# Role of Antioxidant Vitamins on the Expression of HNF-1 Alpha Sterol Regulatory Element Binding Protein 1-C in PCB-Induced Experimental Diabetic Rats

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**Abstract**: **Introduction:** PCB ( polychlorinated biphenyl ) is one among the environmental pollutants or toxicants and in addition to that it is also one of the neurologically toxic compounds. PCB functions by inducing the production of free radicals which in turn leads to the formation of oxidative stress. In the human body, antioxidants are classified into two groups based on the source of availability into endogenous and exogenous antioxidants. Vitamin C and vitamin E are well-known antioxidants . Vitamin C (ascorbic acid) is a water soluble vitamin, vitamin E (tocopherol) is a fat soluble vitamin. Vitamin inhabit the membrane disintegration effects of ROS reactive oxygen species which is induced by lipid peroxidation. Vitamin C is found in plasma and it helps in the production of collagen synthesis.

**Materials and Methods:** Adult male albino rats were divided into 3 groups. Group I: served as vehicle control (corn oil alone); Group II: Control rats were injected intraperitoneally daily with PCB (Aroclor 1254) at a dose of 2mg/k.b.wt , for 30 days to induce diabetes; Group III: PCB induced rats treated with vitamins E (50 mg/kg body weight) and C (100 mg/kg body weight), once daily through gastric intubation for 30 days. On completion of treatment, animals were anesthetized, blood was collected, sera were separated, gastrocnemius muscle was dissected out and subjected to assessment of gene expression analysis. Fasting blood glucose, serum insulin levels were measured in the serum whereas, HNF-1 alpha mRNA and SREBP-1c proteins were measured in the skeletal muscle using Real Time-PCR analysis using gene specific primers and ELISA methods. The data were statistically analyzed using One-Way-ANOVA followed

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by Duncan's multiple range test were used to check the statistical significance and considered at the levels of $p<0.05$.
**Results and Discussion:** PCB-induced animals showed a significant increase ($p<0.05$) in both fasting blood glucose (FBG) and fasting serum insulin levels compared to control rats. Vitamin supplementations (Vit C and E) effectively reduced the FBG and serum insulin levels near to that of the control animals. mRNA expression of SREBP1-c was also found to be significantly increased in PCB-induced rats while HNF-1 alpha mRNA levels were found to be reduced in PCB-exposed rats. Antioxidant vitamins treatment normalized the gene expression levels.
**Conclusion**: Our study for the first time providing an evidence that Vitamin C and E have the potency in reducing hyperglycemia and hyperinsulinemia by modulating the expression of HNF-1 alpha and SREBP1-c in the PCB-induced diabetic gastrocnemius muscle. Therefore, Vitamin C and E could be a promising drug candidate for the management of type-2 diabetes.

**Keywords:** Aroclor 1254, PCB, Vitamin C, Vitamin E, innovative technique, Hepatocyte Nuclear Factor-1 alpha, SREBP-1, Diabetes, novel method.

## 1. INTRODUCTION

Vitamin C ascorbic acid, Vitamin E tocopherol, lycopene and beta carotene are notable antioxidants (1). Vitamin C is a vitamin which falls under water soluble vitamins and it is not synthesized by the human body. This is an essential vitamin required for the vital functions of the human body so it has to be ingested through daily dietary consumption. Ascorbic acid won't get diffused easily in between the cell membranes (2). Oxidation of ascorbic acid produces dehydro ascorbic acid which readily diffuses through cell membranes. Vitamin C deficiency leads to a disease called scurvy which is characterized by bleeding gums, loosening of teeth, capillary hemorrhage, collage weakening (3).

Ascorbic acid is directly involved in stimulation of collagen synthesis through the process of activation of multiple genes. The general function of ascorbic acid is to strengthen the collagen (4). Vitamin C also serves as an enzyme cofactor for certain enzymes, such as hydroxylase enzymes, example Polly hydroxylase and lysyl hydroxylase. These two enzymes are highly essential for hydroxyproline and hydroxylysine formation. (5)
The daily dietary source includes eight different kind of vitamin E supplementation and eight related molecules synthesized by biologically available plants. (3)
The biological function of vitamin E is mostly dependent upon regulatory mechanisms that helps retain or hold on the alpha tocopherol compound and eliminate the non alpha tocopherol compound. Alpha tocopherol transfer protein (TTP) is crucial for a stable health because mutations in this alpha tocopherol transfer protein leads to Siddhi Er vitamin a deficiency which is diagnosed by the symptom of neurological abnormalities especially ataxia and gradually leads to the death of an individual (6).

