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Research Article
Resveratrol: A Potential Protector Against Benzo[a]pyrene-Induced Lung Toxicity

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Abstract

Background and Objective: Benzo[a]pyrene (B[a]P), a major component of lipophilic pollutants then can be translated to diffluent substances. The aim of t he present article was to investigate protective activity of resveratrol against lung toxicity induced by B[a]P. **Material and Methods:** Male Sprague-Dawley rats were randomly assigned to 6 groups (6 animals/group): 3 negative control groups, control positive, B[a]P (20 mg kg^{-1} b.wt., resveratrol (50 mg kg^{-1} b.wt.)-B[a]P and vitamin C (1 g kg^{-1} b.wt.)-B[a]P groups. **Results:** The daily oral administration of the resveratrol (50 mg kg^{-1} b.wt.) and vitamin C (1 g kg^{-1} b.wt.) for 30 days to rats treated with B[a]P (20 mg kg^{-1} b.wt.) resulted in a significant improve plasma cholesterol, triglyceride and HDL-C as well as serum TNF-α, TBARS, IL-2,IL-6, haptoglobin, histamine, IgA, Ig E,Ig G and Ig M in B[a]P treated rats. On the other hand oral administration of resveratrol elevated the SOD, GPx and GR gene expression in lung rats treated with B[a]P. Furthermore, resveratrol and vitamin C nearly normalized these effects in lung histoarchitecture. **Conclusion:** The obtained biochemical, molecular biology and histological results of this study proved the lung protective activity of resveratrol against B[a]P induced lung toxicity in rats.

Key words: Resveratrol, B[a]P, oxidative stress biomarkers, haptoglobin, histamine IgA and Ig E

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Polyaromatic hydrocarbons (PAHs) are a group of chemicals that are formed during the incomplete combustion of organic substances, examples, burning of coal, oil, gas, wood, garbage or other organic substances, such as tobacco and charcoaled meat[1]. The acute exposure induced health effects of PAHs in humans are not fully understood. Occupational exposure to mixtures of PAHs causes eye irritation, inflammation, nausea, vomiting, diarrhea, etc. Anthracene, B[a]P and naphthalene are direct skin irritants[2].

Chronic exposure to PAHs mixtures decreased the function of respiratory and immune systems. Some of the PAHs are known human carcinogens and also cause reproductive, neurologic and developmental effects[3,4].

The lung is the most susceptible organ primarily due to its large surface area and high vasculature and its interaction directly with the atmosphere. The mechanism of which includes alterations in the epithelium of lungs, which may lead to disruption in the architecture as well as the function of the cell[5].

In addition, Reactive Oxygen Species (ROS) are produced[6,7] in the phase I and II, via B[a]P biotransformation and, can react with DNA, leading to DNA strand breaks[8,9].

ROS can deplete endogenous antioxidants by induced oxidative stress[10-13] leading to lipid peroxidation as well as nucleic acid and protein oxidation[14]. Although the cellular oxidative damage, resulting from the exposure to B[a]P and association between oxidative stress and disease[15,16].

Natural products are known to scavenge free radicals, modulate antioxidant defense system and promote carcinogen detoxification. Various epidemiological studies have suggested natural products rich in flavonoids to be effective in diabetes, neurodegenerative disorder, cardiovascular disorder and cancer[17,18]. Compounds of natural origin have been extensively used to prevent the toxicity induced by various chemicals and xenobiotics.

Resveratrol belongs to the stilbene class of compounds and is a phytoalexin. It is produced by plants in response to pathogens[19]. Not surprisingly, phenolic compounds such as resveratrol, a strong anticholestatic[20], antioxidant[21], anti-inflammatory[22], antiestrogenic[23] and anticarcinogenic agent[24], are excellent scavengers of reactive oxygen species[25]. It is nontoxic and did not have any negative effects when administrated at high dose in rats[26]. Inrats, 20 % of resveratrol are absorbed intestine[27]. The objective of this article was to evaluate the protective effect of resveratrol against lung toxicity induced by benzo[a]pyrene.

MATERIALS AND METHODS

The current study was carried out at the Faculty of Applied Medical Sciences, October, 6 University, Egypt collaboration with Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Menoufia, Egypt during March, 2020.

Chemicals and dosage: Resveratrol, benzo[a]pyrene and tween 80 were obtained from Sigma Chemical Co. (St. Louis MO, USA). All other chemicals used in this study were of the analytical grade.

Experimental animals: This study was carried out according to guidelines drawn up by the Faculty of Applied Medical Sciences, University of October 6, Egypt, on Animal Care and Utilization. A 180±10 g of rat adult were purchased from the National Cancer Institute, University of Cairo. In a room with air conditioning and 22±2°C, relative moisture of 60% and a light cycle from 8:00 am to 20:00 pm, they were individually housed in cages. Each animal was raised to the regular diet of *ad libitum* during the acclimatization period.

Experimental set up: This experiment was performed to examine resveratrol's biochemical effect on b[a]p induced lung toxicity. For 30 days, the following were treatment for 6 group of animals, each consisting of 6 rats each (Table 1).

At 31st day, blood was collected, centrifuged and plasma was used fresh for estimation of plasma cholesterol, triglycerides and cholesterol-High Density Lipoprotein (HDL) using commercially available kits (Asan and Youngdong Pharmaceutical Co., Korea)[30-32]. Serum IL-2 and IL-6 were also measured using ELISA kits from RayBiotech, Inc., USA, Quest Diagnostics Nichols Institute, San Juan Capistrano, California and R and D Systems Inc., Minneapolis, MN, USA, respectively.

