Original Article

Effect of Tocotrienol on Liver Enzymes, Fatty Liver and Liver Stiffness in People with Type 2 Diabetes and NAFLD : A Pilot Study Based on Biochemical and Transient Elastography Parameters

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Background and Aims : Very few studies are available on the effect of Tocotrienol on Non-alcoholic Fatty Liver Disease (NAFLD) in people with diabetes in India. Hence our aim was to study the effect of tocotrienol on elevated liver enzymes, fatty liver and liver stiffness in people with Type 2 Diabetes.

Methods : A pilot randomized interventional study was conducted in a Tertiary Care Centre for Diabetes in Chennai during July, 2018 to March, 2020. A total of 34 individuals were recruited based on the inclusion criteria (age <65 years, elevated liver enzymes and an ultrasound finding of fatty liver) and randomized into two groups, group 1 (n=17) (control group) and group 2 (n=17) treated with Tocotrienol and followed up for 3 months. The data was analysed using SPSS version 28.

Results : There was no difference in any of the parameters among the groups at baseline except the levels of fasting glucose and globulin. The tocotrienol group showed significant reduction in the levels of SGOT (baseline *versus* follow up; 30.6±10.7 *versus* 25.1±10.1: p=0.035), SGPT, (46(21,104) *versus* 26.5(16,84): p=0.020), GGT, (35.5(16,103) *versus* 31.5(13,106): p=0.012), liver stiffness, 10.1(7.4,17.0) *versus* 9.1(5.5,17.6): p=0.046) and fatty liver (291.2±25.9 *versus* 272.6±20.4: p=0.023) after the intervention. The control group did not show improvement in any of the parameters post-intervention. The comparison of effect of intervention between the groups were found to be similar.

Conclusion : There was an improvement in fatty liver, liver stiffness and also the levels of SGOT, SGPT and GGT in group treated with Tocotrienol compared to control group.

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Key words **: Type 2 Diabetes, Tocotrienol, NAFLD, Intervention, India.**

The prevalence of Non-alcoholic Fatty Liver Disease (NAFLD) in Asia and the Pacific countries is markedly increasing over the last decades compared to the western countries[1-4]. The prevalence of NAFLD in India varies from 9-32% in the general population and 44 to 72% in people with diabetes[5]. Vitamin E is an essential nutrient as the human body cannot produce on its own. It is fat soluble in nature and has an anti-oxidative and anti- inflammatory properties against chronic diseases[6-8]. Tocopherols and Tocotrienols are the major derivatives of the Vitamin E family. Tocotrienols has the high potential of neuroprotective, anti-cancer properties and also reduces plasma cholesterol levels[9]. Suzuki, *et al* elucidated that Tocotrienol that contains unsaturated side chain would allow more efficient penetration into the brain and liver tissues compared to tocopherols that have saturated fatty layers[10]. A review that compiled the different functions and properties of tocotrienols in animal and human studies showed that there was an improvement in liver function in NAFLD among people without diabetes[8]. The literature on effect of administration of Tocotrienols for NAFLD among people with diabetes is limited. Hence, our aim

Editor's Comment :

- Tocotrienol reduced liver enzymes significantly in people with type 2 diabetes and NAFLD.
- A marked improvement was also noted in liver stiffness and fatty liver in the participants who were on tocotrienol for 3 months.
- The findings can be confirmed for any further effectiveness by conducting a longitudinal study with a larger sample.

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was to study the effect of tocotrienol on liver enzymes, fatty liver and liver stiffness in people with Type 2 Diabetes and NAFLD.

