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Protective effect of Soya Bean oil against Bisphenol A induced interstitial lung fibrosis in mice.

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ABSTRACT… Objective: The objective of this study was to evaluate cytoprotective effects of soyabean oil supplementation against interstitial fibrosis induced in lungs of adult mice by Bisphenol A. **Study Design:** Experimental Study. **Setting:** Department of Anatomy, Army Medical College, Rawalpindi. **Period:** Nov 2015 to Nov 2016. **Material & Methods:** Forty healthy adult BALB/c mice of 9 - 11 weeks of age and weighing from 30-37gms were included in this study and were housed in controlled environment of animal house of NIH, Islamabad. Group A animals (10) served as controls. Group B animals (10) were given BPA orally at a dose of 50mg/kg body weight/day and Group C animals (10) were given soya bean oil at a dose of 500mg/day and Group D animals (10) were given BPA and Soya bean oil at doses of 50mg/kg body weight/day and 500mg/day respectively. All doses were administered orally once daily for a period of eight weeks. Animals were dissected 24hrs after receiving the last dose. Lung tissue specimen processing and Masson trichrome staining was done for the histological study. Lung interstitial fibrosis was morphometrically and statistically analysed using SPSSv21. **Results:** Microscopic examination showed Grade 2 interstitial fibrosis in 80% specimens in Group B, whereas only 60% specimens of Group D had Grade 1 interstitial fibrosis. **Conclusion:** It was concluded from results that BPA produces interstitial fibrosis and con-concomitant administration of soya bean oil diet protects against development of lung interstitial fibrosis.

Key words: Bisphenol A, Interstitial Fibrosis, Soya Bean Oil.

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INTRODUCTION

Interstitial lung fibrosis, scarring of the lung affects millions of people worldwide. In Europe and North America alone, reported incidence of IPF is between 2.8 and 19 cases per 100000 people per year.[1] It includes idiopathic disease, pneumoconiosis/particle-induced disease or inflammatory processes. Lung fibrosis exhibits a diverse aetiology and prognosis including inhaled agents, environmental or occupational exposure to particles, organic dusts, and family history.

Bisphenol A (BPA) or 2'-bis (4-hydroxyphenyl) propane bearing the chemical formula $(CH_3)_2C(C_6H_4OH)_2$ was first discovered by Edward Charles Dodds. BPA exhibits estrogenic properties[2], acts as inflammation promoting factor acting through estrogen receptor β (ERβ).[3] Significant studies have proved effects of low or high doses of BPA in experimental animals, causing considerable structural and functional impairment in lungs as well as prostate, testis, mammary gland, uterus, cardiovascular system and malformations of genitalia, along with modifications in brain structure, chemistry and behaviours.[4,5] BPA disrupts STAT3, MAPK, and PI3K/AKT oncogenic signaling pathways leading to carcinogenic effects.[6] These revelations raised safety concerns about use of BPA in the manufacturing of beverage and food contact containers resulting in prohibition of use of BPA in plastic feeding bottles and infant powdered formula packaging materials in 2013[5] by the Food and Drug Administration USA (FDA). TDI (tolerable daily intake) of BPA has been reduced from 50μg/kg/day to 4 μg/kg/day, with the highest level of dietary exposure being documented to be three to five times lower than the new TDI. Michałowicz[7]

investigated human exposure to BPA by consumption of Bisphenol A contaminated food or water in addition to exposures through digital receipts, electronic equipment, automobiles, safety equipment and paints. Occupational exposures are predominantly via inhalation and dermal routes.[8] Chronic BPA exposure especially to its metabolite 4-Methyl-2,4-bis(4-hydroxyphenyl) pent-1-ene alters lung function and growth of surfactant producing pneumocyte cells, leading to pulmonary inflammatory diseases and lung interstitial fibrosis.[9,10] BPA modifies development, maturation and functioning of lungs during prenatal and postnatal life, leading to asthma and allergic lung inflammation especially during childhood.[11,12]

Soyabeans contain abundant bioactive plant compounds including phytic acid, isoflavones and saponins. Isoflavones are antioxidant polyphenols (also known as phytoestrogens)[13], having weak estrogenic and antioxidant activity.[14] Research has substantiated that Phytic acid in soyabean oil also has antioxidant and chelating properties.[15] Saponins are cholesterol lowering agents.[16] In addition, molybdenum, vitamin K1, folate, copper, manganese, phosphorus, and thiamine are also present in significant amounts.

