LPO increased by 5.9-fold in the group of nicotine as compared to control animals (Fig. 5). Exposure of the rats to resveratrol and nicotine had decreased the LPO by 57.4% as compared to nicotine group.

As shown in Fig. 6, the effects of the resveratrol on alleviating oxidative damage caused by nicotine were assessed. Rats had significant high levels of TOS and NO as compared with the control group. However, the group treated with resveratrol and nicotine was able to suppress the TOS and NO levels by 53% and 48.5% as compared to nicotine group, respectively.

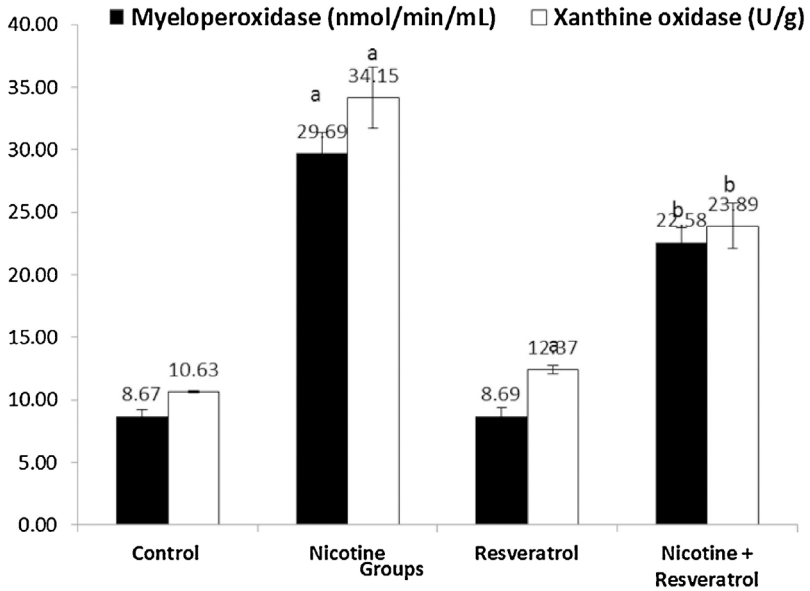


**Fig. 3.** Activity of MPO and XO of lung tissue of rats that exposed to nicotine (2.5 mg/kg/b.w) or/and resveratrol (20 mg/kg b.w) for 4 weeks.
Values are expressed as means ± SE; n = 10 for each treatment group.
[a] Significant different as compared to control rats and [b] significant different as compared to nicotine group.

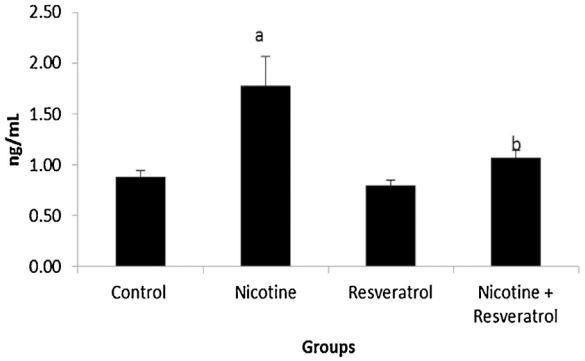


**Fig. 4.** Lung 8-OHDG level of rats that exposed to nicotine (2.5 mg/kg/b.w) or/and resveratrol (20 mg/kg b.w) for 4 weeks.
Values are expressed as means ± SE; n = 10 for each treatment group.
[a] Significant different as compared to control rats and [b] significant different as compared to nicotine group.