The lipid peroxidation products, malondialdehyde (MDA) equivalents, were quantified using a thiobarbituric acid reactive substance assay kit (ZeptoMetrix)[33]. In brief, serum was mixed with 0.1 M sodium phosphate buffer (pH 7.4). One hundred microliters of mixture were added with 2.5 mL of reaction buffer (provided by the kit) and heated at 95°C for 60 min. After the mixture had cooled, the absorbance of the supernatant was measured at 532 nm with a spectrophotometer. The lipid peroxidation products are expressed in terms of MDA equivalents. Also, Rapid, Bio. Laboratories, Inc. has been used to determine plasma content of haptoglobin and histamine ELISA kit[34,35]. Reactions were allowed to develop at room temperature in the dark and

Table 1: Description of treatment groups

Groups	Treatment description	Treatment description
I	Normal control A (distilled water-treated) 3 mL kg^{-1}	3 mL of distilled water, orally for 30 days
II	Normal control B (tween 80-treated) 3 mL kg^{-1}	3 mL of tween 80, 1%, orally for 30 days
III	RES (50 mg kg^{-1} b.wt.)	Was treated with RES (50 mg kg^{-1} b.wt.) suspended in propylene glycol orally for 30 days (22).
IV	B[a]P (20 mg kg^{-1} b.wt.)	Was given benzo[b]pyrene orally (20 mg kg^{-1} b.wt. in propylene glycol) in a single daily dose for 30 days[28]
V	RES (50 mg kg^{-1} b.wt.)	Was pretreated with RES (50 mg kg^{-1} b.wt.)+benzo[b]pyrene (30 mg kg^{-1} b.wt.) for 30 day
VI	Vitamin C (1 g kg^{-1} b.wt.) +B[a]P (20 mg kg^{-1} b.wt.)	Was simultaneously given vitamin C (1.0 g kg^{-1} b.wt.) and benzo[b]pyrene (30 mg kg^{-1} b.wt.) for 30 days[29]

stopped by adding 2 N H_2SO_4 solution and the absorbance was measured at 450 nm.

Determination of plasma immunoglobulin A (IgA), immunoglobulin E (Ig E), immunoglobulin G (Ig G) and immunoglobulin M (Ig M): Abcam's IgA, IgE, IgG and IgM rat ELISA kit is an *in vitro* enzyme-linked immunos or bent assay (ELISA). The test was conducted based on the supplier's protocol (Rapid, Bio. Laboratories, Inc.). ELISA plates were briefly coated in carbonate buffer diluted antibody (0.05 M, 9.6 pH) and incubated overnight at 4°C. Unbound extract was removed and phosphate-buffered saline (PBS) plates at 37°C blocked from the plates. After washing (0.05 % Tween-20 in PBS). Included plasma samples and IgA, IgE, IgG and IgM standards. Plate washed and anti-rats IgA, IgE, IgG and IgM were added after the incubation. At room temperature, reactions were allowed to develop in the dark and stopped by 2 N H_2SO_4 solution, measured at 450 nm.

Quantitative real-time PCR: The total RNA extract was extracted from the lung of the rats and portions of (10-15 µg) of the isolated RNA were subjected to quantitative PCR analysis in real time, using Sepasol-RNA1Super according to instructions of the manufacturer. The two-step RT-PCR gene expression has been measured. The level of SOD, GPx, GR and TNF-α were quantified with the previously described quantitative real-time PCR[36]. The tests in 50 ml single-plex reaction mixture were conducted. Conditions of reaction were a pre-incubation at 50°C in 2 min, followed by 10 min by 40 cycles of 95°C in 15 s and 60°C in 1 min in 1 min, respectively.

The primer sequences were GPx: F 5′-CACAGTCCACCG TGTATGCC-3′, R5′-AAGTTGGGCTCGAACCCACC-3′. SOD: F 5′-ATGGGGACAATACACAAGGC-3′, R5′-TCATCTTGTTTCTCGT GGAC-3. GR: F 5′-CCATGTGGTTACTGCACTTCC-3′, R5′-GTTC CTTTCCTTCTCCTGAGC-3′. TNF-α: F5′-ACT GAA CTT CGG GGT GAT TG-3′, R5′-GCT TGG TGG TTT GCT ACG AC-3′.The internal control used Beta Actin mRNA. Beta Actin-F: 5-AGAGGGA AATCGTGCGTGAC-3′ and Beta Actin-R: 5-CAATAGTGATGA CCTGGCCGT-3′.

Histological assessment: The lungs are sliced and parts have been fixed in histologic solution of 10 % formaldehyde buffered. 5 µm thick was stained with hematoxylin eosin (HE) and examined by light microscopic according to the method of Bankroft and Steven[37].

Statistical analysis: With SPSS/18 software all grouped data was evaluated statistically[38]. The testing of hypotheses included a one-way variance analysis (ANOVA), followed by an LSD test. The statistical significance of p-values less than 0.05 was considered. For six different determinations all results were expressed as mean ± SD.