Ataxia is found in individuals who are deficient in vitamin E. In those cases vitamin E has to be provided in a sufficient amount to overcome the lack of alpha tocopherol transfer protein. Alpha tocopherol acts as a peroxyl radical scavenger that protects

polyunsaturated fatty acids in cell membranes and lipoproteins (7).

Poly chlorinated biphenyls (PCB) have been a problem in civilized areas or urban environments because they exist for many years and it also has the property of bioaccumulation. PCBs are found in the entire ecosystem like soil , air and water (8). Aroclor 1254 is a commercially available poly chlorinated biphenyls and has its constituent distribution of 54 % chlorine by weight. It has been shown to have on several organ systems and developing animals. The central nervous system is sensitive to PCB poly chlorinated biphenyls And are transported by blood to various organs such as muscles and liver. (9).

Commercial PCB mixture Aroclor 1254 is specified as having 54% chlorine by weight . In adult and developing animals, PCBs have an impact on a number of organ systems . One of the target organs for polychlorinated biphenyls is the brain (PCBs). Laboratory animals' motor activity, neurological development, and cognitive function were changed by developmental exposure to PCB combinations or congeners . In both humans and other animals, the hippocampus has long been linked to memory function . Acute exposure to some PCB combinations and particular PCB congeners has been shown to affect synaptic transmission and plasticity in the hippocampus in *in vitro* investigations. The nerve cells of the hypothalamic nuclei, which produce the releasing factors or inhibiting factors, govern the hormone. Our team has extensive knowledge and research experience that has translate into high quality publications(10–19))((20–29).

The hypothalamus is a component of the diencephalon. PCBs are also set to have both neurochemical and neuro endocrine effects. PCB are endocrine disrupting chemicals (30). Aroclor 1254 has serious effects on gonadotropin; it severely affects the level of LH and FSH. It affects the male reproductive system by affecting the levels of sex hormones nd it has effects on receptor concentration of prostate gland (31). Previous study from our laboratory has shown that PCB exposure caused detrimental changes in glucose levels and lycopene treatment retrieved the same. However, the therapeutic role of antioxidant vitamins on insulin signaling mechanisms in the gastrocnemius muscle is obscure. The present study was undertaken to analyze the role of antioxidant vitamins on fasting blood glucose, fasting serum insulin and mRNA expression analysis of HNF-1 alpha sterol regulatory elements binding protein-1 c levels in PCB Induced experimental diabetic Rats.

## 2. 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; Total RNA isolation reagent (TRIR) was purchased from Invitrogen, USA. The reverse-transcriptase enzyme (MMuLv) was purchased from New England Biolabs (NEB), USA and Go Taq Green master mix was purchased from Promega, USA. Insulin receptor (IR), glucose transporter-4 (GLUT4) and β-actin primers were purchased from Eurofins Genomics India Pvt Ltd, Bangalore, India and.

### 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 centre (BRULAC), Saveetha dental college and hospitals, SIMATS, Chennai-77. The Institutional Animal Ethical Committee (IAEC) (Register Number: BRULAC/SDCH/SIMATS/IAEC/8-2021/086) approved all animal-related experimental methods. The animals were housed in a temperature (21 ± 2 °C)-controlled room with a standard 12 h light – 12 h dark cycle and were allowed free access to water and standard pellet diet at BRULAC, Saveetha Dental College and Hospitals, SIMATS, Chennai-77.

**Experimental Design:**

Healthy male albino rats were divided into 3 groups consisting of 6 animals each. Group I: Control rats (Control rate injected with corn oil intraperitoneally (ip) once daily as a vehicle). Group II: Rats received ip injection of A1254 at a dose of 2 mg/kg once daily for 30 days. Group III: Rats received simultaneous treatment of Aroclor 1254+ 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). In the present study, PCB dose was selected based on our previous report of Anne et al. (2013) and Vit C and E doses were selected based on the report of our previous study (Srinivasan *et al.*, 2011). After the treatment period, animals were anesthetized with ether, blood was collected, sera separated and stored at −80 °C. Gastrocnemius muscle from control and treated animals were dissected out and subjected for the assay of various parameters.

