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Original Research Article

EFFECTS OF ANTIOXIDANT VITAMIN C AND E ON TGF-β AND IL-18 GENE EXPRESSION IN KIDNEY TISSUE OF PCB-INDUCED EXPERIMENTAL RATS

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Abstract: ***Introduction***: Polychlorinated biphenyls are synthetic high reactive man made chemical which enter food chain via its extensive use in packing materials and other food materials.TGF-B and IL-18 are diabetic nephropathy markers.Vitamin C&E (electron antioxidant) plays a key role to fight against oxidative stress. ***Aim:*** The role of vitamin C&E in regulating the pro inflammatory markers (TGF BETA &IL-18) in PCB treated experimental rats. ***Materials and methods:*** Adult male wistar rats (180-200g) were divided into three groups (Control, PCB induced and Treated) total RNA was isolated followed by cDNA synthesized using reverse transcriptase.Quantitative Real Time- Polymerase Chain Reaction (RT-PCR) was done for amplification of DNA. The present study used rat β-actin as an invariant control. ***Result:*** From the study, it was evident that exposure of PCB induced group showed an increased expression of pro-inflammatory markers(TGF-B, IL-18) in kidney tissue compared to the control group. The Vitamin C&E treated group showed a marked decrease in the expression of pro-inflammatory markers (TGF-B &IL-18) as compared to control. ***Conclusion:*** Vitamin C&E as an antioxidant has proved to decrease the levels of Proinflammatory markers in turn reduce the complications of diabetic nephropathy.

Keywords: PCB toxicity, oxidative stress, Vitamin C, Vitamin E, TGF-β, IL-18, renal inflammation, gene expression, antioxidant therapy, diabetic nephropathy

INTRODUCTION

Polychlorinated biphenyls (PCBs) are a class of persistent environmental pollutants widely recognized for their toxic effects on both wildlife and humans. Although their industrial production has been banned or restricted in many countries, PCBs remain present in the environment due to their chemical stability and resistance to degradation (1). Exposure to PCBs occurs mainly through contaminated food, water, and air, leading to their accumulation in various organs, including the liver and kidneys(2). Numerous studies have shown that PCBs can induce oxidative stress, inflammation, and cellular damage, which may ultimately impair normal organ function(3,4). The kidney, being a vital organ responsible for filtration and detoxification, is particularly vulnerable to toxic insults caused by environmental contaminants such as PCBs(4).

One of the major mechanisms by which PCBs exert toxicity is through the generation of reactive oxygen species (ROS), resulting in oxidative stress and disruption of cellular homeostasis(5). Excessive ROS production can damage cellular lipids, proteins, and nucleic acids, contributing to tissue injury and inflammatory responses. In the kidney, oxidative stress may activate signaling pathways that regulate the expression of inflammatory and fibrotic mediators. Among these mediators, transforming growth factor beta (TGF-β) plays a crucial role in promoting fibrosis and structural alterations in renal tissue, while interleukin-18 (IL-18) is a pro-inflammatory cytokine involved in immune responses and inflammation(6). Changes in the gene expression of these molecules are often associated with kidney injury and disease progression.

Antioxidant vitamins have attracted considerable attention for their potential protective effects against oxidative stress–related tissue damage(7). Vitamin C (ascorbic acid) and vitamin E (α-tocopherol) are well-known antioxidants that help neutralize free radicals and protect cellular components from oxidative damage. Vitamin C is a water-soluble antioxidant that scavenges reactive oxygen species and helps regenerate other antioxidants within the body. In contrast, vitamin E is a lipid-soluble antioxidant that protects cell membranes from lipid peroxidation. The combined action of these vitamins may enhance the overall antioxidant defense system and reduce inflammation and cellular injury caused by toxic substances such as PCBs(8).