RESULTS

Revealed a significant elevation in plasma cholesterol and triglycerides as well as significant decrease in cholesterol-High Density Lipoprotein (HDL) levels ($p<0.05$) in the fourth group which represents benzo[a]pyrene (20 mg kg^{-1} b.wt.) treated group of rats compared with control group (Table 2). The administration of resveratrol (50 mg kg^{-1} b.wt.) showed significantly decreased in plasma cholesterol and triglycerides as well as significant increase in HDL levels relative to benzo[a]pyrene treated rats after 30 days ($p<0.05$). Also, The administration of vitamin C (1 g kg^{-1} b.wt.) normalized plasma cholesterol, triglycerides and HDL levels when compared to benzo[a]pyrene treated rats ($p<0.05$).The effect was more pronounced in case of simultaneous administration of resveratrol compared to administration of vitamin C-treatment.

The levels of serum lipid peroxides (TBARS), interleukin-2 (IL-2) and interleukin-6 (IL-6) of control and experimental groups of rats was shown in Table 3. Benzo[a]pyrene

Table 2: Levels of plasma cholesterol, triglycerides and cholesterol-high density lipoprotein (HDL) in normal and experimental groups of rats

Groups	Treatment description	Cholesterol (mg dL^{-1})	Plasma triglycerides (mg dL^{-1})	Plasma HDL (mg dL^{-1})
I	Normal control A (distilled water-treated) 3 mL kg^{-1}	120.67±7.32[a]	90.92±7.32[a]	33.23±2.36[d]
II	Normal control B (tween 80-treated) 3 mL kg^{-1}	125.25±8.85[a]	89.68±7.00[a]	32.78±2.00[d]
III	RES (50 mg kg^{-1} b.wt.)	122.42±8.47[a]	91.19±3.52[a]	32.91±3.06[d]
IV	B[a]P (20 mg kg^{-1} b.wt.)	200.28±9.72[d]	145.88±7.48[d]	19.98±1.93[a]
V	RES (50 mg kg^{-1} b.wt.) +B[a]P (20 mg kg^{-1} b.wt.)	148.23±7.78[b]	109.6±8.30[b]	29.00±2.52[c]
VI	Vitamin C (1g kg^{-1} b.wt.) +B[a]P (20 mg kg^{-1} b.wt.)	180.15±7.15[c]	123.64±4.46[c]	22.83±3.25[b]

Values represent the mean±SE (n = 6). Data shown are mean±standard deviation of number of observations within each treatment. Data followed by the same letter are not significantly different at $p \leq 0.05$

Table 3: Levels of lipid peroxides (TBARS), interleukin-2 (IL-2) and interleukin-6 (IL-6)in serum of normal and experimental groups of rats

Groups	Treatment description	TBARS (nmol L^{-1})	IL-2 (ng mL^{-1})	IL-6 (ng mL^{-1})
I	Normal control A (distilled water-treated) 3 mL kg^{-1}	3.53±0.51[a]	0.36±0.04[a]	128.32±8.52[a]
II	Normal control B (tween 80-treated) 3 mL kg^{-1}	3.40±0.43[a]	0.38±0.05[a]	133.29±7.35[a]
III	RES (50 mg kg^{-1} b.wt.)	3.36±0.32[a]	0.40±0.05[a]	134.81±6.55[a]
IV	B[a]P (20 mg kg^{-1} b.wt.)	9.03±0.91[c]	3.24±0.34[c]	198.45±12.40[d]
V	RES (50 mg kg^{-1} b.wt.)+B[a]P (20 mg kg^{-1} b.wt.)	4.01±0.17[a]	0.76±0.08[a]	139.09±6.69[b]
VI	Vitamin C (1g kg^{-1} b.wt.)+B[a]P (20 mg kg^{-1} b.wt.)	7.69±0.67[b]	2.66±0.30[b]	158.91±7.80[c]

Values represent the mean±SE (n = 6). Data shown are mean±standard deviation of number of observations within each treatment. Data followed by the same letter are not significantly different at $p \leq 0.05$

Table 4: Levels of plasma haptoglobin and histamine in normal and experimental groups of rats

Groups	Treatment description	Haptoglobin (µg mL^{-1})	Histamine (ng mL^{-1})
I	Normal control A (distilled water-treated) 3 mL kg^{-1}	66.21±4.23[a]	2.95±0.48[a]
II	Normal control B (tween 80-treated) 3 mL kg^{-1}	65.46±3.69[a]	2.87±0.23[a]
III	RES (50 mg kg^{-1} b.wt.)	67.32±4.55[a]	2.86±0.25[a]
IV	B[a]P (20 mg kg^{-1} b.wt.)	107.42±7.01[c]	7.55±0.63[c]
V	RES (50 mg kg^{-1} b.wt.)+B[a]P (20 mg kg^{-1} b.wt.)	77.52±5.27[a]	4.89±0.54[b]
VI	Vitamin C (1g kg^{-1} b.wt.)+B[a]P (20 mg kg^{-1} b.wt.)	86.54±5.02[b]	5.97±0.35[b]

Values represent the mean±SE (n = 6). Data shown are mean±standard deviation of number of observations within each treatment. Data followed by the same letter are not significantly different at $p \leq 0.05$

(20 mg kg^{-1} b.wt.) orally given to rats markedly increased serum TBARS, IL-2 and IL-6 when compared to negative control rats ($p<0.05$), whereas Benzo[a]pyrene treated rats-treated with the resveratrol restored the altered values to the near normalcy ($p<0.05$). Furthermore, vitamin C a proven lung protecting agent, also significantly inhibited the lipid peroxidation of lung. The effect was more pronounced in the resveratrol treated groups ($p<0.05$).

