

## Study on Polychlorinated Biphenyls-Induced Changes in the Expression of Pro Inflammatory Markers and the Therapeutic Role of Vitamin C And E

Arun Ganesh, R. Gayathri\*, J. Selvaraj, V. Vishnu Priya, Kavitha. S

Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600 077, India

### Abstract

Polychlorinated biphenyls (PCBs) are manmade chemicals which are highly reactive and cause disorders such as diabetes, and cancer and conditions such as breathing difficulties etc., It is endocrine receptors and environmental pollutants. IL1- $\beta$  and TNF- $\alpha$  are pro-inflammatory markers. Vitamin C and E are antioxidants that have therapeutic effects on PCB. They also protect the body from free radicals, pollutants and toxins. They also help in the defence system and protect the body against cancer, arthritis, etc. The harmful effects and the changes induced by PCBs on pro-inflammatory markers and the therapeutic role of vitamin C and E on PCB were studied using Adult male albino rats. Adipose tissues from control and treated animals were dissected out and used for the assessment of pro-inflammatory markers. The IL1- $\beta$  and TNF- $\alpha$  mRNA expression levels were measured using the RT-PCR method. The data were analyzed statistically. The results showed that the IL1- $\beta$  and TNF- $\alpha$  mRNA expression levels of PCB-induced rats were significantly increased ( $p < 0.05$ ). Treatment with Vitamin C and E could effectively reduce the expression of the pro-inflammatory signalling molecules in adipose tissue.

**Keywords:** Antioxidants, Diabetes, Innovative Technology, Novel Method, Vitamins, PCB.

### Introduction

Polychlorinated biphenyls are complex mixtures of contaminants. They are broadly spread in the environment. They are used as dielectric fluid in the making of capacitors, transformers and other appliances [1]. PCBs are harmful and hazardous to human health. Research affirmed that a few PCB congeners debase gradually within the environment and can construct up within the nourishment chain [2]. Poisoning scenes in Asia were first ascribed to PCB – contaminated oil, although consequent investigation proposed that warm corruption items of pcbs were dependable for the watched poisonous quality. Commercial generation of PCBs within the United States was prohibited in 1979 [3, 4]. A few organisations have categorized pcbs as animal

carcinogens; in any case studies of specialists uncovered to high doses of pcbs have not illustrated an expanded cancer risk [5, 6]. Well-being impacts inferable to PCBs include skin and eye bothering. There is no solid proof that pcbs within the environment result in either “endocrine disruption” or mental disintegration in children covered in utero. Since PCB exposures from natural sources don’t posture a noteworthy well-being chance, small advantage to open well-being can result from proceeded remediation of PCB sources [7]. Some of the disorders caused by PCBs are diabetes [8] and cancer [9]. Dehydration and decreased response to pain, coma disruption of health are few effects of PCB. PCB being an endocrine receptor disrupts brain sexual differentiation on prolonged exposure [10].

The IL-1 family comprises three basically related polypeptides [11, 12]. The primary two are IL-1 $\alpha$  and IL-1 $\beta$ , each of which contains a wide range of both advantageous and harmful biological activities [13]. Among the properties of the two shapes of IL-1( $\alpha$  and  $\beta$ ) is the capacity to initiate fever, rest anorexia and hypotension. IL-1 stimulates the discharge of pituitary hormones, increments the amalgamation of collagenases, coming about within the devastation of cartilage, invigorates the generation of prostaglandins, dividing to diminish within the torment limit [14]. The cytokine tumor necrosis factor (TNF- $\alpha$ -mRNA) is a pleiotropic polypeptide that plays a noteworthy part in brain immune and inflammatory activities and acts specifically on vascular endothelium to extend the adhesion of leukocytes amid the inflammatory process [15].