Materials and Methods

This was an open-labelled pilot study conducted in a Tertiary Care Centre for Diabetes in Chennai during July, 2018 to March, 2020. This randomized interventional trial was approved by the Institutional Ethics Committee(IEC/N-003/04/2018). A written informed consent was obtained from all the study participants. A total of 34 participants were recruited based on the inclusion criteria and randomized into two groups, group1 (n=17) (control group without Tocotrienol) and group 2 (n=17), treated with Tocotrienol (200mg twice daily) and both the groups were followed up after 3 months. The inclusion criteria were age <65 years, HbA1c≤8.5%, presence of elevated liver enzymes and an ultrasound finding of fatty liver. Viral markers were done to rule out the presence of viral hepatitis and those with Hepatitis B and C and those with habit of alcohol consumption were excluded from the study. The baseline treatment was not changed during the follow up period. No medicine known to influence the liver pathology was administered at baseline or during the follow up. The socio-demographic details, duration of diabetes, anthropometric details and blood pressure were collected at baseline. The bio-chemical parameters such as fasting and postprandial glucose, HbA1c, Serum albumin, protein, globulin, SGOT(AST), SGPT(ALT), ALP, GGT were collected at baseline and after intervention. BP and BMI were also recorded at follow-up visit. Glycated haemoglobin (HbA1c%) was estimated by immuno- turbidimetric method and liver enzymes by standard enzymatic procedures using fully automated biochemistry analysers. Fibrotouch (FT-100) (Wuxi Hiski Medical Technologies Co, Ltd, China) a non-invasive fibrosis diagnostic system that works on a Transient Elastography technology was used to measure Liver stiffness and also the degree of liver steatosis[11]. A sample of the report generated on Liver stiffness and Fatty liver from Fibrotouch is given as supplement 1. The compliance of medication was ensured by making telephonic calls or at their hospital visit during the study period.

During the study period, 1 participant each from the control and Tocotrienol group had dropped out due to personal reasons. At the end of the follow up, a total of 32 patients, 16 patients in each group were included for analysis. The paired t - test/Wilcoxon signed rank test was used respectively for intra-group comparison. The difference was calculated from baseline to follow up for all the study variables. Student 't' test or Mann Whitney 'U' test was performed appropriately to compare the difference between the groups at baseline and also the effect of intervention using the difference from follow up to baseline using SPSS version 28.

Results

Table 1 shows the baseline characteristics of the study groups. At baseline, the study groups were matched for their age, duration of diabetes, BMI, blood pressure, post prandial glucose levels, HbA1c levels of serum proteins, liver enzymes, measures of liver stiffness and fatty liver except for mean fasting glucose level (group 1 *versus* group 2;138±33.6 *versus* 164±33.3; p=0.043) and the median (min, max) globulin level (group 1 *versus* group 2; 2.8(2.4,3.4) *versus* 3.0(2.6,4.3); p=0.014. Around 94% of the study participants in group 1 were on Oral Hypoglycaemic Agents (OHA) compared to 81% in group 2 and the difference between the groups was not statistically significant.

There were also no changes found in parameters such as weight, BMI, blood pressure, blood glucose levels, HbA1c, total bilirubin, total protein, albumin and globulin levels in both the groups after intervention (Table 2). The tocotrienol group showed significant reduction in the levels of mean SGOT(IU/L) (baseline

Table 1 — *Basic clinical characteristics of the study groups*

Variables	Group I (n=16) (Control)	Group II (n=16) (Tocotrienol)	p value
Age (in years)*	53.4±8.9	54.3±8.1	0.824
Duration of diabetes (in years)*	4.3±2.1	4.6±2.3	0.630
BMI (Kg/m^2)	26.4(22.3,38.5)	27.9(25.4,37.3)	0.051
Systolic BP(mm/Hg)	130(110,140)	125(100,160)	0.461
Diastolic BP (mm/Hg)	80(70,90)	80(60,90)	0.381
Glucose levels(mg/dl)* :			
Fasting	138±33.6	164±33.3	0.043
Postprandial	222.5±77.1	254.4±66.6	0.230
HbA1c(%)*	7.3±1.4	8.0±0.9	0.163
T Bilirubin (mg/dl)	0.9(0.3,1.7)	0.5(0.4,1.5)	0.052
T Protein (gm/dl)*	7.6±0.40	7.9±0.6	0.136
Albumin (gms/dl)	4.9(4.1,5.3)	4.7(4.3,5.1)	0.255
Globulin (gms/dl)	2.8(2.4,3.4)	3.0(2.6,4.3)	0.014
SGOT (IU/L)*	32.3±11.2	30.6±10.7	0.667
SGPT (IU/L)	44(17,98)	46(21,104)	0.880
ALP (IU/L)	207(139,309)	174(79,378)	0.052
GGT (IU/L)	34(20,59)	35.5(16,103)	0.227
Liver Stiffness (kPa)	10.3(6.2,20)	10.1(7.4,17.0)	0.836
Fatty liver (db/m)*	285.1±39.2	291.2±25.9	0.602
Medication details			
OHA	15(93.7)	13(81.2)	0.285
OHA + Insulin	1(6.3)	3(18.8)	