Considering the role of oxidative stress in PCB-induced renal toxicity and the importance of inflammatory mediators in kidney damage, investigating the effects of antioxidant supplementation is of significant scientific interest. Evaluating how vitamin C and vitamin E influence the gene expression of TGF-β and IL-18 in kidney tissue may provide insights into their protective mechanisms against PCB-induced toxicity(9). Therefore, the present study aims to assess the modulatory effects of antioxidant vitamins C and E on TGF-β and IL-18 gene expression in the kidney tissue of PCB-induced experimental rats, thereby contributing to a better understanding of potential therapeutic strategies for reducing environmental toxin–related renal .

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-1β and β-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

Group 1: Control (Vehicle control, rats were intraperitoneally (i.p.) administered with the vehicle (corn oil) for 30 days.

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.

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 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 A260 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).

Quantitative Real Time PCR Principle

The purpose of a PCR (Polymerase Chain Reaction) is to make a huge number of copies of a gene. There are three major steps in a PCR, which are as follows: Denaturation at 94°C for 3 min: During the denaturation at 94°C for 2-5 min, the double strand melts open to single stranded DNA, all enzymatic reactions stop. AAnnealing at 54°C- 65°C for 30 sec: Ionic bonds are constantly formed and broken between primer and the single stranded template to ensure the extension process. Extension at 72°C for 30 sec: Primers that are in positions with no exact match get loose again (because of the higher temperature) and don't give an extension of the fragment. The bases (complementary to the template) are coupled to the primer on the 3' side (the polymerase adds dNTP from 5' to 3', reading the template from 3' to 5' side; bases are added complementary to the template). Because both strands are copied during PCR, there is an exponential increase of the number of copies of the gene.

Reagents used: 1. 2X Reaction buffer: The PCR master mix kit was purchased from Takara

Bio Inc., Japan. Contains TaKaRa Ex Taq HS (a hot start PCR enzyme) dNTP, Mixture, Mg2+, Tli RNase H (a heat-resistant RNase H that minimizes PCR, inhibition by residual mRNA), and SYBR Green I, 2. Forward primer (10µM), 3. Reverse primer (10µM), 4. cDNA- Template, 5. Autoclaved milli Q water, 6. Primers: The following gene specific oligonucleotide primers were used.

Details of primers used in the present study

Rat TGF-β
FW- 5’ -AGCTCTGGAACCTCCTGAAGA – 3’
RW- 5’ – AGCTCCAGTCTAGGGTCGTGA – 3’
Rat IL-18
FW: 5’-AGACGCTTGAGTTGAGAGCC-3’
RW: 5’-CTTCAGTGTGCGGCTTAGGA-3’
Rat β-actin
FW – 5’- TACAGCTTCACCACCACAGC - 3’
RW– 5’- TCTCCAGGGAGGAAGAGGAT - 3’

Procedure

Procedure Real Time PCR was carried out on CFX 96 Real Time system (Bio-Rad). The reaction mix (10 µl) was prepared by adding 5 µl of 2X reaction buffer, 0.1 µl of sense and antisense primer, 1 µl of cDNA and 3.8 µl 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 β-actin

Statistical analysis

The triplicate analysis results of the experiments performed on control and treated rats were expressed as mean ± 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 were considered to be statistically significant.

RESULTS

Effect of PCB on IL-18-mRNA

In the relation to mRNA expression of IL-18 , there is no significant change in the level in PCB induced group and no significant change with the group treated with Vitamin C and Vitamin E when compared to the Control group [Figure-1].