**Determination of fasting blood glucose (FBG) and serum insulin**

The animals were starved overnight the day before sacrifice after receiving treatment for 30 days.The next day, blood was drawn from the rat tail tip to estimate glucose levels using On-Call Plus blood glucose test strips. The data were displayed in milligrammes per decilitre on the meter display window. INS GENLISA ELISA kit from Krishgen Biosystem, Mumbai-400018, India, was used to measure insulin levels in rat serum. According to the manufacturer's guidelines, the detection range and coefficient of variation were set. The serum insulin concentration was measured in pg/ml.

**Total RNA, cDNA Synthesis and Real-Time PCR**

RNA was taken out from the livers of the rats in the present study. The reverse transcriptase RT kit was used to reverse transcript 2µg of RNA (Seraing, Belgium). The primers utilized in this study are listed in Table 1. Using housekeeping gene (β-Actin) as a reference gene, 40 cycles of 95°C, 59–60°C, and 72°C for 30s each were amplified in a Stratagene MX 3000P qRT-PCR system under the subsequent reaction conditions: initial denaturation at 95°C for 5 minutes, followed by 40 cycles of 95°C, 59–60°C and 72°C for 30s each. The melt and amplification curves analyses were used to calculate relative quantification.

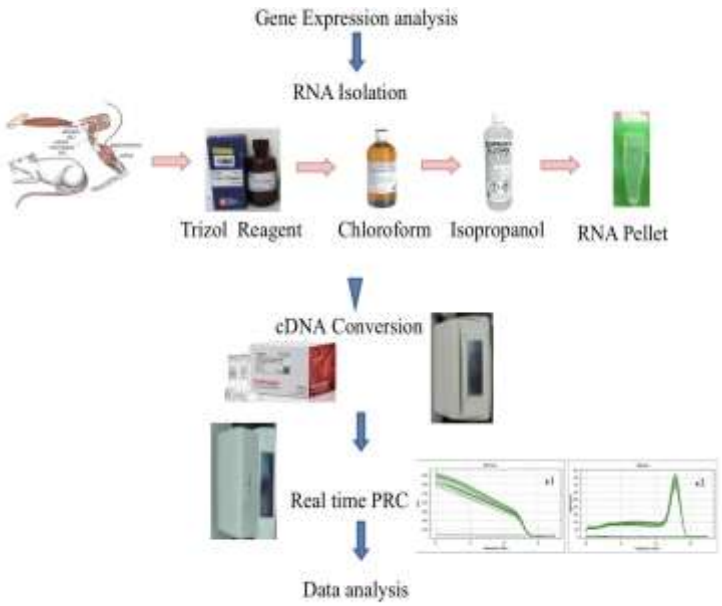



Figure 1: Summarisation of the activities done before data collection and analysis are made

## 3. RESULTS

**Effect of antioxidant vitamins (Vitamin C and E ) on fasting blood glucose and fasting serum insulin in PCB induced rats**

PCB exposure significantly raised fasting blood sugar levels and serum insulin concentration ($p<0.05$) when compared with control suggesting that PCB-administration causes hyperglycemia and hyperinsulinemia in experimental rats (Figure 1 & 2). However, Vitamin C and E administration for a period of 30 days, reduced hyper glycaemin and hyperinsulinemia significantly and this study clearly indicates that antioxidant vitamins play significant role in diabetes mellitus.

