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Boswellic Acid Synergizes With Low-Level Ionizing Radiation to Modulate Bisphenol Induced-Lung Toxicity in Rats by Inhibiting JNK/ERK/c-Fos Pathway

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Abstract

Bisphenol A (BPA) is a low molecular weight chemical compound that has a deleterious effect on the endocrine system. It was used in plastics manufacturing with injurious effects on different body systems. Occupational exposure to low-level ionizing radiation (<1 Gy) is shown to attenuate an established inflammatory process and therefore enhance cell protection. Therefore, the objective of this study was to investigate the protective effect of boswellic acid (BA) accompanied by whole-body low-dose gamma radiation (γ-R) against BPA-induced lung toxicity in male albino rats. BPA intoxication induced with 500 mg/kg BW. Rats received 50 mg BA/kg BW by gastric gavage concomitant with 0.5 Gy γ-R over 4 weeks. The immunoblotting and biochemical results revealed that BA and/or γ-R inhibited BPA-induced lung toxicity by reducing oxidative damage biomolecules; (MDA and NADPH oxidase gene expression), inflammatory indices (MPO, TNF-α, IL-6, and gene expression of CXCR-4). Moreover, BA and or/γ-R ameliorated the lung inflammation *via* regulation of the JNK/ERK/c-Fos and Nrf2/ HO-1 signaling pathways. Interestingly, our data demonstrated that BA in synergistic interaction with γ-R is efficacious control against BPA-induced lung injury *via* anti-oxidant mediated anti-inflammatory activities.

Keywords

boswellic acid, bisphenol, ionizing radiation, JNK, ERK, c-Fos

Introduction

Bisphenol A (BPA) is one of the widely used chemical compounds in the manufacture of polyester resins, epoxy resins, polycarbonate plastics, and flame retardants. In food and beverage packaging, polycarbonate plastics are used; resins are used as lacquers to paint metallic items such as food containers, bottle tops, and water pipes. Some polymers used within dental sealants and dental coatings often include BPA.[1] More than 6 billion pounds of BPA are produced worldwide each year, and more than 100 tons are released into the atmosphere each year.[2] Human exposure is believed to be predominantly dietary, because BPA-containing polymers can be hydrolyzed under high temperatures and acidic or basic conditions, leaching into food and drink containers.[3] Thermal printer paper could also be a source of dermal exposure to BPA.[4] BPA is an endocrine-disrupting, estrogenic agent.[5]

Even at very low doses, the mimicry of estrogen by BPA results in several health problems, including prostate[6] and breast cancer,[7] and induces reproductive disorders.[8] Furthermore, BPA exposure is associated with decreased lung function.[9] In addition to, BPA has other consequences, such as inflammatory cytokine dysregulation,[10] and enhance oxidative

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stress,[11] which is independent of estrogenic activity. BPA can interfere with and disrupt the immune system through a variety of cytokine signals.[12] Therefore, dysregulation of cytokine signaling can cause a variety of diseases including allergies, autoimmune diseases, inflammation, and cancer.[13] As well as, BPA activates the transcription and translocation of MAPK/ERK/c-fos and p-JNK signaling pathway member.[14]

Environmental or occupational ionizing radiation exposures resulted in various cellular responses relying on the dose and exposure rate.[15] The damaging effects of high-level ionizing radiation on the biological system are caused mainly by excessive generation of reactive oxygen species (ROS) that exceed antioxidant levels, resulting in cellular oxidative stress that induces lipid peroxidation, protein oxidation, and antioxidant depletion.[16,17] On the contrary, low-level ionizing radiation (<1 Gy) improves cell protection by activating the pathway AKT / nuclear factor kappa B (NF-κB), which controls apoptosis and cell proliferation.[18,19] Mitogen-activated protein kinase (MAPK) superfamily (extracellular signal-kinase (ERK), c-N-kinase (JNK), and p38 MAPK) regulated by ionizing radiation and affect essential roles in cell survival or death.[20]

Natural compounds have gained considerable attention in recent years for their use in the prevention and treatment of various chronic diseases because they are free of significant toxicity. Boswellic acid (BA) derived from *Boswellia carteri* and *Boswellia serrata* gum resin. It has proven to be an effective agent against many chronic conditions such as asthma, diabetes, arthritis, inflammatory bowel disease, Alzheimer's disease, Parkinson's disease, cancer, etc. The molecular targets associate with its broad range of biological activities include growth factors, receptors, enzymes, kinases, and transcription factors.[21] BA usually reduces the oxidative status and able to lower malondialdehyde (MDA) production due to its antioxidant and anti-inflammatory effects, in addition to it can enhance the anticancer activities, based on the suppression of proinflammatory interleukins and growth factors.[22] Therefore, in this study, we hypothesized that Boswellic acid with whole-body low-dose gamma radiation (γ-R) may improve the BPA-induced lung toxicity in rats through its anti-inflammatory effects by directly targeting inflammatory signals, while also targeting indirectly inflammatory molecules.