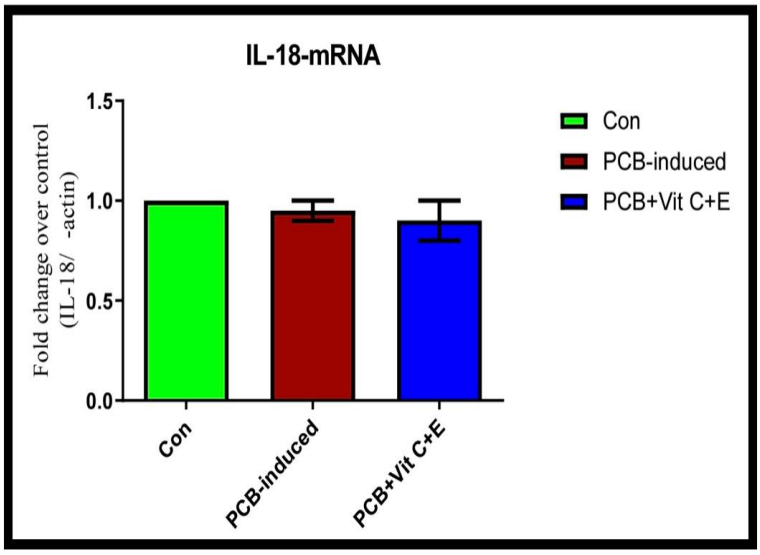

Figure 1: Effect of Vit C & E on mRNA expression of IL-18 in type-2 diabetic rats. The mRNA expression of TNF-**β** and IL-18 were assessed by Real Time-PCR. Each bar represents mean ± SEM (n=6).Significance at P<0.05 The x-axis represents the groups and the y-axis represents the changes in the groups when associated with IL-18.Green bar represents expression level of control rats, red bar represents expression level of PCB-induced rats, and blue bar represents expression levels of PCB-induced rats which were later treated with Vitamin C and E.

Effect of PCB on TGF-β - mRNA

mRNA of **TGF-β** was studied, there was a significant increase in the level in the PCB induced group, but when treated with Vitamin C and Vitamin E, there was a significant decrease in the expression, which became equivalent

to the control group [Figure-2]

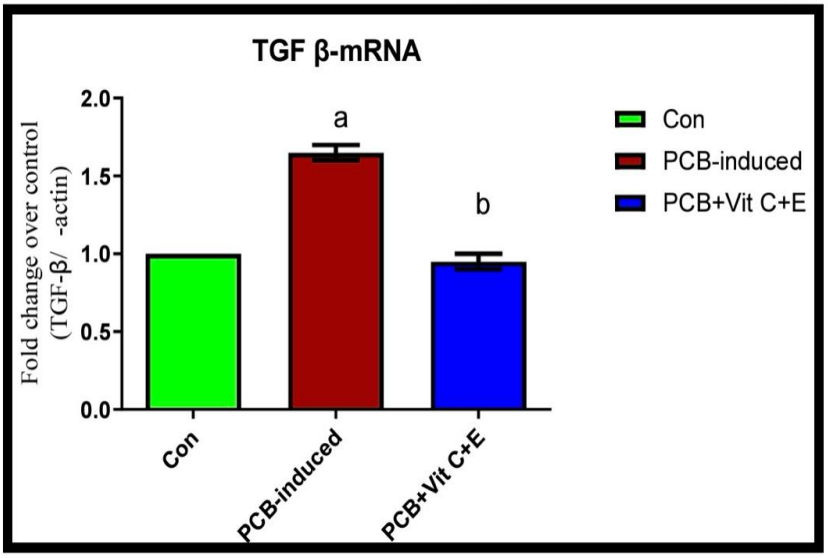

Figure 2: Effect of Vit C & E on mRNA expression of IKKB in type-2 diabetic rats. The mRNA expression of **TGF-β** and IL-18 were assessed by Real Time-PCR. Each bar represents mean ± SEM (n=6). Significance at *P <0.05*, [a]Significantly different from the control group. [b]Significantly different from diabetic control. ± SEM (n=6). Significance at *P <0.05*, [a]Significantly different from the control group. [b]Significantly different from PCB-induced diabetic rats.Green bar represents expression level of control rats, red bar represents expression level of PCB-induced rats, and blue bar represents expression levels of PCB-induced rats which were later treated with Vitamin C and E.