**Figure 1**

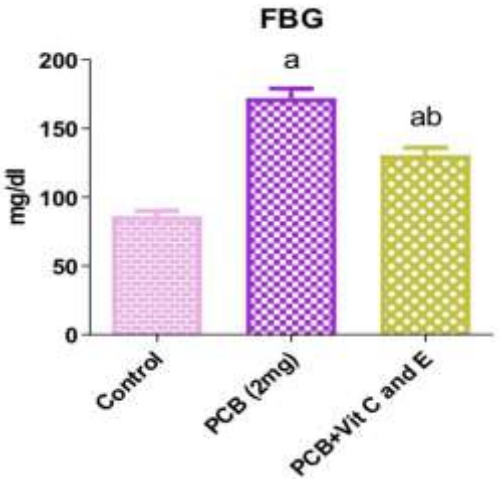



**Figure 2**

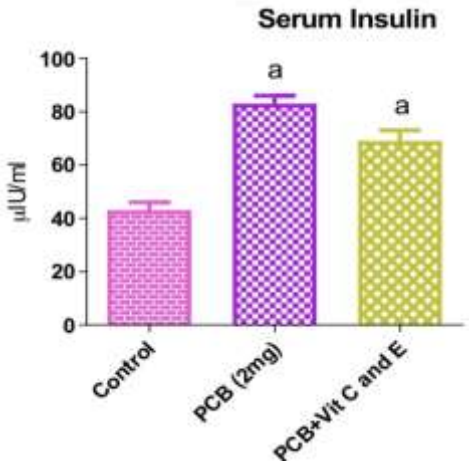



Fig. 1 & 2: . Effect of PCB and vitamins (C and E) supplementation on FBG and serum in sulin in experimental diabetic rats Each bar represents mean±SEM of six animals (n=6). Significance at p<0.05, a—compared with control, b—compared with PCB induced type-2 diabetic rats. X-axis indicates group while Y-axis represents concentration of glucose given as mg/dl concentration while serum insulin has been expressed as micro international unit per ml.

**Effect of antioxidant vitamins (Vitamin C and E ) on SREBP-1 concentration the gastrocnemius muscle of PCB induced rats**

SREBP 1c refers to sterol regulatory element binding protein and it is a transcription factor which helps in the regulation of genes in glycolysis pathway and Lipogenesis pathway. Here in this graph. The expression of SREBP1-c has been represented in terms of pg/ml.. In this study, SREBP1-c protein levels were found to be significantly ($p<0.05$) higher in PCB-induced rats compared to control rats and this might be due to the increased levels of glucose concentration due to PCB administration (Figure 3). However, administration of a combination of vitamin C & E reduced the concentration of SREBP1-c whose effects were found to be significantly equal to that of the healthy control rats.

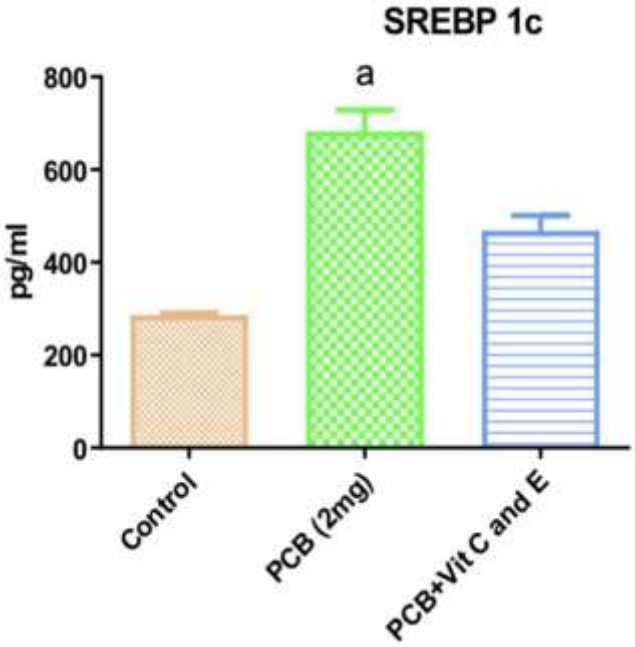
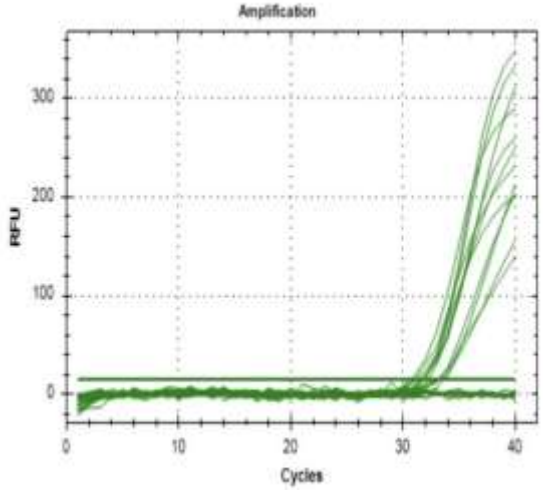



Fig. 3: . Effect of PCB and vitamins (C and E) supplementation on SREBP1-c protein levels in experimental diabetic rats Each bar represents mean±SEM of six animals (n=6). Significance at $p<0.05$, a—compared with control, b—compared with PCB induced type-2 diabetic rats. X-axis indicates group while Y-axis represents concentration of glucose given as mg/dl concentration while serum insulin has been expressed as micro international unit per ml.