Material and Methods

Chemicals

Bisphenol A (BPA) from Sigma Chemical Co., Nasr City, Cairo, Egypt was obtained in the form of rutin hydrate. All other chemical substances and reagents used in this analysis were of analytical grade. BPA was dissolved in ethanol, then complete the volume by corn oil (ethanol is 5% of the total volume).

Animals

In this study, 48 male Wister rats (weighing 120–150 g) were used, obtained from the National Centre for Radiation Research and Technology (NCCRT), Cairo, Egypt. The rats were housed in cages and maintained a 12 h light/ dark cycle. They were allowed to acclimatize to the environmental conditions for 1 week before starting the experiment and were kept on standard food pellets containing all nutritive elements and liberal water ad libitum. All animal procedures were carried out in compliance with the guidelines of the National Center for Radiation Research and Technology, Egypt, and with guidance for the proper treatment and use of laboratory animals (NIH Publication No. 85–23, updated 1985).

Irradiation of Animals

Whole-body ionizing gamma-irradiation (IR) was carried out at the National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority, Cairo, Egypt, using Canadian Gamma Cell-40 biological irradiator (137 Cesium) produced by Canada Limited Atomic Energy, Ontario, Canada. During the time of exposure, the radiation dose was 0.61 Gy / min. The cumulative dose of radiation was 0.5 Gy as a single dose for the whole body measured according to the Dosimeter department in the NCRRT. Animals were not anesthetized before irradiation.

Experimental Design

Animal groups. Animals were divided randomly into 8 groups (6 animals per group):

- **Group 1 (Control):** Healthy animals supplied distilled water by gastric intubation daily for 4 weeks.
- **Group 2 (BA):** According to Mishra et al. (2011),[23] Rats were treated orally with boswellic acid (BA) by gastric intubation in a dose of 50 mg/kg body weight a day for 4 weeks.
- **Group 3 (IR):** Rats were exposed to a single dose (0.5 Gy) of ionizing radiation as a low dose of radiation according to Shimura & Kojima (2014).[24]
- **Group 4 (BA + IR):** Rats were treated orally with BA every day at the same dosage as group 2 for 4 weeks, and were exposed to a single dose of IR (0.5 Gy).
- **Group 5 (BPA):** According to Amaravathi et al. (2012),[25] Rats were treated orally with 500 mg/kg body weight Bisphenol A (BPA) daily over 4 weeks.
- **Group 6 (BPA + BA):** Rats were orally treated with BPA daily for 4 weeks at the same dose given to group 5 then treated with BA daily for another 4 weeks at the same dose given to group 2.
- **Group 7 (BPA + IR):** Rats were orally treated with BPA daily for 4 weeks at the same dose given to group 5 then exposed to a single dose of IR (0.5 Gy).
- **Group 8 (BPA + IR+ BA):** Rats were orally treated with BPA daily for 4 weeks at the same dose given to group 5 then exposed to a single dose of IR (0.5 Gy), then treated with BA daily for 4 weeks at the same dose given to group 2.

Table 1. Primer Sequences for the Genes Amplified.

Gene	Strand	Sequence 5′-3′	Product length (bp)	Ref. Seq.
Ho-1	F	GAAGAGGAGATAGAGCGAAACAAGC	177	NM_012580
	R	CTCGTGGAGACGCTTTACGTAGTGC		
NADPH oxidase	F	GGAAATAGAAAGTTGACTGGCCC	199	XM_008767566
	R	GTATGAGTGCCATCCAGAGCAG		
CXCR-4	F	TCCTGCCCACCATCTATTTTATC	226	NM_022205
	R	ATGATATGCACAGCCTTACAT		
Nrf-2	F	CACATCCAGACAGACACCAGT	121	NM_031789
	R	CTACAAATGGGAATGTCTCTGC		
IκBα	F	ACCTGGTCTCGCTCCTGTTG	173	NM_001105720
	R	GCTCTCCTCATCCTCACTCTCG		
β-actin	F	TTGTCCCTGTATGCCTCT	220	NM_031144
	R	TAATGTCACGCACGATTTCC		

After the last dose of BA administration, rats fasted overnight. Blood samples were withdrawn from the heart of each animal, under light anesthesia by diethyl ether. Blood was allowed to coagulate and then was centrifuged at 3000 rpm for 15 min. Immediately after collecting the blood, animals were sacrificed via cervical dislocation; lung tissues were immediately dissected, rinsed in ice-cold saline, plotted to dry, and weighed. Lung tissue's left lobes were fixed in 10% formalin prepared in phosphate-buffered saline (PBS) for use in histopathological examination. A weighed part of each lung was homogenized with ice-cooled PBS to prepare 20% w/v was homogenate. The homogenate was then centrifuged at 4000 rpm for 5 min. at 4°C using a cooling centrifuge to remove cell debris. The aliquots were then kept at −80° C until analysis day.