Beneath typical circumstances, free radicals that are delivered through organic forms and in reaction to exogenous stimuli are controlled by different chemicals and cancer prevention agents within the body [16, 17]. Research facility proof recommends that oxidative push which happens when free radical arrangement surpasses the capacity to ensure against them, may frame the natural premise of a few intense therapeutic issues such as tissue harm after injury and incessant conditions such as atherosclerosis and cancer [18, 19]. A potential part for the antioxidant micronutrients (Vitamin C, Vitamin E and carotenoids) in altering the risk for conditions which will result from oxidative stress has invigorated strong investigative endeavours, expanded intrigue in micronutrient supplements, and increased customer intrigue in these compounds.[20, 16]. Much remains to be learned, be that as it may, around the bioavailability, tissue take-up, digestion system, and natural exercises of these micronutrients [17, 21] These natural characteristics will eventually drain .This study was designed to check the toxic effects of PCB and the changes it induces in the genetic expressions. It also explains the therapeutic role

of Vitamin C and E. The results of this study can be shared as it creates awareness amongst the public on the harmful effects of PCB.

## Materials and Methods

### Chemicals

All chemicals and reagents used in this study were purchased from Sigma Chemical Company St. Louis, MO, USA; Invitrogen, USA; Eurofins Genomics India Pvt Ltd, Bangalore, India; New England Biolabs (NEB), USA; Promega, USA. PCB was procured from Sigma Chemical Company St. Louis, MO, USA; Total RNA isolation reagent (TRIR) was purchased from Invitrogen, USA. The reverse-transcriptase enzyme (MMuLv) was purchased from Genet Bio, South Korea purchased from Promega, USA. Interleukin-1 $\beta$ , TNF- $\alpha$  and  $\beta$ -actin primers were purchased from Eurofins Genomics India Pvt Ltd, Bangalore, India and.

### Animals

The present experimental study was approved by the institutional animal ethics committee (IAEC no.: BRULAC/SDCH /SIMATS /IAEC/ 12.2019/ 048). Adult male Wistar albino rats, weighing 180–200g, were obtained and maintained in clean propylene cages at the Biomedical Research Unit and Laboratory Animal Centre (BRULAC), Saveetha Dental College and Hospitals, Saveetha University, India) in an air-conditioned animal house, fed with standard rat pelleted diet (Lipton India Ltd., Mumbai, India), and clean drinking water was made available ad libitum. Rats were divided into 3 groups, each consisting of 6 animals.

### Experimental Design

1. Group 1: Control (Vehicle control, rats were intraperitoneally (i.p.) administered with the vehicle (corn oil) for 30 days.
2. Group 2: Rats received PCB (PCB was dissolved in corn oil at a dose of 2mg/kg body weight (b. wt)

intraperitoneally daily at 10:00 a.m. for 30 days).

3. Group 3: PCB and vitamin E (dissolved in olive oil at a dose of 50 mg/kg body weight), and vitamin C treated (100 mg/kg body weight dissolved in distilled water daily at 10 AM through gastric intubation for 30 days).

At the end of treatment, animals were anesthetized with sodium thiopental (5 mg/kg, i.p), and 20 ml of normal saline was perfused through the left ventricle, to clear blood from the liver, and other organs. Visceral adipose tissue was dissected out and used for the assay of various parameters.

### **Gene Expression Analysis by Real Time Pcr**

#### *Isolation of Total RNA*

Total RNA was isolated from control and experimental samples using a TRIR (total RNA isolation reagent) kit. Briefly, 100 mg fresh tissue was homogenized with 1 ml TRIR, and the homogenate was transferred immediately to a microfuge tube and kept at -80°C for 60 min to permit the complete dissociation of nucleoprotein complexes. Then, 0.2 ml of chloroform was added, vortexed for 1 min and placed on ice at 4°C for 5 min. The homogenates were centrifuged at 12,000 x g for 15 min at 4°C. The aqueous phase was carefully transferred to a fresh microfuge tube and an equal volume of isopropanol was added, vortexed for 15 sec. and placed on ice at 4°C for 10 min. The samples were centrifuged at 12,000 x g for 10 min at 4°C. The supernatant was discarded, and RNA pellet was washed with 1 ml of 75% ethanol by vortexing and subsequent centrifugation for 5min at 7,500 x g (4°C). The supernatant was removed, and RNA pellets were mixed with 50 µl of autoclaved Milli-Q water and dissolved by heating in a water bath for 10 min at 60°C.

