Original Article

PROTECTIVE EFFECT OF SOYABEAN OIL ON ALVEOLAR SEPTAL THICKNESS INDUCED BY BISPHENOL A

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ABSTRACT

Objective: To assess the protective effect of soyabean oil on alveolar septal thickness induced by Bisphenol A in alveoli of adult mice.

Study design: Laboratory based randomized control trial.

Place & duration of study: This study was conducted at Department of Anatomy, Army Medical College Rawalpindi in collaboration with National Institute of Health (NIH) Islamabad from November 2015 to June 2016.

Materials and Methods: Forty male and female BALB/c mice were chosen and divided into four groups, each with 10 animals. Group A served as control. Group B was given BPA at a dose of 50milligram/kilogram body weight/day. Group C was treated with soyabean oil at a dose of 500 milligram/day. Group D received BPA at a dose of 50milligram/kilogram body weight/day and soyabean oil at a dose of 500milligram/day. All treatments were given once daily for a period of 8 weeks. Animals were dissected 24hrs after the last dose. Lung tissue processing and H&E staining was done for routine histological study. Alveolar septal thickness was morphometrically and statistically analysed.

Results: Alveolar septal thickness was significantly increased (>3 times normal) in group B in comparison to groups A, C and D and slightly increased in group D in comparison to groups A, B and C.

Conclusion: BPA increases the alveolar septal wall thickness in lungs of adult mice but subsequent administration of soyabean oil protects against thickening of septa.

Keywords: Bisphenol A, Alveolar septal thickness, Soyabean oil.

This article can be cited as: Shaukat S, Hamid S, Saadia A. Protective effect of soyabean oil on alveolar septal thickness induced by bisphenol A. Pak J Pathol. 2016: 27(3): 119-123.

INTRODUCTION

Polycarbonate plastics are widely used because of their malleability, imperviousness to water and economical prices. Plastic additives are toxic and range from 0% to 50% including fillers, plasticizers and colorants. Bisphenol A (BPA) a plasticizer, is produced universally in substantial magnitudes. BPA can leach out of food utensils and dental sealants, routinely entering our food and drinks. Substantial levels of BPA are detected in air, water, dust, oil, tinned and canned foods [1]. BPA exerts deleterious effects via numerous exposure routes i.e ingestion, inhalation, and dermal absorption [2]. Most substantial route for human exposure is via diet [3]. BPA levels have been measured in human placental tissue and fluids like urine, cord blood as well as fluids in amniotic and follicular cavities.

BPA accumulates significantly in human and rodent tissues [4] including lung, liver, kidneys, thyroid, heart, muscle, brain, adipose tissue, reproductive organs and GIT contents, resulting in high exposure levels. It exerts toxic, endocrine disrupting and mutagenic effects leading to carcinogenesis in in vitro and in vivo studies [3]. These effects cause deleterious effects on humans and experimental animals like breast cancer, endometriosis and infertility [5]. Biotransformation to reactive metabolites, causes oxidative stress and augments estrogenicity [3].

Highest concentrations of BPA have been reported in lungs and placenta following intravenous administration [6]. Prenatal BPA exposure modifies lung development and maturation, leading to experimental asthma in mice [7, 8]. Chronic, low dose exposures of BPA disrupt lung architecture with collapsed alveoli, congested blood vessels, fibrin deposition, intra-alveolar hemorrhage and inflammatory cellular infiltration around bronchi or blood vessels. A substantial increase in the number

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Received: ***2 Aug 2016****; Revised:* ***28 Aug 2016****; Accepted:* ***22 Oct 2016***

of alveolar macrophages and iNOS immunoreaction are also documented [9]. For BPA, lowest observed adverse effect level (LOAEL) dose is 50 mg/kg/day, which represents environmentally relevant dose, acknowledged and used as reference dose by EPA [10].

Soyabean/soybean, a species of legume grown in East Asia is considered a cheap source of protein. Soyabean act as phytoestrogens (plant estrogen) containing significant amounts of phytic acid, linoleic acid and isoflavones [11]. It has cholesterol lowering, antioxidant and anti-inflammatory properties [12, 13]. Soyabean oil diet decreases inflammatory cell counts (both total and differential) as well as recruitment of these cells in perivascular and peribronchial areas thus preventing allergic inflammatory processes in respiratory airways [12].However, Consumption of partially hydrogenated oil may lead to obesity, asthma, autoimmune disorders, diabetes, coronary artery disease, bone degeneration and cancer.