A significant increases in plasma haptoglobin and histamine in Benzo[a]pyrene (20 mg kg^{-1} b.wt.) treated groups of rats when compared with the normal control group ($p<0.05$) was shown in Table 4. Treatment of animals with RES (50 mg kg^{-1}) significantly decreased the haptoglobin and histamine levels in plasma as compared to Benzo[a]pyrene-treated group. In addition, Vitamin C treatment decreased both plasma haptoglobin and histamine significantly ($p<0.05$) as compared to Benzo[a]pyrene-treated group.

A significant elevation in plasma immunoglobulin E (Ig E) and immunoglobulin G (Ig G) and as well as significant decrease in immunoglobulin A (IgA) and immunoglobulin M (IgM) levels ($p<0.05$) in the fourth group which represents Benzo[a]pyrene (20 mg kg^{-1} b.wt.) treated rats when compared with control group was revealed in Table 5. The administration of resveratrol(50 mg kg^{-1} b.wt.) and Vitamin C (1 g kg^{-1} b.wt.) showed significantly decreased in Ig E and Ig G as well as significant increase in Ig A and Ig M levels relative to Benzo[a]pyrene treated the group of rats after 30 days ($p<0.05$).

Benzo[a]pyrene (20 mg kg^{-1}) inhibited the lung superoxide dismutase (SOD), glutathione peroxidase (Gpx) and Glutathione Reductase (GR) gene expression in benzo[a]pyrene-treated the group of rats compared with control group was displayed in Fig. 1-3. Administration of resveratrol at 50 mg kg^{-1} b.wt. and Vitamin C (1 g kg^{-1} b.wt.) respectively, led to a statistically significant promoted of SOD, GPx and GR protein expression relative to benzo[a]pyrene treated rats after 30 days ($p<0.05$). Agarosegel electrophoresis images of SOD, GPx and GR and β-action by RT-PCR support the present results (Fig. 5).

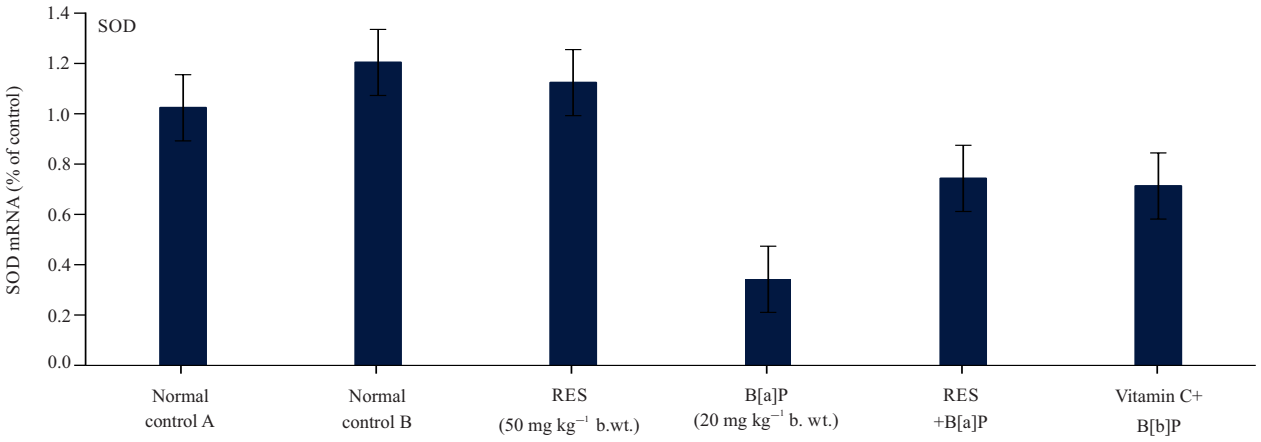

Fig. 1: Effect of resveratrol and vitamin C on lung superoxide dismutase (SOD)gene expression in Benzo[a]pyrene (b[a]p) induced lung toxicity in rats. Representative bar diagram of three independent experiments are presented

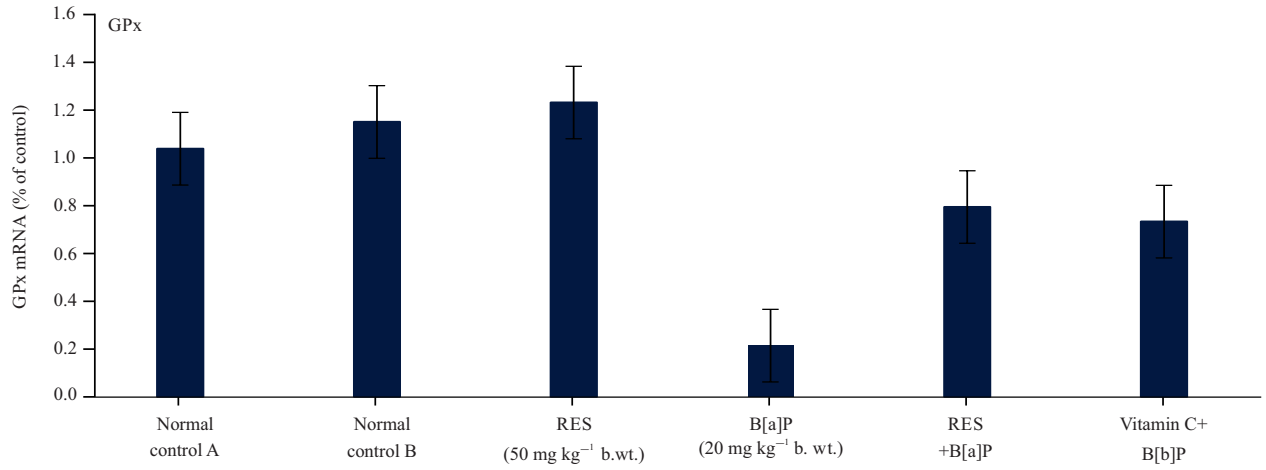

Fig. 2: Effect of resveratrol and vitamin C on lung glutathione peroxidase (GPx)gene expression in Benzo[a]pyrene (b[a]p) induced lung toxicity in rats. Representative bar diagram of three independent experiments is presented