Biochemical investigation. The levels of tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), matrix metallopeptidase 9 (MMP-9), myeloperoxidase (MPO), and ribonuclease P / MRP protein subunit POP1 (POP1) in the lung tissue were measured by ELISA kit supplied by MyBioSource, Inc. MDA in lung tissue was measured by Colorimetric kit supplied by biodiaganostic, 29 El-Tahrer St., Dokki-Giza, Egypt.

Molecular investigation

Determination of hemoxygenase-1 (Ho-1), nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase), chemokine receptor type 4 (CXCR-4), nuclear factor (erythroid-derived 2)-like 2(Nrf-2), and the inhibitor of nuclear factor kappa B α (IκBα) genes expression. Total RNA was extracted from lung tissue using RNeasy Mini Kit (Qiagen, Cat. No. 74104) according to the manufacturer's instructions. First-strand complementary DNA (cDNA) synthesis was performed using QuantiTect Reverse Transcription Kit (Qiagen, Cat. No. 205311) according to the manufacturer's instructions using 1μg RNA as a template. RT-PCR was performed in a thermal cycler step one plus (Applied Biosystems, USA) using the Sequence Detection Software (PE Biosystems, CA). The oligonucleotides utilized in these experiments are listed in Table 1. The reaction mixture of total volume 25 μl was consisting of 2X SYBR Green PCR Master Mix (Qiagen, Cat. No. 204143), 900 nM of each primer, and 2μL of cDNA. PCR thermal-cycling conditions included an initial step at 95°C for 5 min; 40 cycles at 95°C for 20 s, 60°C for 30 s, and 72°C for 20 s. The relative expression of the real-time reverse transcriptase PCR products was determined by the ΔΔCt method. This method calculates a relative expression to the housekeeping gene using the equation: fold induction $= 2^{-(\Delta\Delta Ct)}$. Where ΔΔ Ct = Ct [gene of interest (unknown sample)-Ct housekeeping gene (unknown sample)] − [Ct gene of interest (calibrator sample) − Ct housekeeping gene (calibrator sample)].[26]

Western immunoblotting analysis of ERK1/2, JNK, and c-fos proteins in lung tissue homogenate. Lung tissue protein was extracted using TRIzol reagent and protein concentration was quantified according to Bradford.[27] 20μg of protein per lane were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using 10% acrylamide gels and transferred on to PVDA membranes. Membranes were incubated at room temperature for 2 hours with blocking solution (5% non-fat dried milk in 10 mM Tris-HCl, pH 7.5, 100 mM NaCl, and 0.1% Tween 20), then incubated overnight at 4°C with a primary antibody toward ERK1/2 or JNK or c-fos proteins with β-actin as a control. After washing 3 times in washing buffer (10 mM Tris-HCL, pH 7.5, 100 mM NaCl, and 0.1% Tween 20), the membrane was incubated with the secondary monoclonal antibody conjugated to horseradish peroxidase at room temperature for 2 h, and then membranes were washed 4 times with the same washing buffer. The membrane was developed and visualized by chemiluminescence using Invitrogen™ detection kit (Catalog #AHO1202) according to the manufacturer's protocols, then exposed to X-ray film. Quantification of ERK1/2 or JNK or c-fos proteins was carried out using a scanning laser densitometer (Biomed Instrument Inc., USA).

Histopathological study. Specimen from the lung of all examined groups was washed, dehydrated in ascending grades of ethyl alcohol, cleared in xylene, and embedded in paraffin wax. Sections of 5–6 μm in thickness were cut out, deparaffinized, and