As in Fig. 7, the data showed that treatment with nicotine caused a significant decrease in the activity of SOD of lung tissue. Administration of resveratrol (20 mg/kg b.w) did not cause any changes in the activity of SOD of lung as compared with those of control rats. In addition, a

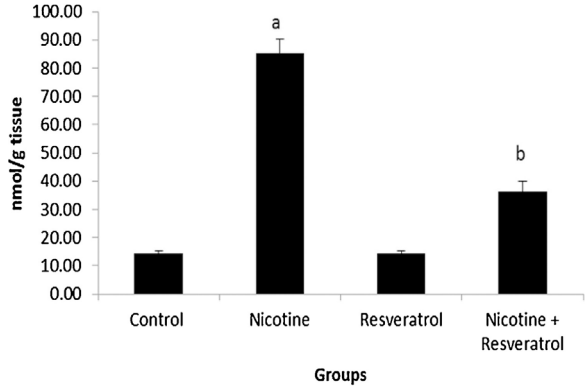


**Fig. 5.** Lung LPO level of rats that exposed to nicotine (2.5 mg/kg/b.w) or/and resveratrol (20 mg/kg b.w) for 4 weeks.
Values are expressed as means ± SE; n = 10 for each treatment group.
[a] Significant different as compared to control rats and [b] significant different as compared to nicotine group.

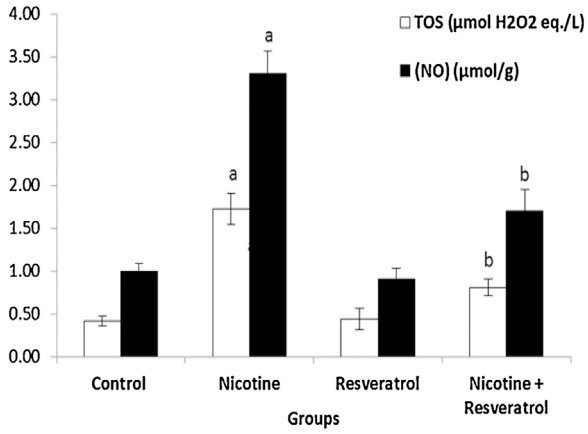


**Fig. 6.** TOS and NO levels of lung tissue of rats that exposed to nicotine (2.5 mg/kg/b.w) or/and resveratrol (20 mg/kg b.w) for 4 weeks.
Values are expressed as means ± SE; n = 10 for each treatment group.
[a] Significant different as compared to control rats and [b] significant different as compared to nicotine group.

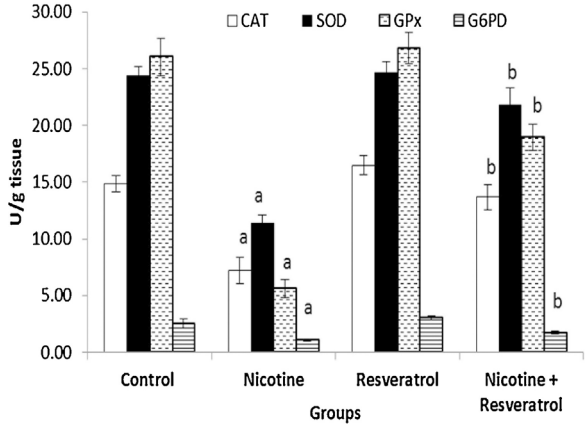


**Fig. 7.** Activities of some antioxidant enzymes of lung tissue of rats that exposed to nicotine (2.5 mg/kg/b.w) or/and resveratrol (20 mg/kg b.w) for 4 weeks.
Values are expressed as means ± SE; n = 10 for each treatment group.
[a] Significant different as compared to control rats and [b] significant different as compared to nicotine group.

significant recovery relating to SOD was observed in response to the presence of resveratrol with nicotine.

