Effects of delta-tocotrienol supplementation on glycaemic control in individuals with prediabetes: A randomized controlled study
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Abstract
Objective: To study the effects of delta-tocotrienol on glycaemic control parameters in individuals with pre-diabetes.
Method: The randomised control trial was conducted at the Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from July 15 to November 15, 2019, and comprised individuals aged 18-60 years having fasting plasma glucose of 5.6 to 6.9 mmol/L or glycosylated haemoglobin of 5.7 to 6.4%. They were randomised into group A receiving 300mg delta-tocotrienol and group B receiving a placebo once daily for 12 weeks. Weight, height, waist circumference, fasting plasma glucose, insulin and glycosylated haemoglobin were measured at the beginning and end of the trial to assess any change. Body mass index and homeostatic model assessment-insulin resistance were also calculated. Data was analysed using SPSS 21.
Results: Of the 77 participants, 40(52%) were in group A and 37(48%) in group B. Group A showed significantly greater reduction in terms of fasting plasma glucose, glycosylated haemoglobin, insulin and homeostatic model assessment-insulin resistance index ($p<0.001$) post-intervention.
Conclusion: Delta-tocotrienol supplementation was found to have a significant effect in improving glycaemic control parameters in persons with pre-diabetes. Futures larger scale clinical trials are needed to confirm these findings.
Clinical Trial Number: SLCTR/2019/024.
Keywords: Delta-tocotrienol, Pre-diabetes, Supplementation, HbA1c, HOMA index. (JPMA 72: 4; 2022)
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Introduction
Pre-diabetes mellitus (PDM) is defined as a state of hyperglycaemia characterised by glycaemic parameters above normal but below the diagnostic cut-off for type 2 diabetes mellitus (T2DM). It comprises impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). It is an asymptomatic condition that leads to T2DM with yearly conversion rate of 5-10%. The worldwide prevalence of IGT is estimated at 316 million; a figure projected to increase to 471 million by 2035. The persons with PDM may develop T2DM in due course of time. According to the World Health Organisation (WHO) 2016 data, 422 million adults had diabetes in 2014. The increase was particularly in African and Asian countries. Diabetes country profile of Pakistan shows a prevalence of 10% and 9.7% in males and females respectively.

The pathogenesis of PDM involves insulin resistance (IR) primarily affecting the skeletal muscle, liver and adipose tissue. IR promotes hypersecretion of insulin from the β-cells of pancreas. Over time, insulin hypersecretion causes beta cell exhaustion that leads to hyperglycaemia.

The American Diabetes Association (ADA) diagnostic criteria defines PDM as IFG 100.8-124.2 mg/dl (5.6-6.9 mmol/L) and IGT 140.4-198 mg/dl (7.8-11 mmol/L) 2 hours after the ingestion of 75g of oral glucose load, or glycosylated haemoglobin (HbA1c) 5.7-6.4%.

At PDM stage, the abnormal glycaemic profile could be reverted to normal if timely intervention is started. Presently, the treatment options for PDM are scarce. Tocotrienols are naturally occurring subtypes of vitamin E. They consist of four homologs; alpha (α), beta (β), gamma (γ) and delta (δ). They are differentiated from each other by the position and degree of methylation. Although studies in humans remain scarce, a multitude of animal studies have shown that tocotrienol rich fraction (TRF), especially γ- and δ-tocotrienol, possess potent anti-diabetic properties. Thus γ- and δ-tocotrienol are shown to significantly improve fasting plasma glucose (FPG) and HbA1c by improving insulin sensitivity in animal models. In addition, they prevent systemic complications of obesity and diabetes by suppressing inflammation and oxidative stress (OS). However, no human study has assessed the anti-diabetic effects of δ-tocotrienol in PDM population.

The current study was planned to ascertain the effects of supplementation of 300mg δ-tocotrienol on glycaemic control parameters in PDMs.

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**Patients and Methods**

The randomised control trial (RCT) was conducted at the Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from July 15 to November 15, 2019, after approval from the institutional ethics review board and Clinical Trial was registered in the Sri Lanka Clinical Trials Registry (SLCTR/2019/024 SLCTR).

Those included were individuals of either gender aged 18-60 years fulfilling PDM diagnostic criteria. Cardiac patients, pregnant or breast-feeding women, critically ill patients, those suffering from acute stress/anxiety, severe renal, liver or respiratory disease, cancer patients, and those taking medication for glucose control or vitamins or dietary supplements were excluded. Written informed consent was obtained from each participant after which they were randomised into group A receiving 300mg delta-tocotrienol and group B receiving a placebo. It was done on the basis of a 1:1 ratio, and by using random draw method in which each participant was assigned a number on a piece of paper. These assigned numbers were randomly picked and added to one group or the other. The placebo group received a matched capsule containing inert compound. Intervention allocation was blinded for both the participants and the investigators during the intervention period. The participants were advised to take one capsule once daily after breakfast for 12 weeks. The δ-tocotrienol (American River Nutrition) was derived from annatto seeds and consisted of 90% and 10% δ-tocotrienol and γ-tocotrienol. All subjects in both the groups were also advised changes in lifestyle and exercises which equally affected all participants as participants might show improvement due to psychological and biological factors. Placebo was given for blinding and for minimising possible bias.