Values are in median (min, max); * - Mean ±SD

Table 2 — *Intra group comparison of basic clinical profile and liver function before and after intervention*

Variables	Group I (n= 16) Control			Group II (n= 16) Tocotrienol		
	Baseline	Follow up	p value	Baseline	Follow up	p value
Weight (kgs)	73.7(57.2,109.1)	72.3(62.4,108.0)	0.470	79.7(60.5,98.1)	79.0(59.7,99.8)	0.311
BMI (Kg/m^2)	26.4(22.3,38.5)	27.1(21.6,39.0)	0.826	28.0(25.4,37.3)	28.5(24.6,39.3)	0.087
Systolic BP(mm/Hg)	130(110,140)	130(100,140)	0.291	125(100,160)	120(110,160)	1.000
Diastolic BP (mm/Hg)	80(70,90)	80(70,90)	0.178	80(60,90)	80(70,100)	0.957
Glucose levels (mg/dl)*						
Fasting	138.6 ±34.6	142.4±45.5	0.658	164±33.3	151.6±29.7	0.118
Postprandial	222.6±79.7	214.2±59.9	0.637	254.4±66.6	231.2±50.9	0.218
HbA1c(%)*	7.4±1.4	7.2±1.1	0.537	8.0±0.9	7.5±1.0	0.093
T Bilirubin (mg/dl)	0.9(0.3,1.7)	0.9(0.3,1.5)	0.730	0.5(0.4,1.5)	0.5(0.3,1.4)	0.314
T Protein (gm/dl)*	7.6±0.40	7.6±0.41	0.348	7.9±0.60	7.8±0.54	0.404
Albumin (gms/dl)	4.9(4.1,5.3)	4.8(3.8,5.2)	0.731	4.7(4.3,5.1)	4.7(4.2,5.1)	0.361
Globulin (gms/dl)	2.8(2.4,3.4)	2.8(2.3,3.3)	0.683	3.0(2.6,4.3)	3.2(2.1,3.7)	0.153

Values are in Median (min, max); * - Mean ± SD

versus follow up; 30.6±10.7 *versus* 25.1±10.1: p=0.035), median SGPT(IU/L), 46(21,104) *versus* 26.5(16,84): p=0.020), median GGT(IU/L), 35.5(16,103) *versus* 31.5(13,106): p=0.012), median liver stiffness (kPa), 10.1(7.4,17.0) *versus* 9.1(5.5,17.6): p=0.046) and mean fatty liver(dB/m) (291.2±25.9 *versus* 272.6±20.4: p=0.023) after the intervention. It was noted that the level of ALP (IU/L) was increased in both the groups after intervention, but the difference was non-significant. The control group did not show improvement in any of the parameters post-intervention (Fig 1 - Panel A & Panel B). None of the study participants reported any adverse events during the study period.

The difference in the levels of all study parameters from baseline to follow up visits were calculated and compared the effect of intervention between the groups. The difference between the groups was similar although there was a relative improvement found in many of the test parameters in the Tocotrienol group at follow up (data not shown).

