Tocotrienols: Exciting Biological and Pharmacological Properties of Tocotrienols and Naturally Occurring Compounds, Part II

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Abstract

δ-Tocotrienol plus AHA Step-1 diet in hypercholesterolemic subjects caused reductions in lipid parameters (14% to 18%) with 250 mg/d dose, and 500 mg/d resulted in induction in these parameters. Although, α-tocopherol is the most bioavailable form of vitamin E. There are few reports on bioavailability of tocotrienols in humans. Pharmacokinetics and bioavailability of δ-tocotrienol was quantified on plasma levels of tocol isomers, cytokines, and microRNAs. Subjects were fed doses of 125 mg/d to 500 mg/d. Plasma samples collected between 0 h to 10 h, levels of tocols estimated by HPLC, which resulted dose-dependent increases in AUC0-10, Cmax0-∞, Tmaxh, t1/2h, Cl-T 1/h, Vd/f, keh-1. Maximum plasma levels of δ-tocotrienol were at 3 h (125 mg/d to 250 mg/d), 6 h (500 mg/d). Effects of 32 compounds were evaluated on TNF-α secretion, nitric oxide production, and gene expression (TNF-α, IL-1β, IL-6, iNOS activity) in PPAR-α knockout mice. Anticancer activities of thiostrepton, dexamethasone, 2-methoxyestradiol, δ-tocotrienol, quercetin, amiloride, quinine sulfate showed significant anti-proliferative properties in Hela cells, pancreatic, prostate, breast, lungs, melanoma, B-lymphocytes, T-cells (40% to 95%). Results of plasma total mRNAs after δ-tocotrienol feeding to hepatitis C patients revealed significant down-regulated gene expression of pro-inflammatory cytokines. A mixture of δ-tocotrienol, resveratrol, vitamin D3 (NS-3) were given two capsules/d or cellulose/olive oil as placebo to individuals with T2DM (24-weeks). Significant down-regulation (15% to 74%) of gene expression in diabetes biomarkers and decreases in serum levels of fasting-glucose, HbA1c, hs-CRP, fasting-insulin, HOMA-IR, MDA (9% to 23%) were observed with NS-3 treated T2DM. Pure plasma mRNAs and miRNAs of pre-dose vs. post-dose of NS-3 treated samples were analyzed by Next Generation Sequencing (NGS). Venn diagrams have established genetic regulatory network images and canonical signaling pathways for mRNA, miRNA, and paired mRNA-miRNA.
Keywords

Tocotrienols; Resveratrol; Inflammation; Type 2 diabetes mellitus; Diabetes biomarkers & lipid parameters; mRNAs; miRNAs; Next generation sequencing

Introduction

My journey of studying tocotrienols has started thirty years ago, when I reported the isolation and biological function of α-tocotrienol as hypocholesterolemic agent from barley first time in 1986 as reported in part I [1,2]. This was acknowledged by late Byron J Richards and Dr. Barry Tan in their articles [3-5]. In part II, the remaining published results of papers 10 to 17 are summarized in vitro and in vivo studies on the impact of various isomers of tocotrienols (Figure 1) and other natural products on inflammation, cardiovascular, cancer, hepatitis C disease, Type 2 Diabetes (T2DM) and pharmacokinetics using several cell lines, experimental animal models and human subjects from 2011 to present day. Most of the information described here, are based on our published papers during last decade (2011–2021). All human studies (6 out of 8 papers in this article) were double-blind, Randomized, placebo-Controlled Trial (RCT). A non-probability convenience sampling technique was used. The protocol of each human study was registered with WHO regional office in Asia (World Health Organization Sri Lanka Clinical Trial Registry, Sri Lanka Center; srilanactr@gmail.com), after ethical approval by the Institutional Review Board of Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan. The registry number and date has been reported in each human study paper. The studies were carried out according to the guidelines provided by the United States Food and Drug Administration (FDA, 2003) at (AFIP), Rawalpindi, and National University of Medical Sciences, Rawalpindi, Pakistan. All participants of human studies have signed an informed consent form before start of the study. All papers were published in refereed journals.