Demographic data and disease history were obtained. Body weight, waist circumference and height were measured using standard protocols. Body mass index (BMI) and HOMA index were determined using the standard formula.

Blood samples were taken at baseline and post-intervention from the peripheral vein following a 10-12 hour overnight fast. Whole blood was used for HbA1c analysis. The samples were processed at 3000xg for 10 minutes to isolate the serum/plasma for analysis of remaining parameters. FPG and HbA1c were analysed on ADVIA 1800 analyser (Siemens, USA). Serum insulin was measured on ADVIA Centaur XP analyser (Siemens, USA). The Primary outcome measures were mean reduction in FPG and HbA1c from baseline to 12 weeks, while secondary outcome measures consisted of reduction in serum insulin and HOMA-IR from baseline to post-intervention.

Since the trial was conducted as a pilot study, sample size was not calculated. However, equal number of participants were enrolled in each group based on the recommendations for pilot trial sample size justification. Data was analysed using SPSS 21. Kolmogorov-Smirnov test was applied to assess data normality. Variables with normal distribution were presented as mean±standard deviation (SD). Qualitative variables were expressed as frequencies and percentages. Paired sample t-test was used to test intra-group differences. An analysis of covariance (ANCOVA) model, adjusted for baseline value as covariate, was used to test inter-groups differences. P≤0.05 was considered statistically significant.

**Results**

Of the 126 volunteers screened, 90(100%) met the eligibility criteria and were randomised into two groups of 45(50%) subjects each. Of them, 77(85.5%) completed the study; 40(52%) in group A, and 37(48%) in group B (Figure). Demographic and biochemical parameters were comparable between the groups at baseline (Table 1).

Intra-group comparison showed significant mean reduction in group A in terms of FPG, HbA1c, insulin and HOMA index (p<0.05). In group B, the difference from baseline values was not significant (p>0.05).

Inter-group comparison showed reduction in FPG, HbA1c, insulin and HOMA index to be significantly greater in group A compared to group B (p<0.001). A pronounced effect on weight, BMI and waist circumference was also evident in group B (p<0.05), but not in group A (Table 2).

Figure: Study flow-chart.
various studies, both in humans and diet-induced glucose uptake in mouse adipocytes and primary human improved postprandial plasma glucose in rats fed on high-fat diet. Annatto-derived tocotrienol improved glucose FPG and insulin in mice fed a high-fat diet. 11
tocotrienol-enriched palm olein normalised FPG and The study demonstrated that δ- tocotrienol was effective in animal models of obesity. 11,13-15 In vitro experiments revealed that γ- tocotrienol restored insulin sensitivity and glucose uptake in mouse adipocytes and primary human adipose-derived stromal cells (hASCs). 11 Palm TRF and δ- tocotrienol-enriched palm olein normalised FPG and improved postprandial plasma glucose in rats fed on high-fat diet. Annatto-derived tocotrienol improved glucose utilisation following intraperitoneal glucose injection in mice fed on high-fat diet. 15 Also, γ- tocotrienol normalised FPG and insulin in mice fed a high-fat diet. 11

Previous studies on anti-diabetic effects of tocotrienols on humans produced heterogeneous results. Some studies demonstrated that tocotrienol supplementation improved glycemic control in diabetics. 16,17 Thus, tocotrienol-enriched canola oil supplementation to 45 diabetics resulted in reduced PFG levels. 16 Another RCT involving mixed tocotrienol supplementation showed an improved glycemic control in the patients with diabetes. 17 The current results support these findings.

In a randomised controlled crossover trial, 32 diabetics, supplemented with 1800mg of TRF or refined palm oil daily for 60 days, did not show any improvement in the glycaemic status or HbA1c levels. 18 Similarly, in another trial conducted on 19 diabetics with hyperlipidaemia, TRF treatment for 60 days did not result in a significant improvement in blood glucose and HbA1c. 19 In another study, palm oil TRF supplemented to 86 patients with diabetes for 8 weeks did not improve plasma glucose, serum insulin, HbA1c and HOMA index. 20 Our results appear to be inconsistent with these findings. The reason for this discrepancy in the results may be the purity and composition of the supplement used in these studies, indicating that only certain tocotrienol isoforms, like δ-tocotrienol, exhibit significant hypoglycaemic activity. 7

Mechanistically, tocotrienol improves glycemic control by enhancing insulin sensitivity. Tocotrienol enhances insulin sensitivity by restoring the expression of insulin receptor substrate 1, glucose transporter type 4, and Akt signalling in skeletal muscles. 21 Tocotrienol also enhances insulin sensitivity by up-regulating peroxisome proliferator-activated receptor-γ (PPAR). PPAR activation induces the expression of several genes involved in the insulin signalling, thereby improving insulin sensitivity. 22 Besides, tocotrienols also reduced OS and inflammation which could contribute to improvement in insulin sensitivity. 22

The current study has some limitations. First, the duration of the trial was too short to provide a complete assessment of the clinical utility of tocotrienol in PDM subjects. Second, the sample size was small, which decreases the generalisability of the results.

