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Effects Of Antioxidant Vitamins On The Expression Of Nrf2/Keap1 Signaling Molecules In Liver Against PCB Induced Experimental Diabetic Rats

Shivam Madan[1], Selvaraj Jayaraman[2*], Kavitha.S V.[3*], Vishnu Priya[4], R. Gayathri[5]

Abstract

Background: Polychlorinated biphenyl, or PCB, consists of biphenyl rings with a fluctuating number of chlorine compounds attached to it; approximately 209 such compounds have been found to date. Antioxidant vitamins are really important for the body. Vitamins C and E are two of them. While vitamin C acts as a water-soluble antioxidant and vitamin E acts as a major chain-breaking antioxidant, they have been continuously exploited for their beneficial effects against oxidative stress. Oxidative stress is the imbalance between the production and accumulation of oxygen-reactive species, which play several pathological roles in cell signaling. Aim: The study was designed

[1]Saveetha Dental College and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University,Chennai, India
[2]*Department of Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University,Chennai, India Email:- selvarajj.sdc@saveetha.com
[3]*Department of Biochemistry,Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India Email:- kavithas.sdc@saveetha.com
[4]Department of Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University,Chennai, India Email:- vishnupriya@saveetha.com
[5]Department of Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India gayathri.sdc@saveetha.com

***Corresponding author:-** Selvaraj Jayaraman* Kavitha.S
*Department of Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University,Chennai, India Email:- selvarajj.sdc@saveetha.com
*Department of Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India Email:- kavithas.sdc@saveetha.com

to assess the effects of antioxidant vitamins on the expression of Nrf2/Keap1 signaling molecules in PCB induced experimental rats.
Methods: Adult male Wistar rats weighing 180–200 g were used for this study. The experimental groups were divided into three groups (control, test group, and treatment group). Total RNA isolation was followed by reverse transcriptase-aided RNA to cDNA conversion and mRNA expression, which were analyzed by RT-PCR. The statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test to determine statistical significance among the groups.
Results: The findings of this study confirmed that exposure to PCB can alter the FBG level and serum insulin levels. Furthermore, mRNA expression results proved that expression of Nrf2 was significantly ($p<0.05$) lower in the PCB and vitamin E and C treated groups but gradually increased in the control group.
Conclusion: The treatment of rats with a combination of vitamin C and vitamin E will reduce the oxidative stress in PCB-exposed rats by facilitating NRF2 and KEAP1 molecules.

Keywords: PCB, Oxidative stress, ROS, Liver, Albino rats

INTRODUCTION

Polychlorinated biphenyl, or PCB, consists of biphenyl rings with a fluctuating number of chlorine compounds attached to them; approximately 209 such compounds have been found to date. Antioxidant vitamins are really important for the body. Vitamins C and E are such vitamins. While vitamin c acts as a water soluble antioxidant and vitamin E acts as a major chain breaking antioxidant, both have been continuously exploited for their beneficial effects against oxidative stress.

Oxidative stress is the imbalance between the production and accumulation of oxygen reactive species, which play several pathological roles in cell signaling and are normally generated as a by-product of oxygen metabolism (1). Environmental stressors and xenobiotics contribute greatly to ROS production, causing an imbalance that further leads to cell and tissue damage, which is commonly known as oxidative stress (2).

Antioxidants such as Vitamin E and Vitamin C have continuously been exploited for their beneficial effect against oxidative stress. Oxidative stress is being exploited continuously in order to be used as a therapeutic approach in spite of the fact it has been termed harmful (3). The antioxidant stress is achieved by both enzymatic and non-enzymatic reactions, they reduce the rate of initiation or prevent the propagation of free radicals and are not able to contain non-enzymatic antioxidants (4). Antioxidants and vitamins have shown action against even cancer cells such as colorectal adenoma (5) (6). Similarly, oxidative stress, which is released during placental development,

requires an adequate amount of antioxidant micronutrients. However, there could be complications in pregnancy with decreased intake of micronutrient antioxidants. Vitamin C plays an important role in the diet of humans, acting as a terminal enzyme in numerous pathways. iron transport is another important function in which vitamin c is involved (7)