DISCUSSION

The findings of the present study demonstrate that exposure to polychlorinated biphenyls (PCBs) alters the expression of inflammatory and fibrotic markers in kidney tissue, as evidenced by the increased gene expression of TGF-β and IL-18 in PCB-treated rats(10). PCBs are well-known environmental toxicants capable of inducing oxidative stress, inflammation, and cellular damage in various organs. Previous toxicological studies have reported that PCB exposure leads to excessive generation of reactive oxygen species (ROS), which disrupts cellular homeostasis and promotes inflammatory signaling pathways(11). The kidney, due to its high metabolic activity and role in detoxification, is particularly susceptible to oxidative damage caused by environmental contaminants. The upregulation of TGF-β and IL-18 observed in this study therefore supports earlier reports that PCB toxicity is closely associated with inflammatory and fibrotic responses in renal tissue(12).

The increased expression of TGF-β in PCB-exposed animals observed in the current study is consistent with findings reported in earlier investigations examining toxicant-induced renal injury(13). TGF-β is recognized as a key mediator of tissue fibrosis and plays a central role in the progression of chronic kidney damage. Previous experimental studies have shown that exposure to environmental toxins and xenobiotics can stimulate TGF-β signaling pathways, leading to increased extracellular matrix deposition and structural alterations in renal tissue. Such changes contribute to the development of renal fibrosis and impaired kidney function(14). Therefore, the elevated TGF-β expression observed in PCB-treated rats in the present study may indicate the activation of fibrogenic mechanisms triggered by oxidative stress and inflammatory processes.

Similarly, the increased gene expression of IL-18 in the kidney tissue of PCB-treated rats aligns with findings from previous studies investigating inflammatory cytokines in toxicant-induced renal damage. IL-18 is a pro-inflammatory cytokine involved in immune activation and the amplification of inflammatory responses(15). Earlier research has demonstrated that elevated IL-18 levels are associated with acute and chronic kidney injury, as well as with exposure to various environmental pollutants. The upregulation of IL-18 observed in the present study suggests that PCB exposure may stimulate inflammatory signaling pathways that contribute to renal tissue injury and dysfunction.

The present results also indicate that supplementation with antioxidant vitamins C and E can modulate the expression of these inflammatory and fibrotic markers(16). This observation is supported by several previous studies that have demonstrated the protective

role of antioxidants against oxidative stress-induced tissue damage. Vitamin C and vitamin E are well-established antioxidants that function by neutralizing reactive oxygen species and protecting cellular components from oxidative injury. Vitamin C primarily acts as a water-soluble antioxidant that scavenges free radicals and helps regenerate oxidized vitamin E, whereas vitamin E protects cell membranes by preventing lipid peroxidation. Studies investigating antioxidant therapy in toxicant-induced organ damage have reported that combined antioxidant supplementation can significantly reduce oxidative stress, inflammation, and cellular injury.

Furthermore, earlier experimental studies using animal models have shown that vitamins C and E can attenuate the expression of inflammatory cytokines and fibrotic markers in tissues exposed to environmental toxins or chemical stressors. These studies suggest that antioxidant treatment may help restore the balance between pro-oxidant and antioxidant systems, thereby preventing the activation of signaling pathways associated with inflammation and fibrosis.(16,17) The reduced expression of TGF-β and IL-18 observed in antioxidant-treated groups in the present study is therefore consistent with the protective effects reported in previous research.

Overall, the findings of this study support the growing body of evidence indicating that oxidative stress plays a central role in PCB-induced renal toxicity and that antioxidant therapy may provide protective benefits. By reducing oxidative damage and modulating inflammatory and fibrotic gene expression, vitamins C and E may help mitigate kidney injury caused by environmental pollutants. However, further studies are necessary to better understand the molecular mechanisms involved and to evaluate the long-term therapeutic potential of antioxidant supplementation in preventing toxin-induced renal damage.

CONCLUSION

Vitamin C&E as an antioxidant has proved to decrease the levels of Proinflammatory markers in turn reduce the complications of diabetic nephropathy.

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