### **Quantification of RNA**

Diluted RNA samples were quantified spectrophotometrically by measuring the absorbance (A) at 260/280 nm. 40 µg of RNA in 1 ml gives one absorbance at 260 nm. Therefore, the concentration of RNA in the given sample can be determined by multiplying its A<sub>260</sub> by 40 and dilution factor. The purity of RNA preparation can be calculated using the ratio between its absorbance at 260 and 280 nm. A ratio of absorbance at 260/280 nm > 1.8 is generally considered as good quality RNA (Fourney et al., 1988). The purity of RNA obtained was 1.8.

### **Reverse Transcriptase – Polymerase Chain Reaction (RT – PCR)**

RT-PCR is an approach for converting and amplifying a single-stranded RNA template to yield abundant double-stranded DNA products.

1. First strand reaction: Complementary DNA (cDNA) is made from the mRNA template using Oligo dT, dNTPs & reverse transcriptase.
2. Second strand reaction: After the reverse transcriptase reaction is complete, standard PCR (called the “second strand reaction”) is initiated. Principle RT-PCR is a method used to amplify cDNA copies of RNA. It is the enzymatic conversion of mRNA into a single cDNA template. A specific oligodeoxynucleotide primer hybridizes to the mRNA and is then extended by an RNAdependent DNA polymerase to create a cDNA copy. First strand DNA synthesis The RT kit was purchased from Eurogentec (Seraing, Belgium). Reagents 1. 10X RT buffer: One vial containing 1.4 ml of 10X RT buffer. 2. EuroScript reverse transcriptase: One tube containing 75 µl of Moloney Murine leukemia virus reverse transcriptase (3750 U at 50 U/µl). Primers details are follows Rat IL-1 β-FW-5'-CCAGGATGAGGACCCAAG-3'; RW-5'TCCCGACCATTGCTGTTT -3'; **Rat TNF-α**- FW -5'-ACGCTCTTCTGTCTACTG-3'; RA -5'GGATGAACACGCCAGTCG-3'-  
**Rat β-actin-** FW – 5'-

TACAGCTTACCACCACAGC - 3'; RW-  
5'- TCTCCAGGGAGGAAGAGGAT - 3'.

## Procedure

Real Time PCR was carried out on the CFX 96 Real Time system (Bio-Rad). The reaction mix (10  $\mu$ l) was prepared by adding 5  $\mu$ l of 2X reaction buffer, 0.1  $\mu$ l of sense and antisense primer, 1  $\mu$ l of cDNA and 3.8  $\mu$ l of sterile water. The thermal cycler protocol was as follows: Initial denaturation at 95°C for 3 min, followed by 40 cycles of PCR, denaturation at 95°C for 10 sec, annealing at 60°C for 20 sec and extension at 72°C for 20 sec. All reactions were performed in triplicate along with no template control (NTC). Melt curve analysis was performed using the thermal cycling programmed at 50-95°C for each sample to determine the presence of multiple amplicons, non-specific products and contaminants. The results were analysed using CFX 96 Real Time system software (Bio-Rad). As an invariant control, the present study used rat  $\beta$ -actin.