Oxidative stress plays an important role in developing a wide range of diseases such as diabetes, cancer, and liver disease (8). Vitamin E, a lipophilic antioxidant, is important in the treatment of hyperglycemia, and combined therapy is also effective in the treatment of streptozotocin-induced diabetes (9). The importance of antioxidant vitamins is highly valued in our body and needs to be maintained well. Nrf2 / Keap-1 signaling pathways have evolved in defense mechanisms to cope up with toxicant, carcinogenic-induced oxidative stress, or electrophiles. Nrf2 which is nuclear factor erythroid 2- related factor 2 and Keap-1 which is Kelch-like erythroid cell-derived protein protect normal cells and has shown even protects against cancer cells. Nrf2 is induced during the course of drug resistance. Collectively it has been studied that it shows resistance against chemoresistance (9,10). In the current study, we have shown the possible role of antioxidant vitamins in reducing hyperglycemia by regulating the expression of Nrf2/Keap1 signaling molecules in the liver of PCB-induced experimental rats.

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; and Promega, USA; The total RNA isolation reagent (TRIR) was purchased from Invitrogen, USA. The reverse-transcriptase enzyme (MMuLv) was purchased from New England Biolabs (NEB), USA, and the GoTaq Green master mix was purchased from Promega, USA. nrf2/keap1 and β-actin primers were purchased from Eurofins Genomics India Pvt Ltd, Bangalore, India.

Animals

Adult male Albino Wistar rats weighing 150–180 g were used in our study. They were maintained as per the guidelines of the Indian National Law on Animal Care and Use at Biomedical Research Unit and laboratory animal center (BRULAC), Saveetha dental college and hospitals, SIMATS, Chennai-77. The Institutional Animal Ethical Committee (IAEC) (Registration Number: BRULAC/SDCH/ SIMATS/I

AEC/8-2021/086) approved all animal-related experimental methods. At BRULAC, Saveetha Dental College, and hospitals SIMATS in Chennai-77, the animals were housed in a temperature-controlled room with a standard 12-hour light/12-hour dark cycle and were given free access to water and a standard pellet diet.

Experimental Design:

Healthy male albino rats were divided into three groups of six animals each (n = 6).

Group I: Control rats, injected with corn oil intraperitoneally (ip) once daily as a vehicle.

Group II: PCB was administered orally for 30 days (PCB was dissolved in water at a dose of 2 mg/kg//day at 8 a.m.).

Group III: PCB+ Vitamin E (50 mg/kg b.w.t.) and Vitamin C (100 mg/kg b.w.t.) treated orally for 30 days.

After the treatment period, the animals were anesthetized with ether, blood was collected, sera were separated, and they were stored at -80°C. The livers of control and treated animals were dissected and tested for various parameters.

Fasting blood glucose (FBG)

Blood glucose was estimated using On-Call Plus blood glucose test strips (ACON Laboratories Inc., USA) after overnight fasting. Blood was collected by pricking the tip of the rat tail, and results are expressed as mg/dl.

Serum insulin

Serum insulin was assayed using an ultrasensitive rat insulin ELISA kit obtained from Crystal Chem Inc (Illinois, USA). Elisa kit as per the manufacturer's instructions. Results were expressed as IU/ml.