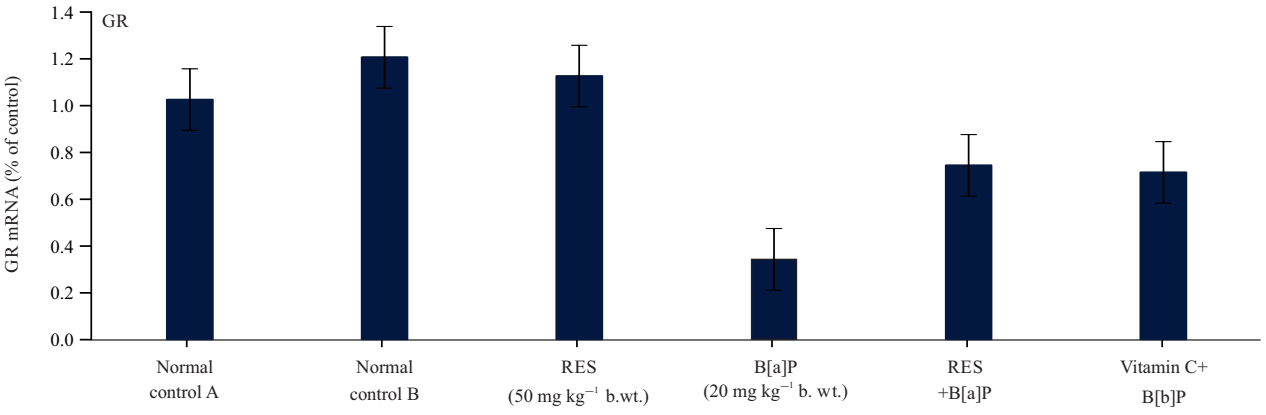

Fig. 3: Effect of resveratrol and vitamin C on lung glutathione reductase (GR)gene expression in Benzo[a]pyrene (b[a]p) induced lung toxicity in rats. Representative bar diagram of three independent experiments are presented

Table 5: Levels of plasma immunoglobulin A (IgA), immunoglobulin E (Ig E), immunoglobulin G (Ig G) and immunoglobulin M (Ig M) in normal and experimental groups of rats

Groups	Treatment description	IgA (ng mL^{-1})	Ig E (ng mL^{-1})	Ig G (ng mL^{-1})	Ig M (ng mL^{-1})
I	Normal control A (distilled water-treated) 3 mL kg^{-1}	49.24±5.24^{d}	4.02±0.36^{a}	383.09±17.92^{a}	77.16±5.28^{c}
II	Normal control B (tween 80-treated) 3 mL kg^{-1}	50.39±3.88^{d}	3.95±0.29^{a}	289.97±10.92^{a}	74.66±4.70^{c}
III	RES (50 mg kg^{-1} b.wt.)	48.51±4.91^{d}	4.12±0.25^{a}	392.51±15.66^{a}	76.67±6.25^{c}
IV	B[a]P (20 mg kg^{-1} b.wt.)	29.57±3.38^{a}	7.46±0.60^{b}	527.13±17.81^{d}	39.02±2.54^{a}
V	RES (50 mg kg^{-1} b.wt.)+B[a]P (20 mg kg^{-1} b.wt.)	41.30±3.44^{c}	4.62±0.33^{a}	426.97±13.06^{b}	70.44±6.43^{b}
VI	Vitamin C (1g kg^{-1} b.wt.)+B[a]P (20 mg kg^{-1} b.wt.)	37.31±3.06^{b}	5.29±0.31^{a}	451.86±14.71^{c}	58.22±5.98^{d}

Values represent the mean±SE (n = 6). Data shown are mean±standard deviation of number of observations within each treatment. Data followed by the same letter are not significantly different at $p \leq 0.05$

The reverse transcription-polymerase chain reaction (RT-PCR) results (Fig. 4) showed that significant increase in the expression levels of lung tumor necrosis factor-α (TNF-α) in group of treated rats with benzo[a]pyrene (20 mg kg^{-1}) when compared with normal control group of rats. Also, Administration of resveratrol at 50 mg kg^{-1} b.wt. and Vitamin C (1 g kg^{-1} b.wt.) respectively, led to a statistically significant decrease of TNF-α gene expression relative to benzo[a]pyrene treated rats ($p<0.01$). Agarose gel electrophoresis images of TNF-α and β-actin by RT-PCR support the present results (Fig. 5).