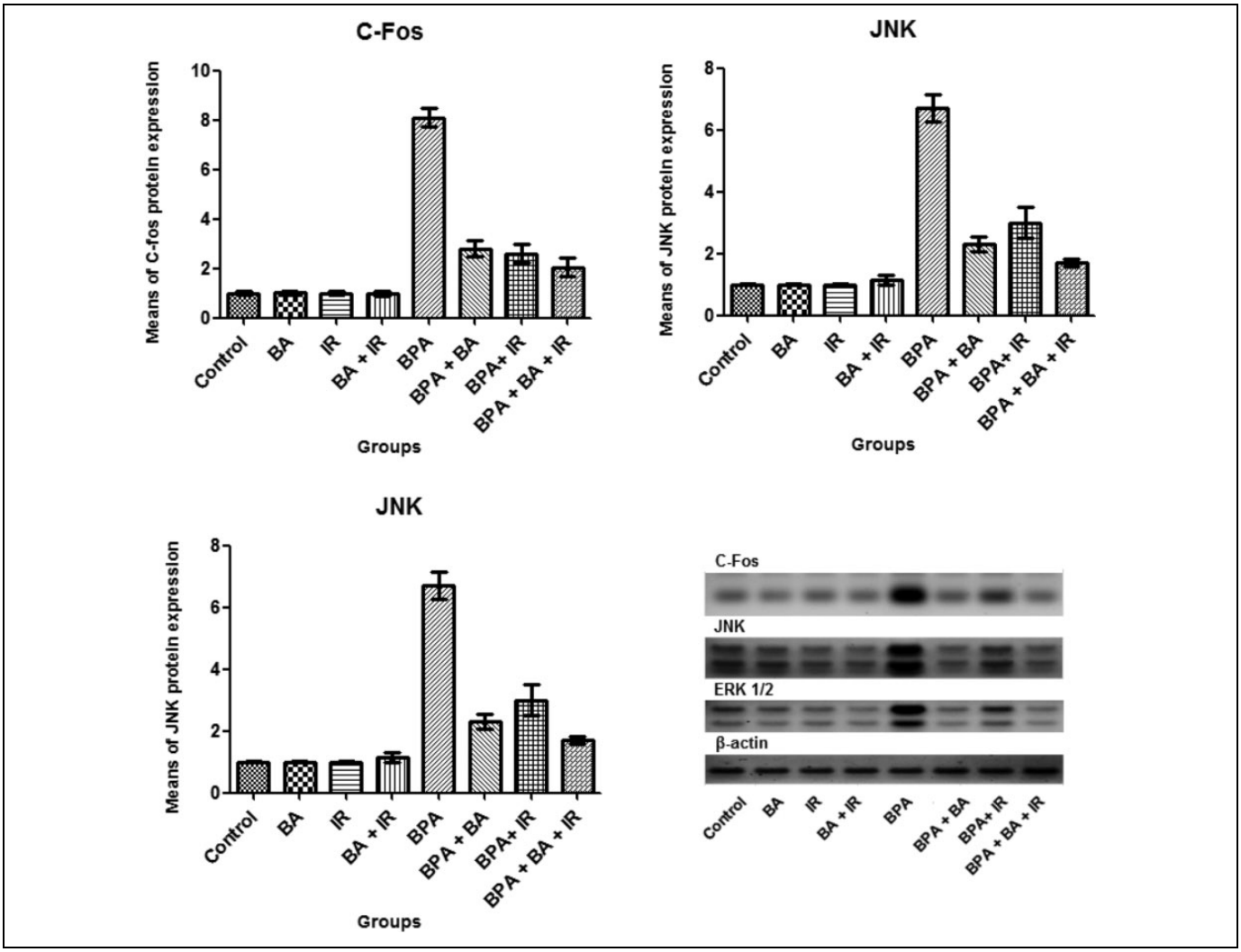

Figure 1. Western blotting analysis of c-Fos, JNK, and ERK1/2 protein expressions in BPA-treated rat groups, each column presents mean ± SD.

stained with Hematoxylin and Eosin (H & E) for examination under the light microscope.[28]

Statistical Analysis

The data were presented as means ± standard error of the mean (S.E.) they were analyzed using One-Way ANOVA followed by Tukey-Kramer multiple comparison test. Graph Prism software, version 5, Inc., USA was used to perform the statistical analysis and graphical presentations. The level of significance was fixed at $P \leq 0.05$ with respect to all statistical tests.

Results

Biochemical Studies

The effect of BA+IR on the MAPK family members (ERK1/2, JNK, and c-Fos) activities. Immunoblot detection of MAPK family members proteins (ERK1/2, JNK, and c-Fos) in the lung tissue of different treated groups and the statistical analyses are shown in Figure 1. Western blot analysis was performed with antibodies against c-Fos, JNK, ERK1/2, and β-actin proteins as mentioned in the Materials and Methods section. The expression of β-actin acts as a reference loading control. The lung tissue levels of MAPK family proteins were significantly increased with BPA toxicity (ERK1/2: 3.5 fold, JNK: 5.7 fold, and c-Fos: 7.1 fold in respect to normal control) but its level significantly modulated with BPA-combined treatments while the synergistic interaction between BA and γ-R significantly inhibited its expression (ERK1/2: −0.73 fold, JNK: −0.75 fold, and c-Fos: −0.75 fold in respect to BPA group).

BA and γ-R suppress cytokines levels in BPA-treated rats. The effect of BPA intoxication upon pro-inflammatory cytokines (TNF-α and IL-6) and gene expression of chemokine (CXCR-4) were evaluated in lung tissue as shown in Table 2. It was found that BPA treatments induced a significant increase in the levels of TNF-α and IL-6 and CXCR-4 gene expression with respect to normal control (2.9 fold, 3.5 fold &7.7 fold respectively).