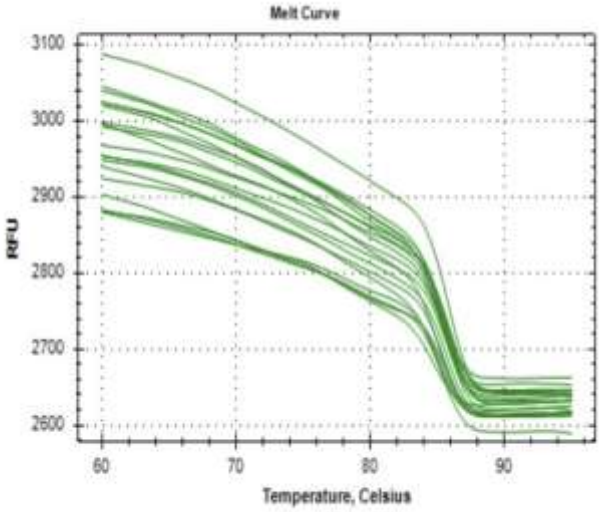
**Effect of antioxidant vitamins (Vitamin C and E ) on HNF-1 alpha mRNE expression in the gastrocnemius muscle of PCB induced rats**

HNF1A is a gene which acts as a switch that turns on and off other genes in the body. Changes in the HNF1A gene cause diabetes by lowering the amount of insulin that is produced by the pancreas. It allows insulin to be produced normally in childhood but the amount of insulin. HNF-1 alpha mRNA expression is represented in fold change over control. In this study, HNF-1 alpha mNRA expression was found to be significantly ($p<0.05$) reduced in PCB-induced rats compared to control rats and this might be due to the increased levels of HNF-1 alpha due to PCB administration (Figure4 A-C). However, administration of a combination of vitamin C & E activated and increased HNF-1 alpha mRNA significantly equal to that of the healthy control rats.

**A**


Amplification
RFU
300
200
100
0
0
10
20
30
40
Cycles

**B**


Melt Curve
RFU
3100
3000
2900
2800
2700
2600
60
70
80
90
Temperature, Celsius

**C**

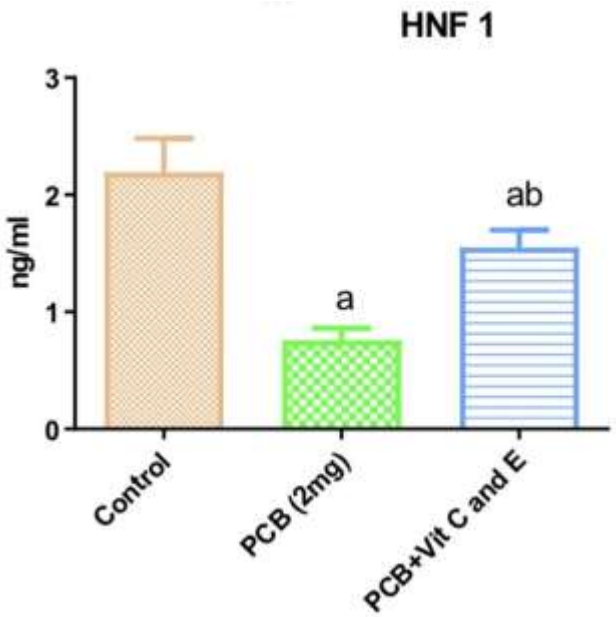



Fig. 4A-C: . Effect of PCB and vitamins (C and E) supplementation on HNF-1 alpha mRNA expression in the gastrocnemius muscle of experimental diabetic rats Each bar represents mean±SEM of six animals (n=6). Significance at $p<0.05$, a—compared with control, b—compared with PCB induced type-2 diabetic rats. X-axis indicates group while Y-axis represents concentration of glucose given as mg/dl concentration while serum insulin has been expressed as micro international unit per ml. Figure 4 A is showing amplification plots while Figure 4B is representing melt curve analysis of HNF-1 alpha expression.