Microscopic pictures of H and E stained lung sections in Fig. 6 showing prominent diffuse lung lesion consisted of congested blood vessels (red arrows), perivascular and peribronchial lymphoid enlargement (yellow arrows), narrowed alveolar lumen due to thickening of alveolar walls (black arrows) with congested capillaries (red arrows), edema and leukocytic cells infiltration (blue arrow) (A-C) in group received benzo[a]pyrene (20 mg kg^{-1}). In contrast, mild focal lung lesion consisted of thickening of alveolar walls (black arrows) with mild edema and few leukocytic cells infiltration (blue arrow) is seen in group received resveratrol (50 mg kg^{-1} b.wt.)+benzo[a]pyrene (20 mg kg^{-1}) (D and E). Moderate diffuse lung lesion is seen in group received Vitamin C (1 g kg^{-1} b.wt.)+benzo[a]pyrene (20 mg kg^{-1}) consisted of thickening of alveolar walls (black arrows) with mild edema and few leukocytic cells infiltration (blue arrow) (F and G). (A,B,D,F) X: 100 bar 100 and (C,E,G) X: 400 bar 50.

DISCUSSION

In the present study, the histological findings proved that resveratrol affected the recovery of the lung structure in rats with B[a]P-induced lung toxicity. Indeed, there was remarkable reduction in fibrosis extent and a decrease of stellate infiltration in rats treated with resveratrol groups compared to the control B[a]P treated group. Histological studies confirmed the lung protective effect of resveratrol.

During incomplete combustion of organic matter, B[a]P a polycyclic aromatic hydrocarbon (PAH) is formed and found extensively in tobacco smoke, grilled food and industrial activities. B[a]P is a known human carcinogen, causes teratogenicity, neurotoxicity, immunotoxicity[39], induces acute lung injury, acute respiratory distress syndrome and changes in the structure and function of the alveoli[40].

Indeed, repeated treatment of B[a]P *in vivo* resulted in dyslipidemia in the form of increased plasma TC, TG and decreased HDL-C.

Our results in confirmed with Narvekar *et al.*[41] and Yen *et al.*[42], who reported that increased plasma TC, TG and decreased HDL-C in rats treated with B[a]P.

Also oral administration of resveratrol and Vit. C, respectively, showed significant protection against B[a]P induced increase in plasma cholesterol, triglycerides and HDL.

Current study showed increased plasma cholesterol and triacylglycerols levels in B[a]P-treated rats. Hydrolysis is necessary to release free cholesterol and transport vehicle is lipoprotein into the blood. In view of HDL's importance for reverse cholesterol transport the plasma lipid profile is significantly reduced by resveratrol.

Reduced plasma cholesterol levels in resveratrol treated rats could reflect the altered activity of two cholesterol effective enzymes, HMG-CoA reductase and 7α-hydroxylase cholesterol. HMG-CoA reductase activity was not investigated in the current study but Hussein[43,44] suggested that a cholesterol-lowering effect of resveratrol could be mediated by the stimulation of hepatic cholesterol-7α-hydroxylase activity. The same result was investigated by Jung *et al.*[45], who reported that, reducing effect of the activity of polyphenols on liver HMG-CoA in type 2 diabetic model of mice.

TBARS, IL-2 and IL-6gene expression[22] are involved in the regulation of cell proliferation and death by the generation of free radicals, oxidative stress and lipid peroxidation[45,46]. B[A]P leads to changes in the antioxidant-prooxidant balance, which favour the latter because the production of hepatic MDA increases and free-radical activity spreads. The increased lungs levels of TBARS, IL-2 and IL 6 have been shown to induce mitochondrial toxicity and free radical generation with chronic pulmonary toxicity[47].

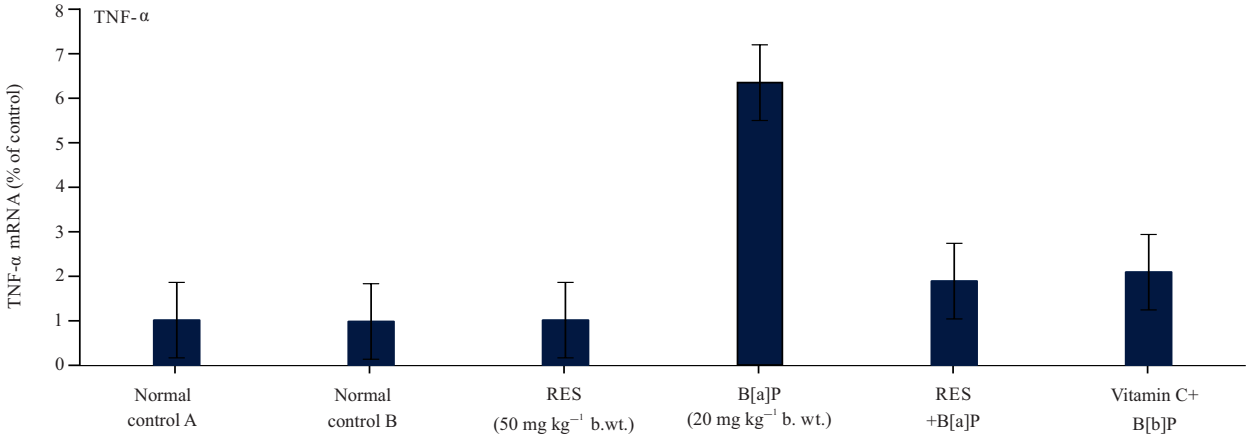

Fig. 4: Effect of resveratrol and vitamin C on lung tumor necrosis factor-a (TNF-a)gene expression in Benzo[a]pyrene (b[a]p) induced lung toxicity in rats. Representative bar diagram of three independent experiments are presented