The CAT activity decreased after nicotine treatment (Fig. 7). The administration of resveratrol with nicotine significantly increased the

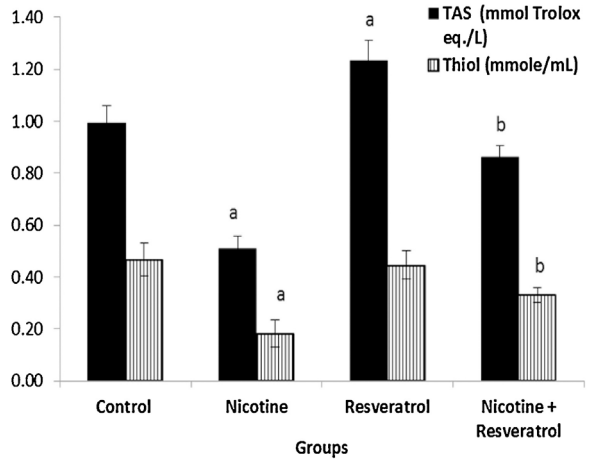


**Fig. 8.** Lung TAS and thiol of rats that exposed to nicotine (2.5 mg/kg/b.w) or/and resveratrol (20 mg/kg b.w) for 4 weeks.
Values are expressed as means ± SE; n = 10 for each treatment group.
[a] Significant different as compared to control rats and [b] significant different as compared to nicotine group.

CAT activity in lung tissue as compared with nicotine group.

The activity of GPx was significantly decreased in lung tissue of rats treated with nicotine by 78.5% as compared with the control group (Fig. 7). Treatment of the animals with resveratrol did not cause any significant changes in the activity of this enzyme, while the presence of resveratrol with nicotine minimized the observed alterations in examined enzyme activity induced by nicotine intoxication in lung tissue.

Treatment of the rats with nicotine caused a significant decrease in the activity of G6PD of the lung. A significant recovery relating to G6PD was observed in response to the presence resveratrol with nicotine by 1.6-fold, as compared with nicotine group (Fig. 7).

The total antioxidant status (TAS) levels were significantly decreased in lung tissue of rats treated with nicotine (Fig. 8). However, the presence of resveratrol with nicotine minimized the observed alterations in the TAS level induced by nicotine intoxication. Co-treatment of resveratrol with nicotine to the rats improved the thiol level by 1.8-fold as compared with nicotine group (Fig. 8).

### 3.3. Histological changes

Lung sections of control rats showed normal lung architecture with thin normal interalveolar septa and clear alveolar sacs with regular air sacs. The interalveolar spaces may be extremely narrow (Fig. 9A). Lung section of animals treated with resveratrol group showed normal perivascular and peribronchiolar cells of the lung tissues (Fig. 9B). The histological examination of lung tissue of rats treated with nicotine for 4 weeks revealed variable degrees of alterations. Nicotine treatment at the dose level of 2.5 mg/kg showed marked and severe congestion of the alveolar lung tissues with scattered congestion of peribronchiolar and perivascular cells as well as inflammatory cells were observed (Fig. 9C). Moreover, septa showed an increment in thickening with mild inflammation compared with control group. The alveoli showed an extensive destruction of their walls resulting in the formation of enlarged, irregular air space. This was evidenced by the disappearance of the alveolar septa in most areas, increased irregularity, and size of air sacs and destruction of normal tissue pattern (Fig. 9D). The section in lung treated with nicotine and resveratrol showed very mild congestion of the alveolar lung tissues (Figs. 9E and 9F).

The histopathological changes in lungs are graded and summarized in Table 1. Histological grading was made according to four grades as mansion above. The presence of several areas of dilation of bronchioles caused pressure on blood vessels leading to congestion with destruction in some blood vessels, which led to hemorrhage among the pulmonary