Majority of studies on BPA focus on either endocrine disruptions or the embryonic reproductive system. However, tissue distribution of BPA in rats is predominantly to the lungs. Hence, objective of this study was to study the evaluate the effect of BPA on the lung interstitium of adult mice and to assess the potential protective role of soyabean oil supplementation in the BPA induced lung fibrosis.

MATERIAL & METHODS

Randomized control study of one-year duration was carried out in Department of Anatomy, Army Medical College (AMC) Rawalpindi in collaboration with Pathology Department and National Institute of Health (NIH) from November 2015 to November 2016. The study was conducted after ethical approval of authorities of Army Medical College, (ERC/SA-16/Dr. Sadia Shaukat). Rawalpindi. The experimental chemical Bisphenol A was purchased from Sigma Eldritch.

Forty adult BALB/c mice, 9-11weeks old and weighing 30-37gm were included in this experiment. Mice were kept in polystyrene cages in a well-ventilated room of NIH with room temperature ranging from 20 - 26°C and were permitted 12hours of dark-light sleep cycle.[11] Mice were fed with Soya bean free lab diet (to remove phytoestrogen content of pallets) and minimize background BPA exposure.[4,17] Water was supplied ad libitum in polystyrene bottles. All doses were administered via oral gavage once daily for a total period of eight weeks.

Mice in Group A served as untreated controls. Experimental Group B was administered with 50mg/kg/day of BPA. Group C was administered with 500 mg/day of soyabean oil and mice in experimental Group D were administered 50mg/kg/day of BPA and 500 mg/day of soyabean oil simultaneously. At the end of 8-week experiment, animals were euthanized; sacrificed, dissected and fresh lung specimens were removed. Lung tissue processing was done in 10 per cent formalin and 5-micron thick sections were cut using rotary microtome. Masson's trichrome staining was used to demonstrate deposition of supporting collagen fibre in alveolar epithelium, capillary endothelium as well as in perivascular and peribronchial areas. Slides were examined under 40X for the presence of fibrosis on a semi quantitative score described[18] as follows:

Grade 0: normal lung, Grade 1: isolated septa with gentle fibrotic changes with septa < 3 times thicker than normal and minimal fibrous thickening of bronchiolar walls, Grade 2: Clear fibrotic changes of alveolar septa being > 3 times thick with knot-like formation not connected to each other, Grade 3: contiguous fibrotic walls of alveolar septa, Grade 4: single fibrotic masses.

SPSS version 21 was used for data analysis. Qualitative variables were expressed as frequency and percentage and compared by Chi-square test. P –value of < 0.05 was considered significant.

RESULTS

In all 10(100%) specimens of control Group A, normal lung tissue was seen, without fibrosis. In experimental Group B, 2(20%) of specimens showed Grade 2 fibrotic changes and 8 (80%) had Grade 3 fibrotic changes (Table-I, Figure-2b). On intergroup comparison, Group B was statistically significant with Group D (p-value=0.001) and highly significant with control Group A and experimental Group C (p-value<0.0001) (Table-I, Figure-1). In experimental Group C, fibrosis was recorded at Grade 0 in 8 (80%) of specimens whereas 2(20%) specimens showed Grade-1 fibrosis (Table-I, Figure-2a). On comparison of experimental Group C with experimental Group B (p-value<0.0001), results were highly significant (p-value<0.0001) and the difference was statistically significant when compared with experimental Group D (p-value=0.001), but insignificant when compared with Group A (p-value=0.474) (Table-I, Figure-1). Whereas in Experimental Group D, 6(60%) of the specimens showed Grade 1 infiltrates, 3(30%) specimens showed Grade 2 (Table-I, Figure-2c) and rest of the 1(10%) had Grade 3 fibrosis. On intergroup comparison, it was found to be statistically significant when compared with control Group A, experimental Group D (p-value<0.0001) and experimental Groups C (p-value=0.001) (Table-I, Figure-1).