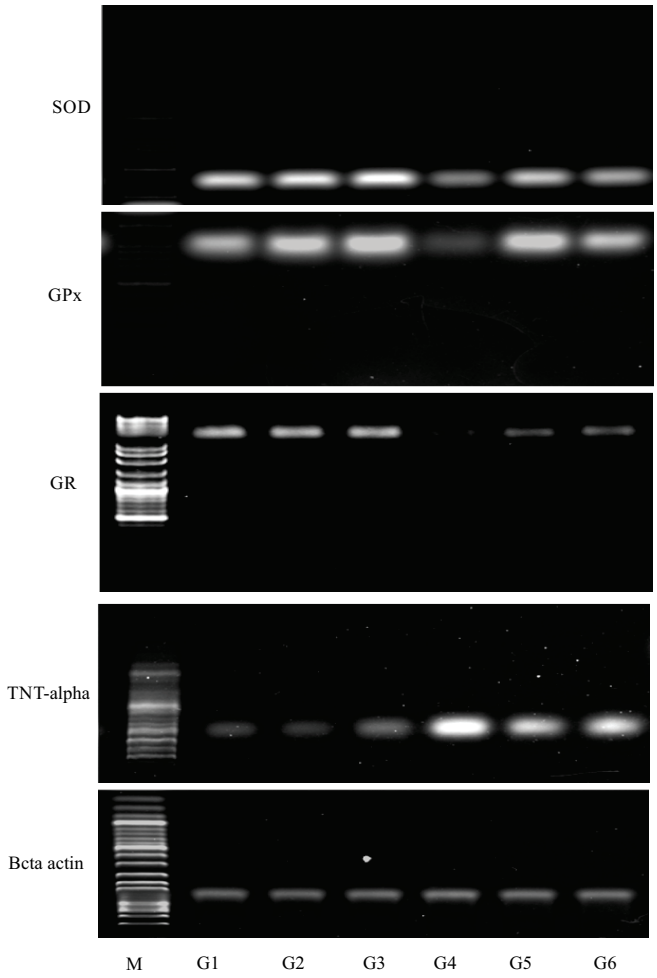

Fig. 5: An agarose gel electrophoresis shows PCR products of lung superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), tumor necrosis factor-α (TNF-α) and beta actin (C) in different studied groups
M: DNA marker with 100 bp

Resveratrol is also able to inhibit proinflammatory cytokine expression[48]. Taken together, these results indicate that the antifibrotic effect of Resveratrol is associated with the blockade of mitogenic and/or fibrogenic signaling. TNF-α was reported to induce NO formation[49]. In addition, resveratrol is a potent reactive oxygen species (ROS) scavenger[50] that normalized the oxidative stress biomarkers SOD, GPx, Akt-1 and MDA.

In this study B[a]P induced lung toxicity in rats and exhibited a significant increase in inflammatory markers (e.g., histamine and haptoglobin), this recorded significant increase was agreement with Wickman[51] and Claessens *et al*.[52] showed that, histamine is involved in the inflammatory response.

Histamine is produced by basophils and mast cells in the nearby functional tissues as part of an immune response to foreign pathogens. It is increases the capillary permeability to (WBCs) and certain proteins so that they are capable of using pathogens in infected tissues.

The present results show that resveratrol inhibits the histamine and haptoglobin levels in B[a]P-treated group's. Our results have been confirmed by the results of Amir *et al*.[53], who reported that the inhibitory effects of resveratrol against histamine, IL-6, IL-8, TPA-1 and TNF-α.

Also, B[a]P play an important role in IgE and IgG production, which is consistent with findings from other epidemiological studies[54,55] and enhanced allergic inflammation[56,57]. An inverse association between levels of B[a]P and IgA as well as IgM was observed, which is primarily responsible for protecting mucosal surfaces (eg, the respiratory and gastrointestinal tracts). Also, IgA and IgM levels was decreased in coke oven workers who had been chronically exposed to B[a]P[58].This study results were appear to agree with findings related to chronic rather than acute exposure to B[a]P. The decrease in IgA and IgM production suggests that B[a]P may compromise protection of mucosal surfaces in the respiratory tract.

Resveratrol inhibits the IgE and IgG mediated release of histamine, leukotrienes and prostaglandin D from bone marrow-derived mouse mast cells[59]. This study found that resveratrol diminished the release of IgE, IgG, haptoglobin and histamine from IgE-sensitized and antigen-exposed mast cells as markers of antigen-induced degranulation. These results suggested that this compound inhibited mast cell degranulation. Consistently oral administration of resveratrol normalizes the plasma IgA and IgM level in IgE-sensitized B[a]P treated rats. Similarly, nonapoptotic resveratrol is effective at inhibiting human eosinophil activation and degranulation at<100 m mol L^{-1} concentrations[60]. Another stilbene-type polyphenol piceatannol inhibits mast cell-mediated mediator secretion, cellular signaling and effector function[61,62]. However, the molecular regulatory mechanism(s) for the resveratrol inhibition of cell degranulation are reported. Nevertheless, resveratrol has been considered as potential targets to mediate antigen-induced release of chemical mediators and cytokines in the type I allergicreactions[63,64]. Mast cell activation is regulated by antigen-triggered FceRI aggregation that sequentially activates receptor-associated protein tyrosine kinases such as Syk and Lyn[64].

Many classes of environmental pollutants are known to enhance the intracellular formation of ROS[65]. The role of ROS in alterations of physiology, growth and survival of aquatic organisms has been reported[66]. Since induction of antioxidants represents a cellular defense mechanism to counteract toxicity of ROS, antioxidants have been used extensively to assess the extent of pollution in rivers, lakes and coastal waters[67]. In this study, the activity changes of the three antioxidant enzymes showed coherence (i.e. the activities increased initially and then dropped to lower than the controls at the end of exposure). When exposed to pollutants such as B[a]P, the self-defense system of the organisms will try to metabolize the compounds. Competition between toxic B[a]P and those produced by B[a]P bio transformation could be responsible for the inhibition of SOD, GPx and GR.