Total RNA isolation, cDNA conversion, and real-time PCR

Using a TRIR kit, total RNA was isolated from control and experimental samples. In brief, to 100 mg fresh tissue, 1 ml of TRIR was added and homogenized. The content was transferred to a microcentrifuge tube instantly and 0.2 ml of chloroform was added, vortexed for 1 min, then kept at 4**°**C for 5 min. Later, the contents were centrifuged at 12,000×g for 15 min at 4**°**C. The aqueous phase (upper layer) was carefully transferred to a fresh microfuge tube and an equal volume of isopropanol was added, vortexed for 15S and placed on ice for 10 min. After centrifugation of the content at 12000×g for 10 min at 4**°**C, the supernatant was discarded, and the RNA pellet was washed with 1 ml of 75% ethanol by the vortex. The isolated RNA was estimated, and the RNA concentration was expressed in micrograms. By using the reverse transcriptase kit from Eurogentec (Seraing, Belgium), complementary

DNA (cDNA) was synthesized from 2 µg of total RNA as stated in the manufacturer's protocol. To perform real-time PCR, the reaction mixture containing 2x reaction buffer-master mix, forward and reverse primers of the target gene and housekeeping gene, water, and β-actin (the primer sequences were listed in Table 1) in total volume of 45 µl expect the cDNA was made, mixed intensively, and spun down. In individual PCR vials, about 5 µl of control DNA for positive control, 5 µl of water for the negative control, and 5 µl of template cDNA for samples were taken and reaction mixture (45 µl) were added. 40 cycles (95°C for 5 min, 95°C for 5 s, 60°C for 20 s and 72°C for 40s) was set up for the reaction and obtained results were plotted by the PCR machine (Stratagene MX 3000 P, Agilent Technologies, 530 l, Stevens Creek Blvd, Santa Clara CA, 95051) on a graph. The melt and amplification curves were analyzed to determine relative quantification.

S.no	Gene name	Sequence
1.	Nrf2	TTGTAGATGACCATGA GTCGC TGTCCTGCT TAT GCT CTT
2.	Keap1	CTGGAGGATCATACCAAGCAGG GGATACCCTCAATGGACACCAC
3.	β-actin	AAGTCCCTCACCCTCCCAAAAG AAGCAATGCTGTCACCTTCCC

Table No. 1: Primer sequence details

Statistical analysis

One-way analysis of variance (ANOVA) and Duncan's multiple range test were used to assess the significance of individual variations between the control and treatment groups using computer-based software, GraphPad Prism version 5. In Duncan's test, the significance was considered to be at the level of $p<0.05$.

Results

Effect of antioxidant vitamins on FBG and serum insulin levels in PCB induced diabetic rats

Fasting blood glucose, serum insulin, are shown in figures 1 and 2. Rats administered PCB significantly ($p<0.05$) increased blood glucose and serum insulin concentration compared to control. However, treatment with antioxidant vitamins improved glycemic control and insulin sensitivity, and this study suggests that antioxidant vitamins (Vitamin C and E) regulate glucose homeostasis.

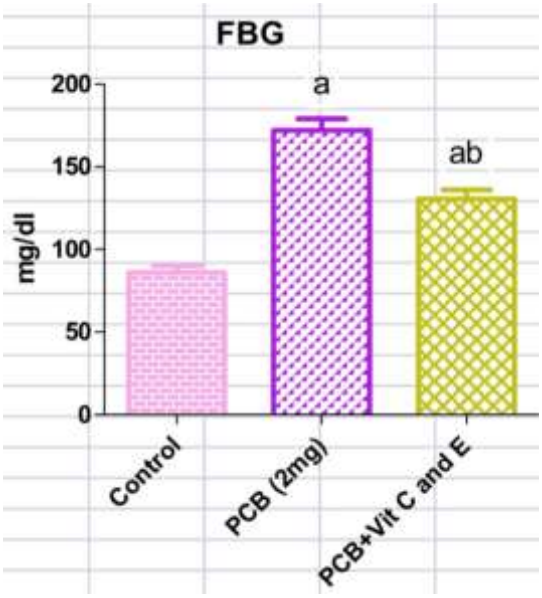

Fig. 1: Fasting blood glucose in PCB and vitamin treated rats. Each bar represents the mean ± S.E.M of 6 animals. a-compared to control; b-compared to PCB-induced rats. Significance was considered at the levels of $p<0.05$.