Table 2. Effect of Ba and γ-R on Cytokines Levels of BPA-Induced Lung Toxicity in Different Groups.

Parameters / groups	TNF-α (ng/mg tissue)	IL-6 (ng/mg tissue)	CXCR-4 (Relative gene expression)
Control	34.3 ± 2.55	17.85 ± 4.12	1 ± 0.074
BA	33.1 ± 8.91	14.95 ± 2.10	1 ± 0.075
IR	43.55 ± 3.18	32.8 ± 3.99[a]	1 ± 0.066
BA + IR	25.6 ± 0.99	26 ± 2.7	1.08 ± 0.09
BPA	132.35 ± 5.87[a]	81.05 ± 13.09[a]	8.65 ± 0.92[a]
BPA + BA	64.95 ± 2.62[a,b]	45.4 ± 4.03[a,b]	3.35 ± 0.31[a,b]
BPA + IR	71.75 ± 5.02[a,b]	58.55 ± 6.44[a,b]	3.91 ± 0.55[a,b]
BPA + BA +IR	45.75 ± 4.03[b]	31.15 ± 3.88[a,b]	2.43 ± 0.34[a,b]

Each value represents the mean ± standard deviation.
[a]Significant difference versus control group at $p \leq 0.05$.
[b]Significant difference versus BPA group at $p \leq 0.05$.

Table 3. Effect of BA and γ-R on Pro-Inflammatory Mediator's Levels of BPA-Induced Lung Toxicity in Different Groups.

Parameters / groups	MMP-9 (ng/mg tissue)	IκB lpha; (relative gene expression)	MPO (ng/mg tissue)
Control	24.30 ± 3.40	1.03 ± 0.03	47.05 ± 10.76
BA	45.80 ± 10.04	1 ± 0.11	37.25 ± 3.60
IR	40.70 ± 6.51	1.01 ± 0.02	66.60 ± 6.56[a]
BA + IR	29.20 ± 2.26	1.03 ± 0.09	84.80 ± 7.70[a]
BPA	120.65 ± 9.69[a]	0.41 ± 0.18[a]	117.45 ± 6.27[a]
BPA + BA	56.65 ± 9.97[a,b]	0.82 ± 0.06[a,b]	65.15 ± 2.61[a,b]
BPA + IR	57.15 ± 4.60[a,b]	0.73 ± 0.12[a,b]	92.15 ± 9.44[a,b]
BPA + BA +IR	39.35 ± 9.83[b]	0.91 ± 0.09[b]	42.35 ± 3.47[b]

Each value represents the mean ± standard deviation.
[a]Significant difference versus control group at $p \leq 0.05$.
[b]Significant difference versus BPA group at $p \leq 0.05$.

Interestingly, BA supplementation in a synergistic interaction with low-level exposure of γ-R showed statistically significant restoration of TNF-α and IL-6 levels and gene expression of CXCR-4 (TNF-α: −0.65 fold, IL-6: −0.62 fold and CXCR-4: −0.72 fold compared to BPA group).

BA and γ-R inhibit pro-inflammatory mediators in BPA-treated rats. Further evaluation of BA and γ-R for their anti-inflammatory activity was shown in Table 3. It's worthy to note that BPA intoxication gave rise to a significant increase in the inflammatory markers (MMP-9: 3.97 fold, MPO: 1.50 fold, and IκB (IKK): −0.60 fold in respect to normal control).On the contrary, the administration of BA with γ-R exposure showed statistically significant improvement in the inflammatory biomarkers against BPA toxicity compared with normal controls (MMP-9: −0.67 fold, MPO: −0.64 fold and IκB 1.25 fold compared to BPA group).

Effect of BA and γ-R on redox status in BPA-treated rats. The current study was conducted to investigate how BA counteracts the lung toxicity induced by BPA either alone or combined with γ-R by investigating the expression of genes involved in the redox balance. Expression of NADPH Oxidase, Nrf-2, and HO-1 genes by qPCR as well as the level of MDA and POP-1 activity were analyzed. It was found that the MDA level, the transcript levels of the NADPH Oxidase gene, and the activity of POP-1 were significantly increased due to the treatment with BPA (5.39 fold, 4.8 fold, and 1.93 fold respectively in respect to normal control). However, the genes expression of Nrf-2 and HO-1 were significantly downregulated (Nrf-2: −0.88 fold, HO-1: −0.80 fold in respect to normal control). On the other hand, the combined treatments showed a significant modulation in the redox status biomarkers (MDA: −0.68 fold, NADPH Oxidase: −0.7 fold, POP-1: −0.51 fold, Nrf-2: 6.6 fold, HO-1: 3.15 fold in respect to BPA group). BA supplementation synergistically with low-level exposure of γ-R showed significant modulation in the transcript levels of NADPH Oxidase, Nrf-2, and HO-1 genes against BPA-induced lung toxicity (Table 4).