Soyabean oil has one of the highest universal consumption for cooking purposes with various benefits as well as adverse effects. This study was carried out to assess the efficacy of soyabean oil in protecting against histological changes induced in alveolar septal thickness by Bisphenol A in alveoli of adult mice lungs.

MATERIALS AND METHODS

This study was approved by Ethical Committee, of the Army Medical College Rawalpindi. Study was carried out in the Department of Anatomy, Army Medical College Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad and Armed Force Institute of Pathology (AFIP) Rawalpindi. Forty adult male and female BALB/c mice, 9-11 weeks of age and weighing 30-37gm were used for this experiment. Mice were housed in separate polystyrene cages to minimize background BPA exposure. They were kept in a well-ventilated room, with temperature range of 20 - 26°C and were allowed 12h dark-light sleep cycle throughout the duration of experiment [14]. Mice were fed with soya bean free laboratory diet (to remove phytoestrogen content of pallets) provided by NIH. Casein was added to diet to achieve 20% of protein content [12]. Water was provided ad libitum in polystyrene bottles. All doses were administered via oral gavage once daily for a period of 8 weeks.

Mice in Group A served as untreated controls. Group B was given BPA 50mg/kg/day and mice in Group C were given soyabean oil 500 mg/day, whereas, Group D was given BPA 50mg/kg/day and soyabean oil 500mg/day. At the end of 8 weeks, the animals were sacrificed, dissected and fresh lungs were removed. Lungs were placed in containers containing 10 per cent formalin and after processing, were cut into 5-micron thick sections using rotary microtome. Staining was performed with haematoxylin and eosin (H&E) for routine histological analysis of lungs under light microscope. Each image was opened in Image J v1.48 [15] and alveolar septal thickness was measured in 3 consecutive high power field (HPF) for each H&E stained specimen. Data was analysed using statistical package for social sciences version 21. Parameter was expressed as mean + standard deviation. Significant difference was determined using one way analysis of variance (ANOVA) followed by post Hoc Tukey test. $p<0.05$ was considered significant.

RESULTS

Histologically, Control Group A and experimental Group C showed normal distended polygonal alveoli, alveolar sacs, bronchioles and blood vessels (Figure-1 A, C). Experimental Group B showed distended alveoli with thickened alveolar septa and reduced alveolar diameter (Figure-1 B). Mean thickness ± SD of alveolar septa of control Group A was 2.14 ± 0.25, which was statistically

significant as compared to experimental Groups B and D (*p*-value=0.00*) but non-significant when compared with experimental Group C (p-value=0.42). Mean thickness ± SD of alveolar septa of experimental Groups B, C and D were 6.44 ± 0.38, 2.42 ± 0.32 and 2.97 ± 0.56 respectively (table-1, Fig-2). On intergroup comparison, experimental Group B showed high statistical significance (p-value=0.000*) with control Group A and experimental Groups C and D (table-2). When experimental Group C was compared with experimental Group D, statistical significance was observed (*p*-value= 0.016)

Table-1: Showing Comparison of mean value of alveolar septal thickness among control group A and experimental Groups B, C and D.

	Group A Mean ± SD (n = 10)	Group B Mean ± SD (n = 10)	Group C Mean ± SD (n = 10)	Group D Mean ± SD (n = 10)
Alveolar septal thickness (um)	2.14 ± 0.25	6.44 ± 0.38	2.42 ± 0.32	2.98 ± 0.57

Table-2: showing Comparison of p values of alveolar septal thickness among control Group A and experimental Groups B, C and D.

	Group A vs B	Group A vs C	Group A vs D	Group B vs C	Group B vs D	Group C vs D
Alveolar septal thickness (um)	0.000*	0.423	0.001	0.000*	0.000*	0.016

p value < 0.05 is statistically significant.