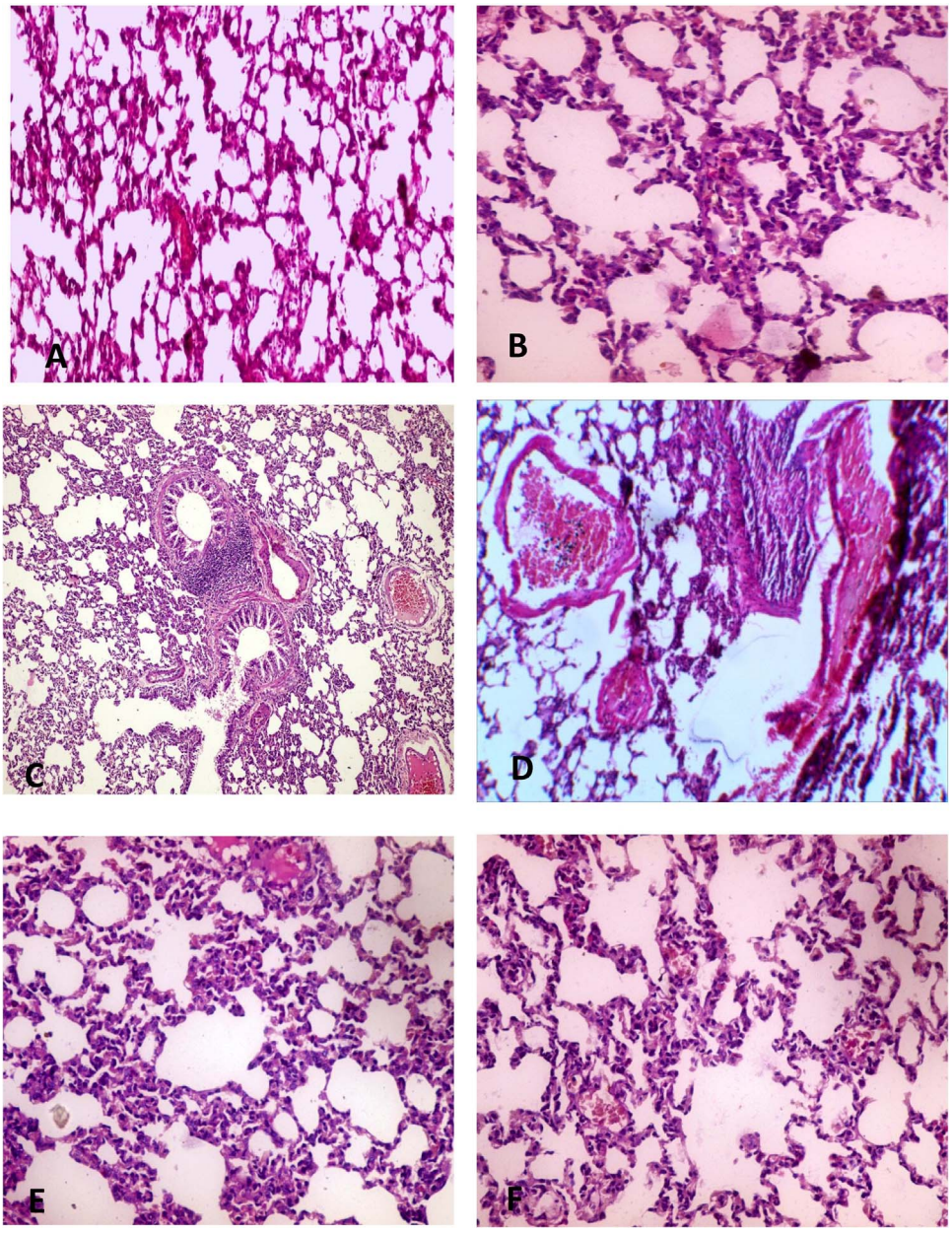


**Fig. 9.** Representative light micrographs of the lung of rats treated with resveratrol or/and nicotine. A: Showed normal control structure of the perivascular cells of the lung tissues (X 400). B: Resveratrol group showed normal perivascular and peribronchiolar cells of the lung tissues (X400). C: Section in lung of nicotine group showed congested alveolar capillaries with aggregates of chronic inflammatory cells (X100). D: Section in lung of nicotine group showed marked and severe congestion of the alveolar lung tissues with scattered congestion of peribronchiolar and perivascular cells (X400). E: Section in lung treated with nicotine and resveratrol showed thickened alveolar wall, scattered intra-alveolar non-specific inflammatory infiltrate (X400). F: Section in lung treated with nicotine and resveratrol showed very mild congestion of the alveolar lung tissues (X400). All the sections have been stained with H & E.