Histopathological Observation

Lung tissues of control and rats receiving BA, γ-R, and BA+γ-R showed normal lung architecture, thin inter-alveolar septa, folded columnar epithelial cells of bronchiole, clearly seen alveolar sacs, normal pulmonary vessels, and normal fibrous tissues distribution. The alveoli appeared inflated with thin inter-alveolar septa (Figure 2A-D). Lung tissues of rats receiving BPA revealed collapse of some alveoli and compensatory emphysematous appeared as giant alveoli, marked thickening of blood vessels wall with perivascular inflammatory infiltration mainly lymphocytes and macrophages. Thickening of alveolar septa was also noticed (Figure 2E). Lung tissues of rats treated with BA after receiving BPA showed moderate improvement in comparison with the untreated group. The lung tissue section displayed congestion of alveolar capillaries, emphysematous areas, and little leukocytic infiltration (Figure 2F). on the other side, lung tissue sections of rats treated with IR after receiving BPA showed mild improvement in compared with the previous group appeared as congestion of perialveolar capillaries, lung edema with scatted foci of inflammatory (Figure 2G). the lung tissue section of rats treated with BA+ IR after receiving BPA showing marked improvement where almost no significant pathological alterations expect few perivascular leukocytic aggregations (Figure 2H).

Discussion

Various natural compounds isolated from different plants have been used for treating numerous chronic diseases, showing notable pharmacological properties. Different cell signaling pathways can interfere with these agents.[29] This study focuses on the effectiveness of BA as a natural product combined with a low dose of ionizing radiation (IR) to modulate BPA-induced-lung toxicity in rats. It is known that the MAPK family includes classic signal transduction pathways mediators associated with inflammatory processes. ERK is activated by

Table 4. Effect of BA and γ-R on Redox Status Biomarkers of BPA-Induced Lung Toxicity in Different Groups.

Parameter / groups	Nrf-2 (relative gene expression)	MDA (nmol/g.tissue)	NADPH Oxidase (relative gene expression)	HO-1 (relative gene expression)	POP-1 (ng/mg tissue)
Control	0.06 ± 1.02	9.7 ± 2.2	1.03 ± 0.07	0.06 ± 1.02	52.6 ± 6.08
BA	1.01 ± 0.02	5.85 ± 1.7	1.00 ± 0.07	1.0 ± 0.05	34.3 ± 4.10
IR	1.03 ± 0.05	13.5 ± 2.7	1.02 ± 0.07	1.0 ± 0.04	67.2 ± 3.32
BA + IR	1.02 ± 0.04	12.1 ± 1.7	1.01 ± 0.09	1.01 ± 0.03	46.1 ± 3.04
BPA	0.13 ± 0.02[a]	61.95 ± 8.4[a]	6.00 ± 0.24[a]	0.21 ± 0.07[a]	154.3 ± 55.9[a]
BPA + BA	0.71 ± 0.05[a,b]	28.1 ± 2.3[a,b]	2.20 ± 0.18[a,b]	0.76 ± 0.04[a,b]	99.5 ± 34.15
BPA + IR	0.40 ± 0.08[a,b]	25.5 ± 4.2[a,b]	2.65 ± 0.39[a,b]	0.64 ± 0.03[a,b]	87.0 ± 6.29
BPA + BA + IR	0.95 ± 0.05[b]	19.6 ± 1.3[b]	1.84 ± 0.32[a,b]	0.85 ± 0.05[a,b]	75.3 ± 3.96

Each value represents the mean ± standard deviation.
[a]Significant difference versus control group at $p \leq 0.05$.
[b]Significant difference versus BPA group at $p \leq 0.05$.

inflammation and growth factors While JNK and p38 are activated by stress and inflammation.[30]

C-Fos is an essential complex element of the activator protein (AP)-1. c-Fos engaged in signal transduction, cell proliferation, and differentiation, cell motility, cancer development, angiogenesis, invasion, and metastasis.[31] Previous studies identified c-Fos as one of the early-response genes for ionizing radiation.[32] Induced expression of c-Fos by radiation, alongside c-jun, Egr-1, and NF-κB activated a series of downstream genes that were necessary for cells and tissues tolerance to radiation-induced stress.[33] In addition, the induction of c-Fos was also observed in cells treated with low doses of radiation (0.5 to 2 Gy),[34] while this induction was transient, reaching a maximum level of 1 h and decreasing by 4 hours to the constitutive level.[35] C-Fos may have played a major role in cellular response to ionizing radiation, according to previous research.