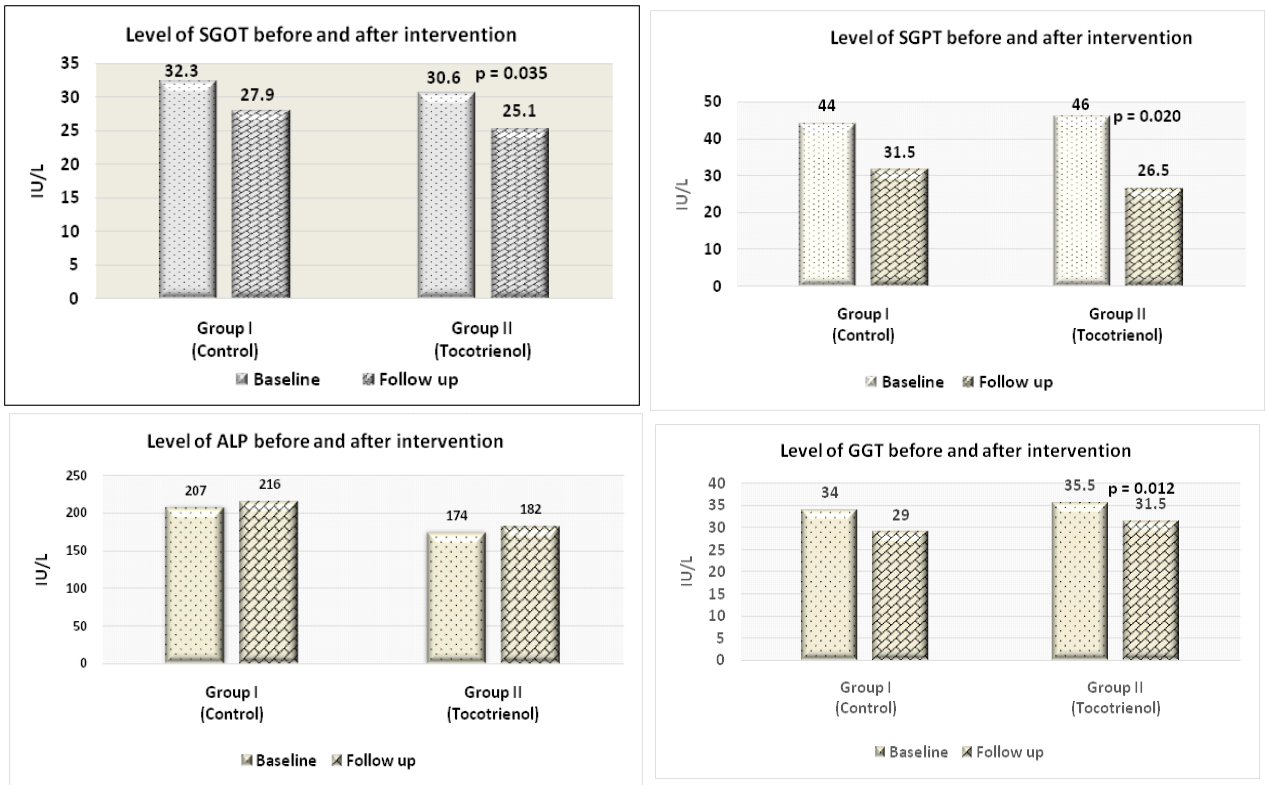

Fig 1 — Panel A: Levels of Liver enzymes before and after intervention

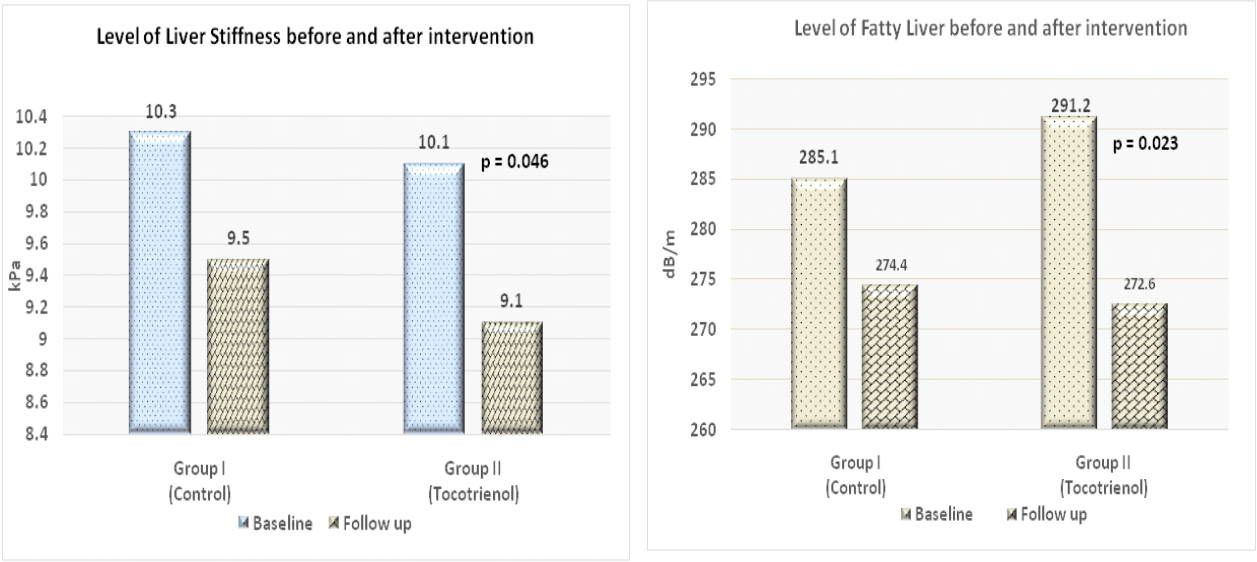

Fig 1 — Panel B: Measure of Liver Stiffness and Fatty Liver

Discussion

Our study findings highlighted that there was a significant reduction in liver enzymes and also measures of fatty liver and liver stiffness in patients who received tocotrienol for 3 months of follow up period. A randomized, double-blind, placebo-controlled study conducted in Pakistan showed significant reduction in the levels of SGOT, SGPT, GGT and ALP enzymes in the group that received d-tocotrienol compared to placebo[12] but ALP did not show any improvement in our study. A meta-analysis by Eder R, *et al* in 2019[13] reported that there was a significant decline in the SGPT level in people with NASH (n=61) who received 12 weeks' supplementation of Tocotrienol (200mg/twice daily).

In PIVENS trial[14], Vitamin E was found to be superior to placebo for the treatment of Non-alcoholic Steatohepatitis in adults without diabetes. There are very few studies conducted in assessing the effect of Vitamin E on NAFLD among people with diabetes. A three arm parallel-group, double-blind, randomized controlled study[15] was conducted among people who were having Type 2 Diabetes with NAFLD and followed them up for a period of 18 months. It showed reductions in SGOT and SGPT in both the intervention groups (that received a combination of pioglitazone(45mg/day) and Vitamin E 400IU b.i.d and only Vitamin E) compared to the placebo group. It also showed a significant improvement in steatosis in the intervention groups. But our findings revealed that there was no significant inter-group difference even though there was a fair reduction in Steatosis/fatty liver found in the tocotrienol group.