## Statistical Analysis

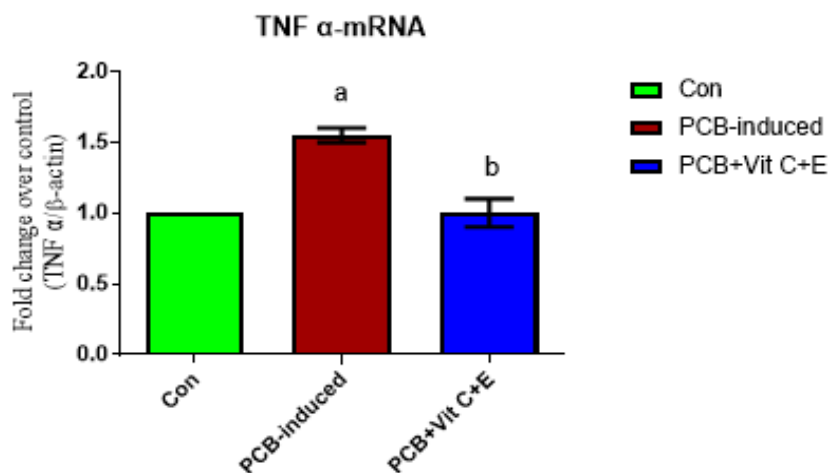
The triplicate analysis results of the experiments performed on control and treated

rats were expressed as mean  $\pm$  SEM. Results were analyzed statistically by one-way analysis of variance (ANOVA) and significant differences between the mean values were measured using Duncan's multiple range test using Graph Pad Prism version 5. The results with  $p < 0.05$  level was considered to be statistically significant.

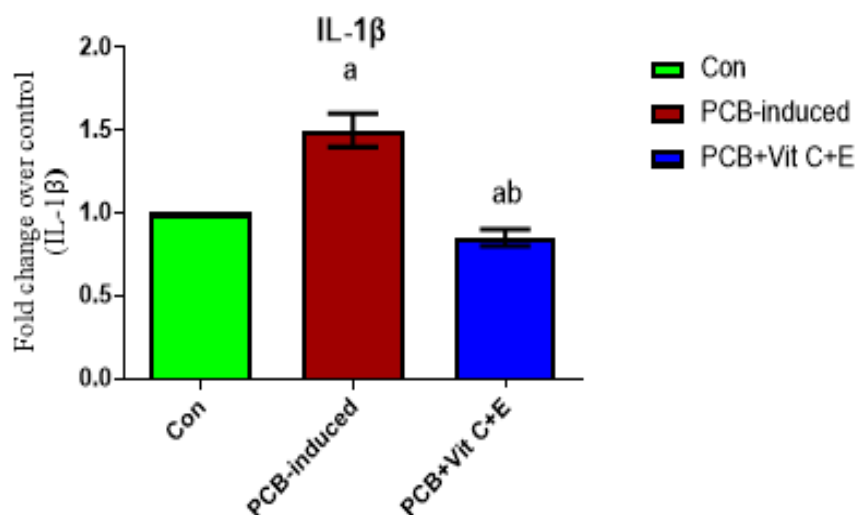
## Results

### Effect of Antioxidant Vitamins on Tnf-A and Il-1 $\beta$ Mrna Expression in Adipose Tissue of Pcb-Induced Rats

In this study the experimental rats induced diabetes with PCB. Their mRNA expression of TNF- $\alpha$  and IL-1 $\beta$  were quantitatively analysed using Real-Time PCR. A group of experimental rats were given vitamin C&E through gastric intubation for thirty days and their mRNA expression levels of TNF- $\alpha$  and IL-1 $\beta$  were also analysed and compared. In comparison, the levels of pro-inflammatory markers seem to be decreased effectively after vitamin C & E administration (fig 1 & 2).



**Figure 1.** Effect of Antioxidant Vitamins on TNF-A And Il-1 $\beta$  MRNA Expression in Adipose Tissue of PCB-Induced Rats