BPA exposure alters cell-signaling pathways by inducing ROS, resulting in increased proliferation, pro-survival upregulation proteins, and increased migration and invasion of other cells.[36] Endogenous ROS in human cells are associated with increased cell proliferation and activation of ERK1/2.[37] BPA activates the transcription and translocation of MAPK/ERK/c-fos and p-JNK signaling pathway member and stimulated estrogen receptor alpha (ER-α) signaling may result in increased ERK / MAPK activation. Such results were reported by the previous studies.[13,38,39] BA or low dose of IR effectively decreased ERK, JNK and c-fos phosphorylated forms. Our findings are agreed with the previous studies.[32,40-44]

MAPK pathways stimulate a cellular response via nuclear transcription factors including NF-κB, a key regulator of inflammatory gene expression that is stimulated in response to different inflammatory stimuli and environmental stressors. Once activated, NF-κB translocates to the nucleus, which is a key process for regulating the transcription of certain cytokines like TNF and IL-6.[45] After exposure to BPA the release of inflammatory cytokines was increased. In addition, elevated (ER)-α expression levels are correlated with changes in oxidative stress, expression of inflammatory genes, and changes in cell proliferation signals. These findings support the oxidative stress induced by BPA and activate inflammatory signals.[46]

In addition, the immunotoxicant BPA can cause toxic effects on organs and systems by altering the cytokine and chemokine secretion. Our present results showed that BPA generally increased the secretion of TNF-α and IL-6 levels accompanied by up-regulation of the gene expression of chemokine receptor type 4 (CXCR-4). These results provided further confirmation that BPA exacerbates inflammation and airway symptoms. Such results were reported by the previous studies.[35,47,48] BA reduced the production of the inflammatory cytokines and chemokines. The ability to suppress proinflammatory cytokines and antioxidant status regulation indicate that the protective effect of BA in rats could be mediated by immune system modulation. We found that BA or low dose of γR effectively decreased TNFα, IL-6 levels, and downregulated CXCR-4 gene expression. Such results were reported by the previous studies.[24,39,49,50-52]

BPA exposure is notably associated with high chronic inflammatory response, resulting in DNA damage due to oxidative stress.[53] ROS Overproduction, usually due to excessive stimulation of reduced NADPH by pro-inflammatory cytokines like TNF-α, leads to oxidative stress. Inflammatory target protein such as MMP-9 is associated with NADPH oxidase activation and ROS overproduction in response to pro-inflammatory mediators. Oxidative stress is a deleterious process that leads to airway and lung damage and consequently to several inflammatory diseases or injuries. Oxidative stress also regulates the key pathways for inflammatory signal transduction and target proteins associated with the pulmonary airway and lung inflammation.[54] The exposure to BPA up-regulated the NADPH oxidase expression, Also, BPA treatment caused a significant increase in MPO, MMP-9 activities, and MDA level. In contrast, BPA treatment induced down-regulated in phosphorylation of IκBα expression. Such results were reported by the previous studies.[53,55-58] The present results demonstrated that BA or low dose of IR downregulated the NADPH oxidase expression as well as a significant decrease in MMP-9, MPO activities and MDA level was observed