A multi-centric randomized double blinded placebo controlled study among children and adolescents having diabetes with NAFLD that observed the improvements occurred in ALT, histological features and resolution of NASH using 800 IU of Vitamin E in 58 patients, 1000mg of metformin in 57 patients and placebo in 58 patients. There was significant reduction in ALT in 24 weeks, 48 weeks in the Vitamin E group compared to placebo and metformin[16]. Madan, *et al*[17] compared lifestyle interventions, lifestyle interventions + UDCA and lifestyle interventions + UDCA + vitamin E in the management of NAFLD. They reported that the people who were in lifestyle interventions + UDCA + vitamin E group showed normal ALT levels and the difference was highly significant when compared to other two groups. Studies have shown both monotherapy of Vitamin E (tocopherol or tocotrienol) or combination of either or other agents improved the condition of NAFLD[18,19]. But the difference has been observed across the studies discussed in the method and design includes treatment allocation, randomization and duration of the study.

There are few limitations in the present study. First, it was pilot study with small sample size. Therefore, the findings have to be confirmed by further evaluating the effect of Tocotrienol in a larger sample. Second, the prolonged use of this may have beneficial or probable side effects which was not assessed in this study. Hence a longitudinal study will be planned to study the efficacy of Tocotrienol based on the current study.

Conclusion : In this pilot study, Tocotrienol was found to be effective in reducing liver enzymes and

showed improvement in fatty liver and liver stiffness. However, its' long term use needs to be evaluated for any further effectiveness.

Conflict of Interest : None

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