There were more major findings in the resveratrol study. firstly, IL-2 and IL-6 can be inhibited by resveratrol[68]. SOD, GPx and GR protein expression also elicited endothelial activation and this effect also could be attenuated by resveratrol. It is significant that resveratrol also attenuated H_2O_2-induced monocyte adhesion to HCAECs in a similar concentration range. The second important finding is that TNF-α is inhibited by treatment with resveratrol[69]. The present study was in confirmed with other studies (70, 71) suggested that resveratrol was effective against iNOS protein expression, IL-10 and TGF-β1-induced NF-κB activation in intact blood vessels as well.

Experimental results show that after B[a]P treatment, lung antioxidant ability is reduced, the level of oxidative stress is increased, myocardial apoptosis is aggravated, and it is obvious that SOD, GPx, GR and MDA levels[72,73]. In this study, rat with lung toxicity induced by B[a]P, the lung SOD, GPx, GR levels were significantly restrained by treatment with resveratrol. Cao *et al*. suggested that the neuro protection of resveratrol attenuates oxidative of global cerebral ischemia/reperfusion injury[74] and SOD and MDA levels of reperfusion-induced damage in rats[75].

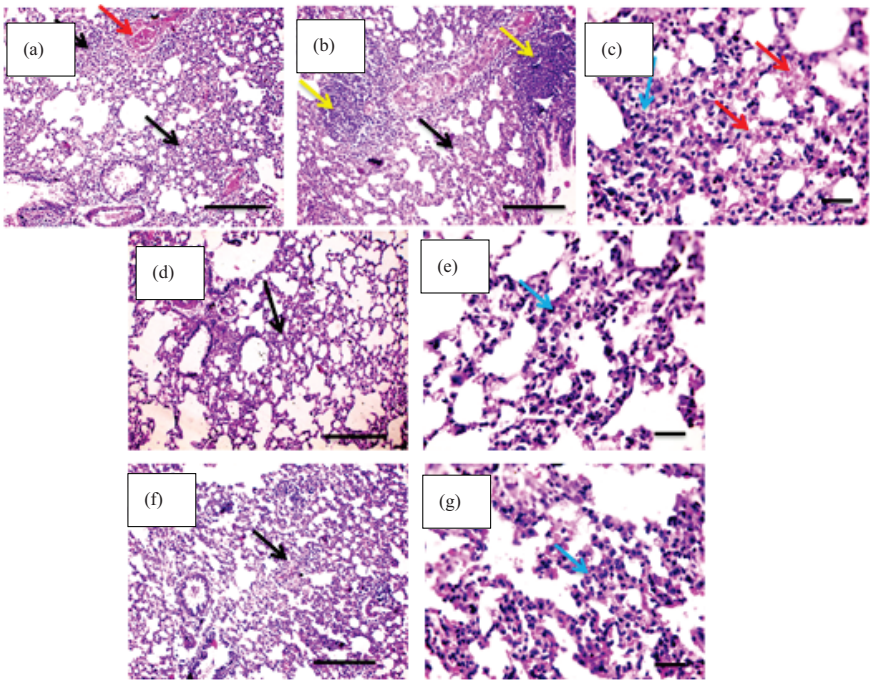

Fig. 6(a-g): Sections stained with hematoxylin and eosin (H and E, 400 X) histological examination of rats lungs of different groups compared to control group, (a), Normal control group A, (b), Group II: Normal control B (tween 80-treated) 3mL kg^{-1}. (c), Group III: Was administrate RES (50 mg kg^{-1} b.wt.). (d), Group IV: Was administrate B[a]P (20 mg kg^{-1} b.wt.), (e), Group V: Was administrate RES (50 mg kg^{-1} b.wt.)+B[a]P (20 mg kg^{-1} b.wt.) and (f) Group VI: Was administrate vitamin C (1 g kg^{-1} b.wt.)+B[a]P (20 mg kg^{-1} b.wt.)

Treatment with resveratrol nearly normalised these effects in lung histoarchitecture. In addition, serious lung changes in rats treated with B[a]P. Therefore, the obtained biochemical, molecular biology and histological results of our study proved the resveratrol could be a lung protective activity against B[a]P induced lung toxicity in rats.

In addition, the most novel and relevant finding was that resveratrol supplementation was accompanied by the alleviation of lung proliferation and oxidative stress and inflammatory reaction in this model. Resveratrol was also able to reduce newly formed lung tissues (Fig. 6). Since the proliferation of lung is an early event in toxicity-related changes, the attenuation of lung injury and fibrosis in rats by resveratrol might be associated with alleviation of inflammatory reaction.

Free radical scavenging activity of phenolic compounds is believed to be influenced by the number and position of phenolic hydrogen in their molecules[76]. It is also proposed that the higher antioxidant activity of resveratrol is related to the greater number of hydroxylgroups[77-78].

Prophylactic effect of resveratrol against B[a]P-induced lung toxicity has not been reported earlier to my knowledge and this study is perhaps the first observation of its kind.

CONCLUSION

In conclusion, the present study showed that resveratrol has powerful lung protective activity against B[a]P-induced lung toxicity, via normalize the levels oxidative stress bio markers and gene expression of inflammatory mediators.

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