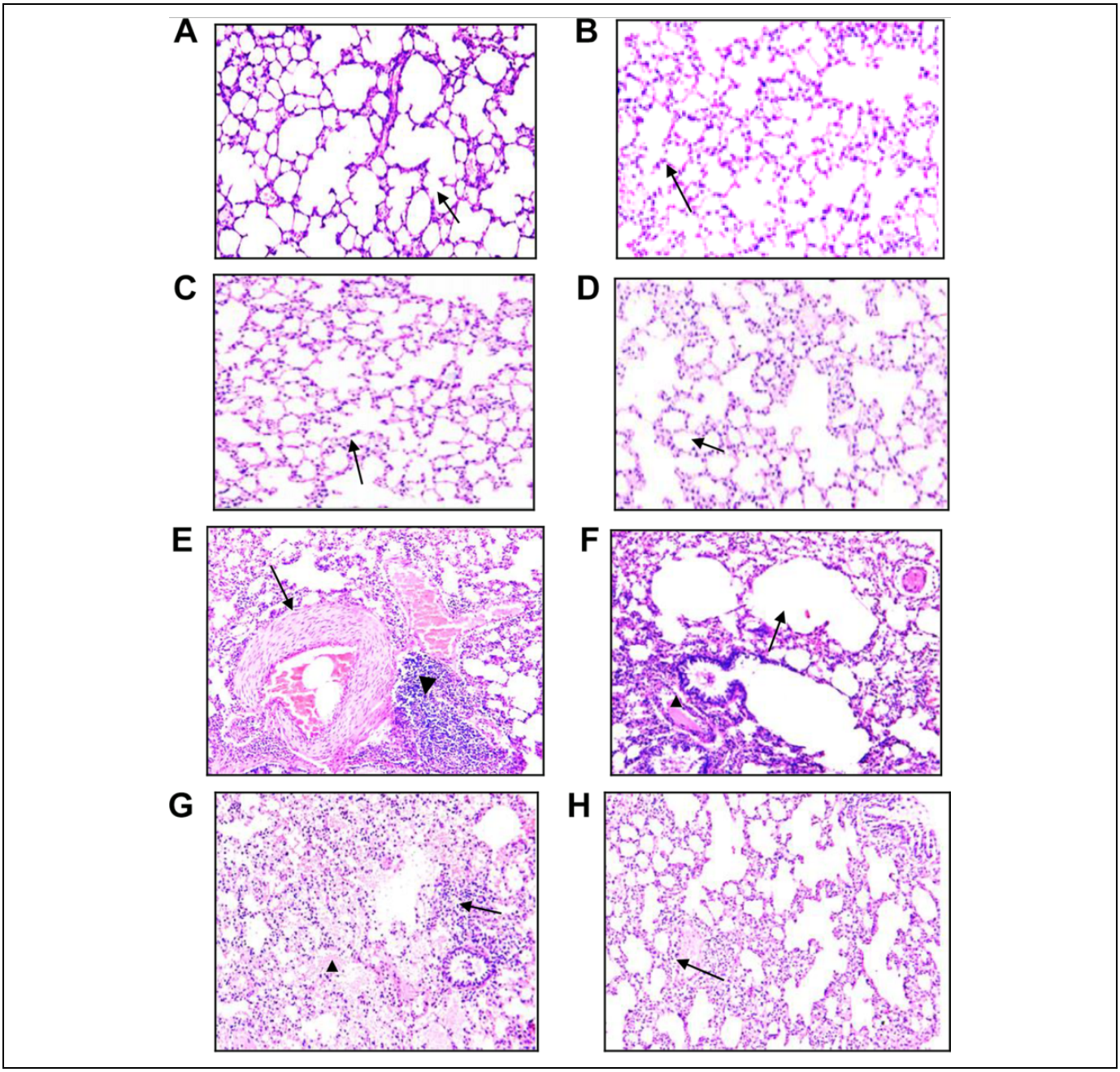

Figure 2. Photomicrographs of T.S. of rat lung (H & E X 200) showing: (A) Normal rat lung arrow. (B) Lung of rat receiving BA showing unremarkable changes arrow. (C) Lung of rat receiving IR showing unremarkable changes arrow. (D) Lung of rat receiving BA+ IR showing unremarkable changes arrow. (E) Lung of rat receiving BPA showing thickening blood vessels are thick-walled arrow with perivascular inflammatory cells aggregation arrow head. (F) A lung of rat treated with BA after receiving BPA showing congested blood capillaries with emphysematous areas arrow. (G) Lung of rat treated with IR after receiving BPA showing congestion of blood capillaries, alveolar oedema and few inflammatory cells infiltration arrow head arrow. (H) Lung of rat treated with BA+IR after receiving BPA showing marked improvement with perivascular few leukocytic infiltration arrow.

accompanied with up-regulation the gene expression of IκBα. Our results agree with those of previous studies.[48,24,50,59-66]

The antioxidant family transcription factor, Nrf2 binds to the antioxidant response element (ARE) sequences in the target gene promoter regions. This, in turn, leads to the expression of ARE-driven cytoprotective genes, including those encoding antioxidants and detoxifying enzymes, such as heme oxygenase-1 (HO-1) and NADPH oxidase.[67] Activation of the regulatory gene pathway driven by Nrf2-ARE through a variety of natural compounds provides chemical prevention against various oxidative stress-related diseases.[68] BA's antioxidant effectiveness may result from its Nrf2 and HO-1 pathway modulation.[64] The significance of Nrf2 and its downstream proteins like NADPH oxidase and HO-1 has been demonstrated in the

defense of several organs against chemically stimulated oxidative stress-inducing cellular attack.[69] BPA was down-regulated the Nrf2 and HO-1 expression. Such results were reported by previous studies.[70] The present results demonstrated that BA or low dose of IR up-regulated the Nrf2 and HO-1 expression. Such results were reported by the previous studies.[64,71]

Overall, BA supplementation with low-level exposures of ionizing radiation exhibited free radical scavenging properties affording protection against BPA intoxication *via* their anti-inflammatory activities. Moreover, the present results indicated that BA combined with a low dose of γ-R markedly improved the BPA induced-lung toxicity by regulating JNK/ERK/c-Fos and NrF-2/HO-1pathways. Therefore the combination therapy appears to have great clinical potential against lung toxicity and as a potentially novel approach to enhance the effectiveness of conventional therapy. From the current study, we can recommend dietary approaches with boswellic acid for plastic users at workplaces exposed occupationally and regularly to low-level ionizing radiation.

Declaration of Conflicting Interests

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