**Figure 2.** Effect of Vit C&E on the mRNA Expressions of IL-1beta, in Adipose Tissue of PCB-Induced Experimental Rats

## Discussion

Proinflammatory cytokines are those proteins that induce inflammation. During infection or inflammation, several cytokines are produced in the body. These proinflammatory cytokines produced because of diseased conditions such as diabetes, hypertension, organ failure, and cancer have proven to have deleterious effects. The proinflammatory cytokines analysed in this study are TNF- $\alpha$  and IL-1 $\beta$ . TNF- $\alpha$  plays a role as a polypeptide mediator of the cellular immune response with pleiotropic activity. TNF- $\alpha$  acts specifically on vascular endothelium to extend the adhesion of leukocytes amid the inflammatory process while IL-1 $\beta$  invigorates the release of pituitary hormones, increases the amalgamation of collagenases, demolition of cartilage as well invigorates the prostaglandins. In this study it was evident that there was an increased expression in proinflammatory markers such as TNF $\alpha$  and IL-1 $\beta$  in PCB induced rats. Upon treatment with antioxidants (Vitamin C and E) there was a marked decrease in the levels of proinflammatory markers (TNF- $\alpha$  and IL1- $\beta$ ) which was significantly equivalent to the controls, showing the reversal of oxidative stress. An increase in proinflammatory markers

causes an imbalance from a normal state. Imbalance in antioxidant levels by increase in proinflammatory markers such as TNF- $\alpha$  and IL1- $\beta$  by causing insulin resistance at cellular level, which induces oxidative stress, increases free radicals and ultimately causes diabetes. To restore the oxidative balance, the tissue is treated with antioxidants such as Vitamin C and E [22].

By the present study, studies have shown that exposure of PCB induces carcinogenic properties [23] and effects on urinary metabolites of neurotransmitters [24]. There are studies which were conducted to support vitamin C and E's antioxidant properties and their therapeutic role against PCB, and other free radicals, toxins and pollutants [25]. To the best of our knowledge, this is the first sort of study to show antioxidant vitamins (Vit C & E) reduced the PCB-induced changes in the expression of proinflammatory cytokines TNF- $\alpha$ , IL1- $\beta$ . Further studies on various proinflammatory cytokines need to be analysed.

## Conclusion

The present findings show that antioxidant vitamins (Vitamin C & E) have a significant role in controlling the expression of

proinflammatory signaling molecules in adipose tissue in PCB-exposed rats and hence it may be considered for the development of new antidiabetic therapeutic drugs. Further studies on the expression of downstream inflammatory signaling molecules need to be analysed in order to ascertain mechanisms of action of Vitamin C and E against PCB-induced inflammation.

## References

- [1] Ribas-Fito N (2001) Polychlorinated biphenyls (PCBs) and neurological development in children: a systematic review. *Journal of Epidemiology & Community Health* 55:537–546.
- [2] Williams AA, Selvaraj J, Srinivasan C, Sathish S, Rajesh P, Balaji V, Arunakaran J, Balasubramanian K (2013) Protective role of lycopene against Aroclor 1254-induced changes on GLUT4 in the skeletal muscles of adult male rat. *Drug Chem Toxicol* 36:320–328.
- [3] Jayashree S, Indumathi D, Akilavalli N, Sathish S, Selvaraj J, Balasubramanian K (2013) Effect of Bisphenol-A on insulin signal transduction and glucose oxidation in liver of adult male albino rat. *Environ Toxicol Pharmacol* 35:300–310.
- [4] Indumathi D, Jayashree S, Selvaraj J, Sathish S, Mayilvanan C, Akilavalli N, Balasubramanian K (2013) Effect of bisphenol-A on insulin signal transduction and glucose oxidation in skeletal muscle of adult male albino rat. *Human & Experimental Toxicology* 32:960–971.
- [5] Mullainadhan V, Viswanathan MP, Karundevi B (2017) Effect of Bisphenol-A (BPA) on insulin signal transduction and GLUT4 translocation in gastrocnemius muscle of adult male albino rat. *The International Journal of Biochemistry & Cell Biology* 90:38–47.
- [6] Sivashanmugam P, Mullainadhan V, Karundevi B (2017) Dose-dependent effect of Bisphenol-A on insulin signaling molecules in cardiac muscle of adult male rat. *Chemico-Biological Interactions* 266:10–16.
- [7] Ross G (2004) The public health implications of polychlorinated biphenyls (PCBs) in the

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## Conflict of Interest

The authors hereby declare that there is no conflict of interest in this study.

environment. *Ecotoxicology and Environmental Safety* 59:275–291.