**Table 1**
Grading of the histopathological changes in lung sections of rats treated with Nicotine or/and Resveratrol.

| Findings | Groups | | | |
|---|---|---|---|---|
| | Control | Nicotine | Resveratrol | Nicotine + Resveratrol |
| Normal structure of the perivascular cells | + + + + | ———— | + + + + | + + +- |
| Congested alveolar capillaries | ———— | + + + + | ———— | + +– |
| Chronic inflammatory cells. | ———— | + + + + | ———— | + +– |
| Severe congestion of the alveolar lung | ———— | + + + + | ———— | ———— |
| Thickened alveolar wall | ———— | + + + + | ———— | + +– |
| Intra-alveolar non- specific inflammatory infiltrate | ———— | + + + + | ———— | ———— |
| Mild congestion of the alveolar lung tissues. | ———— | + + +- | ———— | + + +- |

———— Absence of the change in the animals of the studied group.
+ + + + A change which was often found in all the studied animals of a group.
+ + +- A change which was observed in almost all the studied animals of a group.
+ +– A change not so often observed in all animals of a group.
—+ A change which was rare within a group.

tissues.

## 4. Discussion

Lung damage was induced by injection of nicotine at a dosage of 2.5 mg/kg B.W. for 6 days a week for 4 weeks. The results showed an increase in the level of oxidative and decrease in the antioxidant status in nicotine-treated rats. Resveratrol treatment resulted in a decrement in the levels of all the markers of oxidative status, interleukins, α-FP and 8-OHDG while the enzymatic/non-enzymatic antioxidant markers were significantly increased. Thus, the study shows that resveratrol offers protection against the lung damage caused by nicotine.

In the present study, proinflammatory cytokines secreted from activated neutrophils, monocytes and macrophages of nicotine-treated animals to remove dead cells and promote tissue repair, as α-TNF, IL-1, and IL-6 have been elevated. Our observation is in coincident with Cromwell et al. [10] who found increasing the production of α-TNF, IL-1 and IL-6 within the lung of rats is similar to the effect nicotine. This increase in the levels of cytokines and adhesion molecules results in the activation of neutrophils and macrophages to the lung that can lead to tissue destruction [56].

α-FP contents were found to be increased by administration of nicotine in the current investigation. α-FP is a tumor marker for hepatocellular carcinoma, however, Willder et al. [58] found that the elevated serum α-FP with a lung neoplasm is most likely to represent a metastasis.

In the present study, the levels of 8-OHdG increased in nicotine group as compared to control animals which indicated DNA damage as the effect of nicotine. 8-OHdG acts as a biomarker of oxidative stress especially to DNA, which is involved in the instigation of various diseases [18].

In the present study, MPO was found to increase in rats as the effect of nicotine. During the respiratory burst, MPO utilizes $H_2O_2$ to produce hypochlorous acid (HClO) that reacts with proteins, unsaturated fatty acids, and any oxidizable group, to induce protein adducts and genetic mutations, and influence signaling pathways [59].

Excessive production of ROS in the nicotine-treated group leads to LPO, and this results in oxidative stress. The elevation in the level of MDA in the lungs observed in the present study may reflect the inability of the lungs to eliminate ROS produced by nicotine and/or inactivation of the antioxidant enzymes caused by the excess generation of ROS in the lungs. LPO can be used as the index of oxidative stress and change the membrane permeability. Nicotine produced ROS leading to an increase in LPO in lungs of rats as described before by Balakrishnan and Menon [5]. Other previous study has been reported that oxidative stress was the mechanism responsible for toxicity developing in lung tissue due to nicotine [13]. This suggests that the increase may be due to nicotine-associated excessive ROS production. Moreover, decreasing the functions of the defense system caused nicotine toxicity as reported by Chattopadhyay and Chattopadhyay [9].

Moreover, the present results demonstrated that nicotine administration to rats results in elevated levels of NO in lung tissues. The same observations had been reported in brain tissue by Pogun et al. [41] when nicotine has been administrated to the rats either in the acute or chronic study.

