Soyabean Oil Supplementation Effects on Perivascular Inflammation in Lungs Induced by Bisphenol A: A Histological Study

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ABSTRACT

Objective: To determine the effect of soyabean oil supplementation on perivascular inflammation in lungs of adult mice induced by Bisphenol A (BPA).

Study Design: An experimental study.

Place and Duration of Study: Department of Anatomy, Army Medical College, Rawalpindi, in collaboration with the Animal House, National Institute of Health, Islamabad, from June to November 2016.

Methodology: Thirty male and female BALB/c mice were divided into three groups, of 10 animals each. Group A animals served as control. Group B animals were given BPA at a dose of 50 mg/Kg body weight/day. Group C animals were given BPA and soyabean oil at doses of 50 mg/Kg body weight/day and 500 mg/day, respectively. All treatments were given once daily for a period of eight weeks. Animals were dissected 24 hours after receiving the last dose. Lung tissue specimen processing and H&E staining was carried out for routine histological study. Perivascular inflammation was morphometrically graded and statistically analysed using Chi-square test with p<0.05.

Results: Grade 2 inflammation was recorded in two (20%) animals and grade 3 perivascular inflammation in 80% specimens in Group B; whereas 20% specimens of Group C had grade 2 inflammation and eight (80%) showed grade 1 inflammation. None of the control animals showed any inflammation. All groups were significantly different at p<0.001.

Conclusion: BPA produced perivascular inflammation and concommitant administration of soyabean oil diet protected against it in rodent.

Key Words: Bisphenol A (BPA). Soyabean oil. Perivascular inflammation.
However, partially hydrogenated oils lead to obesity, diabetes, autoimmune disorders, asthma, coronary artery disease and even cancer.

Soyabean oil is extensively utilized as an important component of the diet especially in developing countries like Pakistan. Since lack of awareness about health hazards is associated with injudicious use of polycarbonate plastics, this study was conducted to investigate the effects of concomitant administration of BPA and soyabean oil on mice lung alveoli.

**METHODOLOGY**

This was an experimental study approved by Ethical Committee of the Army Medical College Rawalpindi and 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, from June to November 2016.

Thirty adult male and female BALB/c mice, 9-11 weeks old and weighing 30-37gm were used for this experiment. All mice were housed in NIH in a well-ventilated room, with room temperature maintained at a range of 20 - 26°C and were allowed 12 hours of dark-light sleep cycle.11 Mice were kept in polystyrene cages and fed with soyabean free diet (to remove phytoestrogen content of pallets) to minimize background BPA exposure. 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. Experimental Group B was administered with 50 mg/kg/day of BPA and mice in experimental Group C were given 50 mg/kg/day of BPA and 500 mg/day of soyabean oil. At the end of 8 weeks, animals were sacrificed, dissected and fresh lung specimens were removed. Lung tissue processing was done in 10% formalin and cut into 5-micron thick sections using rotary microtome. Routine histological staining was performed with haematoxylin and eosin (H&E). Slides were observed at 40X objective for inflammatory cellular infiltrate in the perivascular interstitium on a semi-quantitative score adopted.12

Grade 0 was described at no detectable inflammation. Grade 1 was occasional cuffing with inflammatory cells. Grade 2 was most bronchi or vessels surrounded by a thin layer (one to five cells thick) of inflammatory cells. Grade 3 was most bronchi or vessels surrounded by a thick layer (more than five cells thick) of inflammatory cells (Figure 1). Data was analysed using statistical package for social sciences version 21. Qualitative variables were expressed in frequency and percentage and intergroup comparison was done using Pearson Chi-square test. P-value of < 0.05 was considered significant.

**RESULTS**

In all 10 (100%) specimens of control Group A, inflammation was not observed in any slide. Two (20%) specimens of experimental Group B showed grade 2 infiltrate and eight (80%) had grade 3 inflammation (Table I). Experimental Group C showed grade 2 inflammatory infiltrate in two (20%) of cases; whereas, eight (80%) showed grade 1 inflammation (Table I). On intergroup comparison, statistically significant difference was observed when experimental Groups B and C was compared with control Group A (p <0.0001*), (Table I).

**DISCUSSION**

The word plastic is derived from a Greek word plastikos, meaning capable of being shaped and cast into various shapes. Use of plastics has surpassed materials like wood, metal, glass, stone and ceramics especially in developing countries like Pakistan. The domination of plastics was challenged as a result of environmental hazards induced owing to its composition of large molecules and slow environmental decomposition.

Low-dose adverse effects of BPA in experimental animals have been demonstrated since early 1900s. Recent studies emphasize on BPA exposure during pregnancy, antenatal period and early infancy,13,14 with...
consequential bans by U.S. Food and Drug Administration (FDA), especially in infant feeding bottles.\textsuperscript{13,15} Recently, BPA has been included in the list of substances of very high concern (SVHC). Despite the concerns, BPA containing polycarbonate plastics are still widely used especially in developing countries like Pakistan, probably because of the low cost and lack of awareness related to its adverse effects.

In the current study, slides were observed for inflammatory cellular infiltrates in perivascular interstitium using a semi-quantitative score\textsuperscript{12} ranging from grade 0 (no inflammation) to grade 3 (surrounded by >5 cell layers). None of the animals of control group showed inflammatory infiltrates, whereas variable degrees of infiltrates were observed in experimental Groups B and C; 80% of specimens in the experimental Group B showing grade 3 infiltrates. The results of this study are similar to the results of other in vitro studies,\textsuperscript{8,16} which indicated that BPA associated inflammation precedents cellular and molecular activation of macrophages and produces cytokines which further leads to production of chemo attractants and elaboration of adhesion molecules.\textsuperscript{17,18} In addition, the granulocytes of inflamed lungs cause increased expression of vascular adhesion protein-1 (VAP-1) thus playing a role in their adhesion to vascular endothelium and producing perivasculare inflammation. VAP-1 is a glycoprotein amine oxidase, which is normally absent around the vessels of non-inflamed organs excluding large and middle-sized pulmonary vessels.\textsuperscript{19} Selectins along with VAP-1 enhance recruitment of lymphocytes and this process is blocked by inhibition of VAP-1 associated monoamine oxidase activity.\textsuperscript{19} Studies have shown that n-6 and n-3 fatty acid-rich diets exert protective effects against allergic airway inflammation and modulate processes which reduce leukocyte migration, eosinophil and neutrophil counts, and IL-4/IL-5/bradykinin levels in broncho alveolar lavage and lungs, as well as increased nitric oxide levels in BAL.\textsuperscript{20} The soyabean oil-rich diet additionally increases levels of lipoxin A4 and corticosterone in the lungs.\textsuperscript{9}

Ameliorative effects of soyabean oil against lung inflammation are documented in this study in Group C, with only 2% of specimens showing grade 2 inflammation and 80% specimens showing only grade 1 inflammation.

**CONCLUSION**

Soyabean oil countered the inflammatory effects caused by oral administration of BPA in perivascular interstitium in the lungs of adult mice.

**REFERENCES**