- [8] Silverstone AE, Rosenbaum PF, Weinstock RS, Bartell SM, Foushee HR, Shelton C, Pavuk M (2012) Polychlorinated biphenyl (PCB) exposure and diabetes: results from the Anniston Community Health Survey. *Environ Health Perspect* 120:727–732.

- [9] Cohn BA, Terry MB, Plumb M, Cirillo PM (2016) Erratum to: Exposure to polychlorinated biphenyl (PCB) congeners measured shortly after giving birth and subsequent risk of maternal breast cancer before age 50. *Breast Cancer Res Treat* 159:591–592.

- [10] Dickerson SM, Cunningham SL, Patisaul HB, Woller MJ, Gore AC (2011) Endocrine disruption of brain sexual differentiation by developmental PCB exposure. *Endocrinology* 152:581–594.

- [11] Bomford R, BH (phd (1989) Interleukin-1, Inflammation and Disease. *Elsevier Science Limited*.

- [12] Leon LR (1996) Interleukin-1 and Tumor Necrosis Factor Receptor Modulation of the Acute Phase Response to Inflammation.

- [13] Finke JM (2001) Interleukin-1[beta] Folding and Aggregation.

- [14] Dinarello CA (2011) Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood* 117:3720–3732.

- [15] Hallahan DE, Spriggs DR, Beckett MA, Kufe DW, Weichselbaum RR (1989) Increased tumor necrosis factor alpha mRNA after cellular exposure to ionizing radiation. *Proc Natl Acad Sci U S A* 86:10104–10107.

- [16] Pehlivan FE (2017) Vitamin C: An Antioxidant

- Agent. Vitamin C.  
<https://doi.org/10.5772/intechopen.69660>
- [17] Ganapathy D, (2021) Awareness of hazards caused by long-term usage of polyethylene terephthalate (PET) bottles. *Int J Dent Oral Sci* 2976–2980
- [18] Niki E (2019) Synergistic Inhibition of Oxidation by Vitamin E and Vitamin C. *Cellular Antioxidant Defense Mechanisms* 111–122.
- [19] Karthik EVG, Priya V (2021) Gayathri. R, Dhanraj Ganapathy. Health Benefits Of *Annona Muricata-A* Review. *Int J Dentistry Oral Sci* 8:2965–2967.
- [20] Priya DV, (2020) Knowledge and awareness on HIV/AIDS among college students in A university hospital setting. *Int J Dent Oral Sci* 1182–1186
- [21] Chan AC (1993) Partners in defense, vitamin E and vitamin C. *Canadian Journal of Physiology and Pharmacology* 71:725–731.
- [22] Ruotsalainen E, Salmenniemi U, Vauhkonen I, Pihlajamäki J, Punnonen K, Kainulainen S, Laakso M (2006) Changes in inflammatory cytokines are related to impaired glucose tolerance in offspring of type 2 diabetic subjects. *Diabetes Care* 29:2714–2720.
- [23] Ealla KKR, Veeraraghavan VP, Ravula NR, Durga CS, Ramani P, Sahu V, Poola PK, Patil S, Panta P (2022) Silk Hydrogel for Tissue Engineering: A Review. *J Contemp Dent Pract* 23:467–477
- [24] Putschögl FM, Gaum PM, Schettgen T, Kraus T, Gube M, Lang J (2015) Effects of occupational exposure to polychlorinated biphenyls on urinary metabolites of neurotransmitters: A cross-sectional and longitudinal perspective. *Int J Hyg Environ Health* 218:452–460.
- [25] Murugesan P, Muthusamy T, Balasubramanian K, Arunakaran J (2008) Polychlorinated biphenyl (Aroclor 1254) inhibits testosterone biosynthesis and antioxidant enzymes in cultured rat Leydig cells. *Reprod Toxicol* 25:447–454.