Cells employ various antioxidant mechanisms to reduce the levels of excess ROS. In the present study, increased oxidative stress can explain the significant reduction of antioxidative enzyme activity in the lung as SOD, CAT, GPx and G6PD, that suggested scavenging of free radicals from the lung tissues exposed to nicotine. G6PD is an essential modulator of antioxidative defense system [34] and for maintenance of redox potential in cells [19]. It also acts as a cofactor for other antioxidant enzymes like glutathione reductase [34]. Therefore, the inhibition of G6PD activity in the current study leads to the production of $H_2O_2$ that induced cell death and a significant increase in apoptosis as mentioned before by Tian et al. [51]. The NADPH and GSH play an important role in protecting cells against ROS. GSH is regenerated from its oxidized form (GSSG) with the help of NADPH produced in the G6PD catalyzed reaction, as well as, protects the cell from oxidative damage by destroying $H_2O_2$ and peroxide free radicals ($OH^{\cdot}$). However, antioxidant enzymes such as SOD and CAT react against radicals such as superoxide and $H_2O_2$, while GPx has a scavenging effect against alkyl, alkoxy and peroxyl radicals that can be formed from oxidized membrane compounds, and uses GSH as a substrate Ceribasi et al., 2012. Low levels of G6PD, SOD, and CAT in nicotine group as compared to the control animals could increaseoxidative stress and may lead to lung damage.

The histopathological finding showed significant morphological alteration in the lung of nicotine-treated rats and supported the biochemical changes. This could be due to the release of α-TNF, different types of IL and free radicals and subsequently the damage of alveolar structure and congestion of the lung. Stanescu et al. [48] found that the presence of neutrophils in the sputum is directly related to impaired pulmonary function, and suggested a close link between neutrophilic inflammation and airway mucus obstruction.

Our result is in parallel with Gawish et al. [21] who reported that many alterations in the pulmonary structures can be caused by four weeks of nicotine (2.5 mg/kg/day) injections as thickening of interalveolar septa caused extensive destruction of alveoli wall and formation of enlarged, irregular air space. Changes in architecture of lung, inflammatory infiltrations, abnormal air spaces, and increase in the thickening of alveolar septa were dominant in lung tissue as the effect of nicotine and these results are in agreement with El-Sokkary et al. [15]. In our study, mild interstitial inflammation and mild hyperplasia were shown in the bronchiolar lymphoid follicles.

Juan et al. [28] found that repeated consumption of trans-resveratrol at 20 mg/kg/day does not adversely affect the hematologic or biochemical parameters as well as the histopathologic examination of the organs of rats. In the present study, pretreatment with resveratrol before nicotine caused normalization of lung levels of oxidative/antioxidant parameters. It also caused significant improvement in inflammatory and tumor biomarkers. Resveratrol proved its antioxidant ability in our study by preserving TAC as well as reducing MDA levels in lungs of the nicotine + resveratrol group compared to the nicotine group. Jiang et al. [27] found that resveratrol has strong antioxidant activity, that may decrease oxidative stress and inhibit free radicals especially LPO products. Results from the present study are in agreement with other previous studies that reported the lower level of serum LPO products in animals fed with different concentrations of resveratrol [30,45]. Toklu et al. [52] found that resveratrol improved the antioxidant status and decreased oxidative damage to the heart and brain of rats with induced hypertension. Palsamy and Subramanian [40] showed that resveratrol effectively protects the kidneys from oxidative damage in diabetic rats. In the previous study, resveratrol decreased MDA levels and oxidative stress by increasing GSH levels Uguralp et al., 2005. However, our data consider the first study on the effect of resveratrol against the nicotine toxicity in lung tissues.

Resveratrol is both a free radical scavenger and a potent antioxidant because of its ability to promote the activities of a variety of antioxidant enzymes Alarcon de la Lastra et al., 2006.