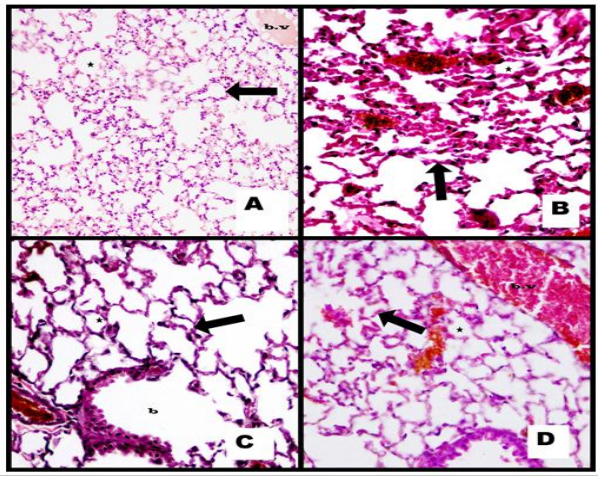

Figure-1: Photomicrograph showing alveolar septa. (A)Animal no.5 of Control Group A showing thin septa (B) Animal no.5 of experimental Group B showing >3 times thickened septa (C) Animal no.2 of experimental group C showing thin septa and (D) Animal no.4 of experimental Group D show <3 times thickened septa. Alveolar septal wall (black arrow), alveoli (*), blood vessels (b.v): 40X, H&E.

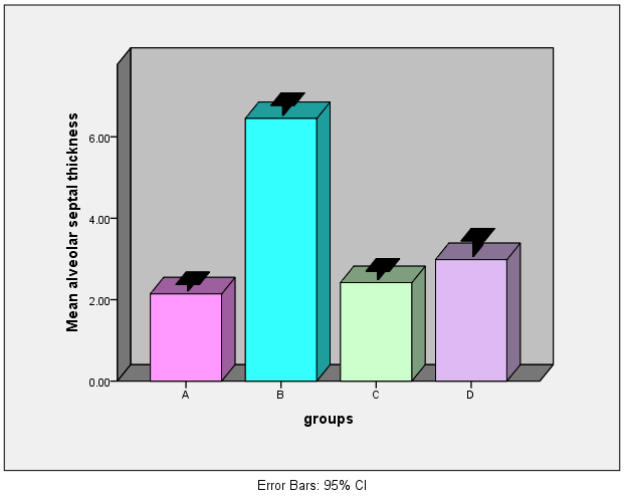

Figure-2: simple bar chart showing comparison of mean value of alveolar septal thickness among the control group A and experimental groups B, C and D.

DISCUSSION

Human exposure to BPA via multiple routes has adverse effects on various tissues and organs, leading to morbidity [1]. Accordingly, this study was designed to investigate the protective effect of soyabean oil diet on the lungs of adult mice when co administered with BPA.

In the current study, chronic exposure to mild doses of BPA altered the lung architecture with increase in thickness of inter alveolar septa. Alveolar

septal thickness remained within normal limits in control group A and experimental group C, where as in experimental group B it was more than 3 times the normal values. Increase in thickness was highly significant as compared to control groups A and experimental groups C and D. These results were in accordance with the previous works [9, 16] showing that collagen fiber deposition increased in inter alveolar walls in BPA-treated mice as compared to the control. It has been observed that BPA stimulates fibroblastic hyperplasia in various organs [17]. Initial phase in the pathogenesis of pulmonary fibrosis signifies epithelial and basement membranes injury, followed by migration and proliferation of inflammatory and immune cells, eventually leading to matrix remodeling and collaged deposition [9].

Histological findings of experimental group D showed minor increase in the thickness of alveolar septa as compared to experimental group B. We found high statistical significance between experimental groups B and D, showing that soyabean oil protected against the harmful effects on inter alveolar septa induced by BPA. Marked reduction in the degree of inflammatory cellular infiltration and collagen fiber deposition signify the beneficial effects of soyabean oil. Earlier data proves that prophylactic intake of soybean oil diet impairs development of allergic inflammation in lungs in rats which were previously sensitized. Inflammation is associated with down regulation of leukocyte migration, white cell counts and IL-4,5 and Bk levels in the BAL and lungs, along with up regulation of NO levels [12]. Furthermore, histo pathological analysis and lung inflammation score have confirmed that soybean oil-rich diet up regulates corticosterone levels and LXA4 in the lungs. Corticosterone, an anti-inflammatory hormone induces the manifestation of mediators, LXA4 receptor and annexin A1 [18].

CONCLUSION

The study confirms that BPA causes an increase in alveolar septal thickness in lungs of adult mice but concomitant administration of soyabean oil will have protective effect on the septal walls.

ACKNOWLEGMENT

I would like to thank all people of Department of Anatomy, Army medical college, Rawalpindi.

AUTHORS CONTRIBUTION

Sadia Shaukat conceived the idea, analyzed the data and drafted the manuscript.

Shabnum Hamid did the critical analysis and revised the manuscript.

Aiza Saadia gave her input in pathological analysis.

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