Parameters	Findings	Group A	Group B	Group C	Group D	P-Value
Interstitial fibrosis	Grade 0	10 (100%)	0 (0.0%)	8 (80%)	0 (0.0%)	<0.0001*
	Grade 1	0 (0.0%)	0 (0.0%)	2 (20%)	6 (60%)	0.474
	Grade 2	0 (0.0%)	2 (20%)	0 (0.0%)	3 (30%)	<0.0001*
	Grade 3	0 (0.0%)	8 (80%)	0 (0.0%)	1 (10.0%)	<0.0001*
	Grade 4	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.001

Table-I. Frequency and percentages of interstitial fibrosis of control group A and experimental groups B, C and D (n=40).
p-value ≤ 0.05 is statistically significant * = highly significant

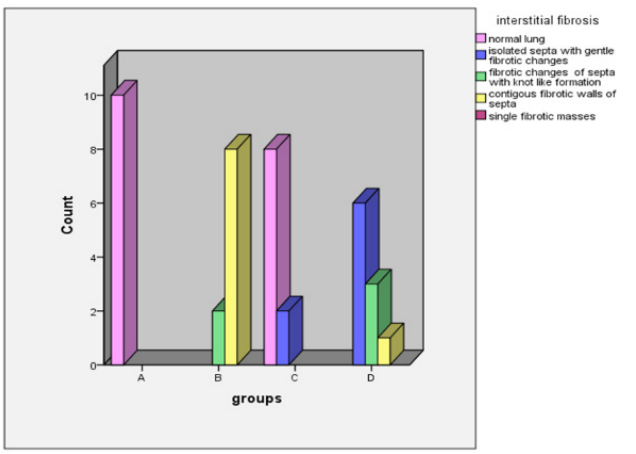

Figure-1. Cluster Bar chart showing comparison of frequency of interstitial fibrosis with scoring among the control group A and experimental groups B, C and D (n=40).

DISCUSSION

The wide spread use of BPA-containing manufactured goods has caused concern because of harmful effects on various vital organs. Accordingly, this study was designed to study the effects of BPA on mice lungs along with administration of soybean oil that can have protective effect.

Masson's trichrome stained slides were examined to observe fibrotic changes in the interstitial space around bronchi, vessels and in the inter alveolar septa using Ashcroft score. In the present study, Group B was found to be highly significant as compared to Control Group A and experimental Group C with p-value<0.0001. Group B was also significant when compared with Group D with p-value=0.001.Similar effects of Chronic exposure to oral BPA on lungs were documented by Kattaia, in 2014, Egypt as a result of inflammation and oxidative stress by producing increased levels of Interleukin-18 (IL-18), malondialdehyde (MDA), and decline in levels of superoxide dismutase (SOD).[19]

The current work proposed increase in positive immunoreaction for INOS and formation of reactive oxygen species (ROS) leading to oxidative stress.