Spanie et al. (2009) found that resveratrol could function as SOD1 and GPx1. These findings supported our results that the LPO levels were significantly lower in nicotine + resveratrol group while antioxidant enzymes increased. The increase in GPx activity in lung tissue observed in this study may also be explained by the excessive production of this antioxidant, in order to scavenge the overproduction of free radicals caused by oxidative stress. The antioxidant activity of resveratrol could be also due to its ability to inhibit reductase in the ribonucleotide and cyclooxygenase 2 transcription ability in DNA polymerase activity as previously reported by Uguralp et al. (2005). Zini et al. (1999) reported that the antioxidant effects of resveratrol could be due to its ability to reduce the oxidative chain reactions of ROS in competition with

coenzyme Q, remove the superoxide radicals formed in the mitochondria; and prevention of LPO.

In the present study, lung tissue TOS and TAS in nicotine + resveratrol group were similar to those of the control group, these observations demonstrated the free radical scavenger and antioxidant effects of resveratrol. The effects of resveratrol on the inflammatory and tumor markers as well as the oxidative/antioxidant parameters may be associated with its constituent compounds (polyphenolic and stilbene phytoalexins) as described by Kolouchova-Hanzlikova et al. (2004).

The decrease observed in 8-OHDG levels, a biomarker of oxidative DNA injury, in the nicotine + resveratrol group compared to the nicotine group, also provided evidence for the antioxidant and free radical-scavenging activities of resveratrol. Our observations are in agreement with previous studies that found resveratrol has been playing an effective role in preventing oxidative DNA injury *in vivo* Tatlidede et al., 2009 and *in vitro* Yan et al., 2012.

Lai et al. [33] found that the immune function was enhanced by resveratrol treatment in immunosuppressive mice. Resveratrol involved alterations of immune cell functions and cytokine production [6]. These results are in coincidence with the present finding that resveratrol in combination with nicotine decreased the inflammatory mediators as IL2, IL6, and α-TNF as well as α-FP in lung tissues when compared to nicotine-treated animals, therefore; it has anti-inflammatory and anticarcinogenic properties. In addition, Yazir et al. [60] found that the plasma levels of α-TNF and IL-1β in the stressed group were increased significantly compared to the control group, effects which were significantly prevented by treatment with resveratrol. This could be proved by the anti-inflammatory activity of resveratrol.

Moreover, the high concentration of NO was produced by inducible NO synthase (iNOS) in inflammation, and the prevention of the expression of iNOS might be an important anti-inflammatory mechanism [11]. Tsai et al. [54] reported that resveratrol inhibited the induction of iNOS and reduced NO generation. Therefore, the anti-inflammatory properties of resveratrol might be mediated also by inhibition of iNOS expression.

Furthermore, the present study found that lung tissues were generally normal in a structure in the nicotine + resveratrol group, in contrast to the abnormal changes seen in the nicotine group. These results were consistent with reports of the strong antioxidant activities of resveratrol (Gulcin, 2010, Atmaca et al., 2014).

In the current study, the activities of SOD, CAT, GPx, and G6PD, which changed after exposure to nicotine, were significantly increased by resveratrol treatment. Previous studies had also shown the antioxidant effects of resveratrol via elevation of the antioxidant status such as GSH and SOD [43,47].

In conclusion, our findings showed that the administration of nicotine caused a significant elevation in LPO and significantly reduced lung antioxidant enzymes activities of male rats. The levels of biochemical mediators implicated in nicotine-induced lung disorder (NO, IL-2, IL6, α-FP and α-TNF), also increase in nicotine exposure animals and thus parallel the changes in pulmonary inflammation and the development histopathological changes that were determined in the present study. The administration of resveratrol followed by nicotine ameliorated lung injury, which was associated with improving all the mentioned parameters. All these findings were confirmed by the histopathological examination, which revealed the ability of resveratrol to maintain structurally and functionally active lung tissues, which is somewhat similar to that of the control if it was administrated before the nicotine.

## Conflict of interest

We declare that there are no conflicts of interest.

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