## 4. DISCUSSION

In general a patient or a person is said to be diabetic if his / her blood glucose level is greater than 200 mg per dl and he or she is said to be prediabetic if his or her blood glucose level is In Between 140 to 199 mg per dl (32). He or she is said to be a normal person if his or her glucose level is below 140 mg per dl. In the same way we also have fasting blood glucose values in accordance with pre-diabetic , diabetic and normal. (33) The fasting blood glucose level of a normal person is less than 100 mg per dl and for a prediabetic patient it is between hundred to 140 mg per dl and for diabetic patients it is above 140 mg per dl.

Generally fasting blood glucose is calculated in a post prandial condition and the test is taken in morning hours before breakfast. AsPCB is induced in liver cells, blood glucose level increases, As we administer antioxidants C and E , FBG value drops to normal. SREBP 1c ( Sterol regulatory element binding protein ) It is a protein that in humans is encoded by SREBP 1 gene . (34). This gene is located within the Smith Magenis syndrome region on chromosome 17. SREBP 1c is mainly found in the liver and muscles. SREBP 1c Plays a key role in the induction of lipogenesis by liver. mTORC 1 is activated by insulin leading to increased production of SREBP 1c which facilitates storage of fatty acids as triglycerides (35). Insulin stimulated SREBP 1c increases glycolysis by activation of glucokinase enzyme and increases lipogenesis. In a similar trend with fasting blood glucose , steroid regulatory element binding protein 1C also showed an increased blood glucose on administration of Aroclor 1254 and later

decreased when administered with vitamin C and E (36).

Serum insulin regulates uptake and utilization of glucose and is also involved in protein synthesis and triglyceride synthesis. There's a condition called hyperinsulinemia which means the amount of insulin in your blood is higher than what is considered normal. (37). Hyperinsulinemia is associated with type 2 diabetes mellitus, as in accordance with SREBP 1c and FBG , Serum insulin levels also follows same trend , it gets higher on administration of PCB Aroclor 1254 but later it get reduced by the administration of antioxidants vitamin C and E. (37,38). Our team has extensive knowledge and research experience that has translate into high quality publications (39), (40), (41), (42), (43), (44,45), (46), (47), (48), (49).

A condition known as hyperinsulinemia occurs when there are excessive amounts of insulin circulating in the blood compared to the amount of glucose. Contrary to popular belief, hyperinsulinemia can be caused by a wide range of metabolic disorders, including diabetes, hyperglycemia, and non-nutritive carbohydrates in the diet. Although hyperinsulinemia is a common sign of type 2 diabetes mellitus in its early stages, it is not the root cause of the disorder. Only when pancreatic beta-cell activity is compromised does type 1 diabetes develop. Numerous disorders, including type 2 diabetes mellitus, neonatal hyperinsulinemia, and drug-induced hyperinsulinemia, can cause hyperinsulinemia. It can also manifest in nesidioblastosis, a kind of congenital hyperinsulinism.

## 5. CONCLUSION

Vitamins C and vitamin E can be used as a potential anti diabetic and antioxidant. Many diabetic patients have low levels of vitamin sea and vitamin E in their cells and could benefit from getting more vitamin C and vitamin E. This provided additional evidence on the beneficial effects of supplementing antioxidant vitamins on type two diabetes mellitus which could improve the clinical condition and prevent diabetic parthenogenesis. This research confirmed that subjects or patients with type two diabetes mellitus after supplementation of vitamins C and vitamin E demonstrated significantly lower levels of hypertension and decreased blood glucose level. In addition, to the best of our knowledge, we are the first to provide an evidence that antioxidant vitamins (Vitamin C and E) play a significant role in reducing diabetic complications at cellular levels by modulating the expression of HNF-1 alpha and SREBP1-c in the diabetic gastrocnemius muscle.

## FUTURE SCOPE OF RESEARCH

There is mounting evidence that antioxidant vitamins have anti-inflammatory and antioxidant properties. In the next five years vitamins C and vitamin E supplementation will become the standard care for diabetes.

**Conflict of Interest :**

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

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**Author Contribution :**

A) Pradeep Veerappan - contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.

B) Dr. Selvaraj - contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.

C) Dr.V.Vishnupriya - contributed in study design, guiding the research work, manuscript correction.

D) Dr. Gayathri R - study design, statistical analysis, manuscript proofreading and correction.

E) Dr. Kavitha S - study design, statistical analysis, manuscript proofreading and correction.

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