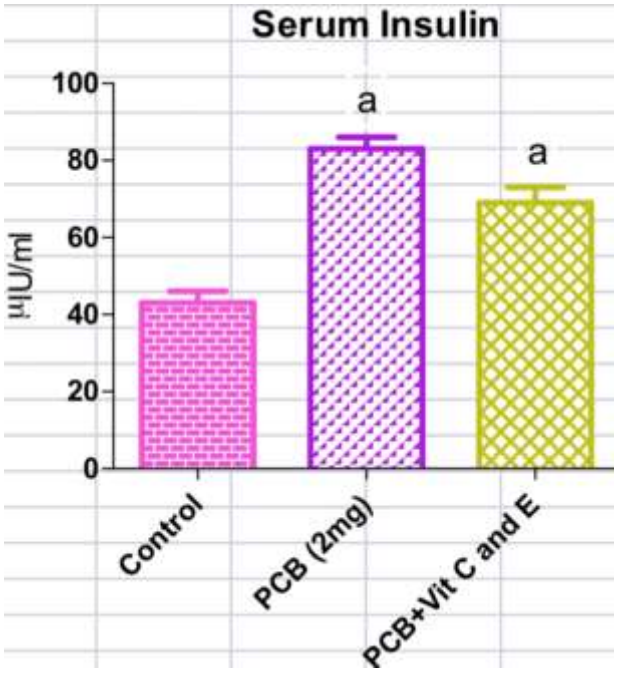

Fig. 2: Fasting serum insulin in PCB and vitamin treated rats. Each bar represents the mean ± S.E.M of 6 animals. a-compared to control; b-compared to PCB-induced rats. Significance was considered at the levels of $p<0.05$.

Effect of antioxidant vitamins on FBG and serum insulin levels in PCB induced diabetic rats

mRNA expressions:

Figures 3 and 4 depict the effects of vitamins E and C on Nrf2 and Keap-1 mRNA expression in the liver. We found that mRNA expression of Nrf2 was significantly ($p<0.05$) lower in the PCB and vitamin E and C treated groups but gradually increased in the control group in the current study. There was a significant ($p<0.05$) increase in Keap-1 compared to the control PCB-induced and vitamin-treated groups.