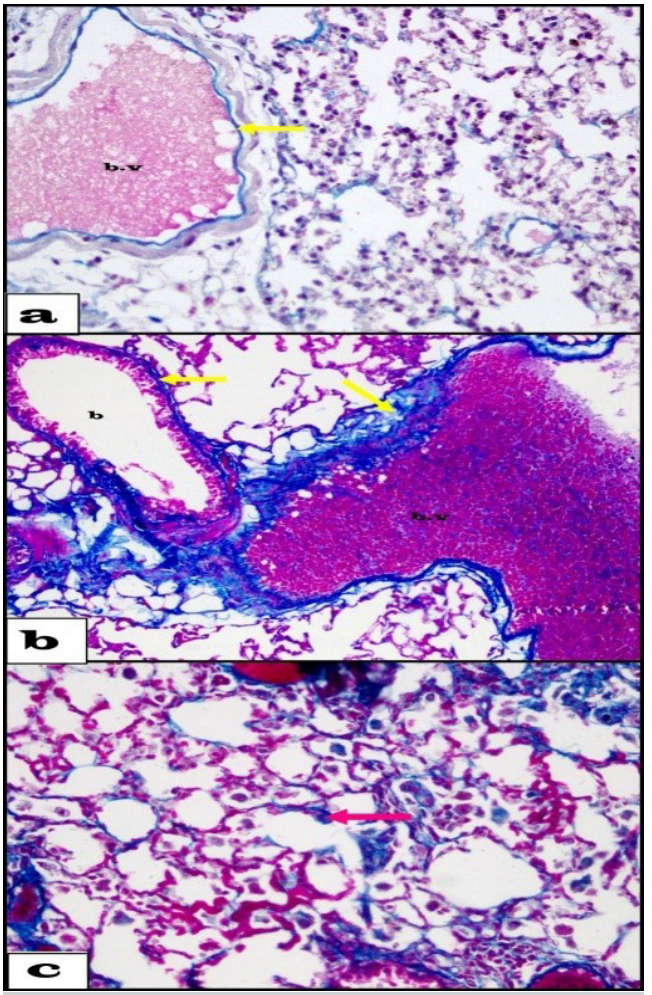

Figure-2. Photomicrograph showing interstitial fibrosis using Ashcroft score. Animal no.7 of experimental Group C showing Grade 1 fibrosis, (b) animal no.1 of experimental Group B showing Grade 3 fibrosis and (c) animal-2 of experimental Group D showing Grade 2 fibrosis. Collagen fibres (yellow arrow), Knot formation (pink arrow): 40X, Masson Trichrome.

Karnam, in 2016, India also suggested oxidative stress leading to various lung conditions including congestion, inflammation, alveolar hemorrhage, and diffuse increase in histocytes in animals treated with BP A. In addition, increased levels of pro-fibrotic IL-13 and/or TGFβ1factors are produced, ultimately disrupting the healing process and culminating into a pathogenic fibrotic reaction.[20] Acute inflammatory processes also result in fibroblastic proliferation and synthesis of extracellular collagen matrix producing fibrosis, which is characterized by thickened alveolar septal walls and diminished air spaces.[18] BPA intensifies collagen fibre deposition in a dose dependant manner involving above mentioned processes and by activation of a transcription factor KB with resultant fibrosis or apoptosis. In addition, genes associated with fibrosis demonstrate increased expression of follistatin-like1 (FSTL1) factors and concomitant decrease in 5 (ADAMTS5) expression levels[9] intermediating proteolysis and loss of aggrecan.

It was further demonstrated by Liuand other researchers in 2016 that 4-Methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene (MBP) a major active metabolite of BPA induces apoptosis of surfactant producing pneumocyte cells and consequently lung dysfunction by enhancing AMP-activated protein kinase (AMPK) phosphorylation and molecular expression of AMPK-regulated ER and endoplasmic reticulum (ER) stress-signalling pathway in pulmonary epithelial cell line L2 of adult rats.[10]

Experimental Group D specimens showed ameliorative effects with 60% falling in Grade 1, 30% in Grade 2 and only 10% in Grade 3, validating ameliorative effects of Soya bean oil. Studies have shown that in non–small-cell lung cancer (NSCLC), adverse effects of radiation including inflammation, pneumonitis and fibrosis can be greatly reduced by soy is flavones consumption during pre and post-radiation period.[21] Portal C, in 2018, France proposed that Soya bean with abundant long-chain polyunsaturated fatty acids (LC-PUFAs) reduce hyperplasia and lung inflammation of bronchial epithelial cells, thus restricting oxidative stress and consequently lung damage.[22]

CONCLUSION

It was observed in present study that oral administration of BPA causes fibrotic changes in lung interstitium of adult mice and that concurrent administration of soyabean oil can counteract the fibrotic changes. Caused by BPA.

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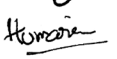
AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Sadia Shaukat	Concept, design of work, Acquisition, analysis and interpretation of data, critical revision of work.	
2	Faiza Umbreen	Contribution to critical revision of work, final approval of revision of work.	
3	Humaira Ali	Contributed in collections, analysis and interpretation of data.	
4	Aamna Khokhar	Dosage calculation and pharmacological aspect of work.