The study has its strengths as well. Few studies have used purified tocotrienols in the form of supplements to evaluate their exclusive effects. Numbers of human studies in this area are scarce, and most of the trials have been conducted on animals. To our knowledge, the present study was the first to ascertain the effects of δ-tocotrienol in humans with PDM.

The potential of δ-tocotrienol to improve glycaemic control needs to be studied with large-scale RCTs.

**Table-1: Baseline characteristics of the study participants (n=77).**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Tocotrienol (n=40)</th>
<th>Placebo (n=37)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24 (60)</td>
<td>20 (54)</td>
<td>0.598</td>
</tr>
<tr>
<td>Female</td>
<td>16 (40)</td>
<td>17 (46)</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>41.60±7.94</td>
<td>42.43±10.94</td>
<td>0.706</td>
</tr>
<tr>
<td><strong>Height (m)</strong></td>
<td>1.68±0.08</td>
<td>1.67±0.076</td>
<td>0.584</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>81.45±9.60</td>
<td>81.60±11.49</td>
<td>0.949</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>28.54±2.77</td>
<td>28.80±2.41</td>
<td>0.656</td>
</tr>
<tr>
<td><strong>WC (cm)</strong></td>
<td>85.07±6.89</td>
<td>86.23±8.11</td>
<td>0.499</td>
</tr>
<tr>
<td><strong>FPG (mmol/L)</strong></td>
<td>6.10±0.36</td>
<td>6.05±0.31</td>
<td>0.491</td>
</tr>
<tr>
<td><strong>HbA1c (%)</strong></td>
<td>13.61±2.56</td>
<td>13.84±2.28</td>
<td>0.679</td>
</tr>
<tr>
<td><strong>HOMA index</strong></td>
<td>3.70±0.79</td>
<td>3.74±0.77</td>
<td>0.822</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation (SD) or number and percentage (%); BMI: Body mass index; WC: Waist circumference; FPG: Fasting plasma glucose; HbA1c: Haemoglobin A1c; HOMA: Homeostatic Model Assessment; Statistical analysis was performed by independent t-test or chi-square test as appropriate.

**Table-2: Comparison of serum biochemical levels and metabolic factors at 12 week.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tocotrienol (n=40)</th>
<th>Placebo (n=37)</th>
<th><strong>p-value</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>81.45±9.60</td>
<td>81.02±9.42*</td>
<td>0.076</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>28.54±2.77</td>
<td>28.39±2.73*</td>
<td>0.062</td>
</tr>
<tr>
<td><strong>WC (cm)</strong></td>
<td>85.07±6.89</td>
<td>84.92±6.83*</td>
<td>0.061</td>
</tr>
<tr>
<td><strong>BP (syst.)</strong></td>
<td>117.79±5.53</td>
<td>117.14±5.82</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>BP (dias.)</strong></td>
<td>77.40±5.48</td>
<td>76.95±4.74</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>FPG (mmol/L)</strong></td>
<td>6.10±0.36</td>
<td>6.05±0.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>HbA1c (%)</strong></td>
<td>6.04±0.21</td>
<td>5.99±0.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Insulin (mIU/L)</strong></td>
<td>13.61±2.56</td>
<td>13.84±2.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>HOMA-IR</strong></td>
<td>3.70±0.79</td>
<td>3.74±0.77</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation (SD); BMI: Body mass index; WC: Waist circumference; BP (syst.): Blood pressure systolic; BP (dias.): Blood pressure diastolic; FPG: Fasting plasma glucose; HbA1c: Haemoglobin A1c; HOMA-IR: Homeostatic Model Assessment-insulin resistance; *p<0.05 vs. baseline (within the same group) by paired t-test; **p-value (between-groups) by analysis of covariance adjusted for baseline.

The supplements were well-tolerated and none of the participants reported any adverse effect.

**Discussion**

The study demonstrated that δ-tocotrienol was effective in improving descriptors of glycemic control in PDM subjects. These findings are similar to earlier results from various studies, both in humans and diet-induced animal models of obesity. 11,13-15 In vitro experiments revealed that γ-tocotrienol restored insulin sensitivity and glucose uptake in mouse adipocytes and primary human adipose-derived stromal cells (hASCs). 11 Palm TRF and δ-tocotrienol-enriched palm olein normalised FPG and improved postprandial plasma glucose in rats fed on high-fat diet. Annatto-derived tocotrienol improved glucose utilisation following intraperitoneal glucose injection in mice fed on high-fat diet. 15 Also, γ-tocotrienol normalised FPG and insulin in mice fed a high-fat diet. 11

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The potential of δ-tocotrienol to improve glycaemic control needs to be studied with large-scale RCTs.
Conclusion
Delta-tocotrienol supplementation at a dose of 300mg daily for 12 weeks resulted in favourable changes in glycaemic homeostasis in human volunteers with PDM.

Disclaimer: The text is based on an M.Phil thesis.

Conflict of interest: None.

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References