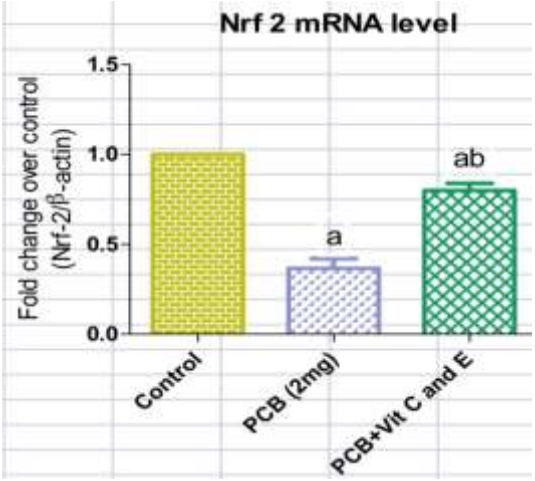

Figure No. 3: Nrf2 mRNA expressions in normal and treated groups

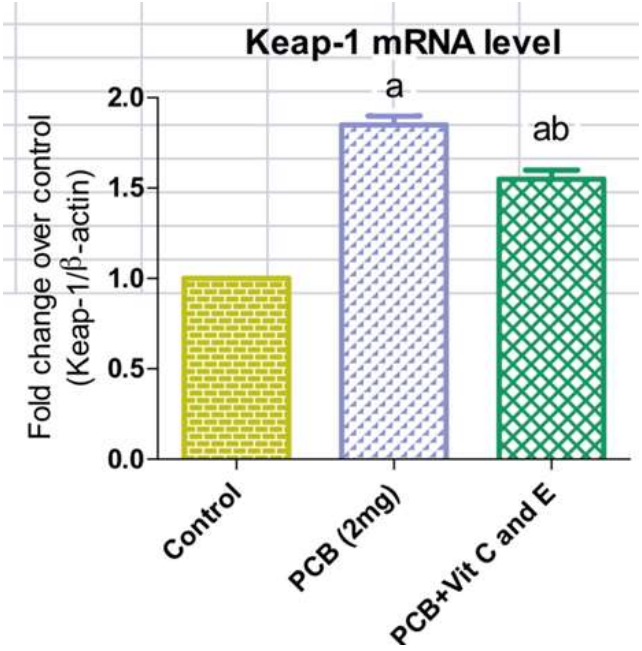

Figure No. 4: Keap mRNA expressions of normal and treated groups

Discussion:

Accidental or occupational exposure to high quantities of toxic compounds has a link between environmental hazards or contamination and diabetes (11) (12). PCB is one of them, PCBs are present all across the ecosystem, including soil, air, and water. Because of their lipophilicity, they are also susceptible to bioaccumulating in the food chain and thus belong to a family of environmental chemicals known as persistent bioaccumulative toxic elements (13). PCB-related issues include hypothyroxinemia, deficiencies in spatial learning and memory, neurochemical and neurobehavioral changes, and hormonal modifications. PCB exposure can lead to type 2 diabetes by changing proinflammatory biomarkers, which elevate insulin resistance (14,15) stated that exposure to PCB is associated with diabetic complications.

From the statistical results, it can be seen that the serum fasting levels and insulin serum levels have decreased with the injection of PCB with vitamin C and vitamin E; the antioxidant vitamins added with PCB to the rats showed a decrease in fasting The blood group levels, which are elevated during diabetes, have decreased. The serum insulin levels, which are normally high in diabetics, have also been shown to decrease with the addition of antioxidants. Although the higher rate than the control is due to insulin resistance, which only causes a decrease in a fasting blood group level. In the present research, the PCB-induced group showed a significant increase in the levels of mRNA expression with Nrf2, and Keap-1 in type 2 diabetic rats, but when treated with vitamin C and E, it showed a decrease in the level.

Graphs 3 and 4 showed decreased expression of Nrf-2 in the PCB-induced rats, indicating the development of oxidative stress in those rats, which may be due to its antioxidant activity. Similarly, the expression of Keap-1 mRNA was found to increase in the PCB-induced rats, but it was reduced on treatment with antioxidant vitamins, which also confirms the antioxidant activity of the vitamins. Nrf2 levels are high in vitamin-induced rats, so antioxidant status will improve, potentially reducing PCB-induced toxicity. The Nrf2 signaling molecule, which is attached to Keap-1 molecular signaling, gets detached from the Keap-1 molecules when it comes in contact with oxidative stress.
PCBs have been shown to promote urea formation, rather than glucose synthesis such as in the study done by (16). The Nrf2/Keap-1 mechanism study has been done by (17), the effect of Nrf-2/Keap-1

molecule on diabetes has been emphasized by (18), and even the emerging role of redox diabetes pathways has been discussed by (19).

Conclusion:

According to the study, exposure to PCB can alter fasting blood glucose levels and serum insulin levels and induce insulin resistance in pancreatic beta cells by elevating lipid peroxidation and reducing the level of antioxidants. Treatment with a vitamin C and E combination reduces oxidative stress in PCB-exposed rats by facilitating Nrf2/keap1 molecules. Furthermore, it has been mechanically demonstrated that it acts in the Nrf2/Keap1 signaling molecules. The findings on PCB effects on insulin secretion amply support the need for a more thorough examination into the specific effects of these environmental pollutants on beta cells and their role in the onset and progression of type 2 diabetes. More research is required in the future to understand the environmental toxicity impact in the animal model and cell line study.

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