Evaluation of Biological Properties, Pharmacokinetics, and Bioavailability of Tocotrienols in Humans

Palm tocotrienol rich fraction (TRF = mixture of tocopherols + tocotrienols) or rice bran TRF preparation low in α-tocopherol concentration (<20%) combined with AHA Step-1 diet were effective in lowering serum total cholesterol, LDL-cholesterol, and triglycerides levels in hypercholesterolemic humans [6-8]. The hypercholesterolemic subjects were administered increasing doses of δ-tocotrienol (125, 250, 500, 750 mg/d) plus AHA Step-1 diet for 4-weeks during 30-weeks study period [9]. The δ-tocotrienol plus AHA Step-1 diet caused significant reductions in lipid parameters in dose-dependent manner with maximum effects on total cholesterol (15%), LDL-cholesterol (18%), triglycerides (14%) with 250 mg/d dose and above 500 mg/d dose resulted induction in the levels of these lipid parameters, without affecting HDL-cholesterol (Figures 2A-2D) [9]. The cytokines associated with cardiovascular disease (plasma TNF-α, IL-2, IL-4, IL-6, IL-8, IL-10) were down-regulated 40% to 67% by δ-tocotrienol treatment (Table 1A). Similar results were obtained with gene expression of these cytokines using blood messenger-RNA (Table 1B) [9]. Circulating miRNA-7a, miRNA-15a, miRNA-20a (anti-angiogenic), miRNA-21, miRNA-21a, miRNA-21b.
miRNA-29a, miRNA-92a (skeletal muscle regeneration), miRNA-200, miRNA-206 were up-regulated by δ-tocotrienol treatment as compared to baseline in hypercholesterolemic subject values (Table 1C) [9]. These results confirmed that consumption of δ-tocotrienol plus AHA Step-1 diet causes significant reduction in serum lipid parameters and several cytokines at a lower concentration with optimum dose of 250 mg/d [9]. The capacity of δ-tocotrienol to modulate inflammation is partly attributable to dose-dependent properties of inhibition/activation, which may play a major role in future treatment of cardiovascular diseases [9].

It is well known that α-tocopherol is the most bioavailable form of vitamin E, but several animal and clinical studies have also demonstrated tocotrienols bioavailability to various tissues. It was also reported that the bio-discrimination of α-tocopherol (vitamin E) influences the rate of tocotrienol absorption, mainly due to high affinity of α-tocopherol with “α-Tocopherol Transfer Protein” (α-TTP), which mediates secretion of α-Tocopherol (100%) from the liver into the circulatory system and is much higher than α-tocotrienol (12%) or other tocotrienols [10,11]. There are few reports on bioavailability of tocotrienols in humans. Most studies were carried out with mixtures of tocotrienols + tocopherols rather than pure tocotrienols. Moreover, dietary α-tocopherol interferes with the bioavailability of tocotrienols and prevents absorption and delivery to organs and tissues [12,13]. Recently, Pharmacokinetics and bioavailability of Annatto-based δ-tocotrienol on plasma levels of α-, β-, γ-, δ-tocotrienol and tocopherols were quantified and in addition, several cytokines and microRNAs were also reported [14]. An open-label, randomized study was reported the pharmacokinetics and bioavailability of δ-tocotrienol in 33 healthy-fed subjects. In which, all subjects (11/dose) were randomly assigned to doses of 125, 250, or 500 mg/d. Plasma samples collected at 0, 1, 2, 3, 4, 6, 8, 10 h intervals and tocols (tocotrienols and tocopherols) were estimated by HPLC [14]. The results reported the effects of δ-tocotrienol on pharmacokinetic parameters of all eight isomers of tocol. Supplementation of 125, 250 and 500 mg/d doses of Annatto δ-tocotrienol have resulted in dose-dependent increases of (a) area under concentration-time curve (AUC_{0-t_{10}}, ng/ml) 2464, 5412, 14986; (b) maximum concentration (C_{max}, ng/ml) 829, 1920, 3278; (c) time to achieve maximum peak (T_{max}; h) 3, 3, 6; (d) elimination of half-life (t_{1/2} h) 1.74, 1.39, 2.54; (e) Time of Clearance (Cl-T, h^{-1}) 0.049, 0.045, 0.030; (f) volume of distribution (Vd/f, mg/h) 0.119, 0.114, 0.113; and (g) elimination rate constant (ke; h^{-1}) 0.412, 0.401, 0.265, respectively (Figures 3A-3D). Similar results were reported for other isomers of tocotrienols and tocopherols (Tables 2A-2D) [14]. Maximum plasma levels of δ-tocotrienol were observed at 3 h with doses of 125 and 250 mg/d, and 6 h with 500 mg/d. γ-Tocotrienol, β-tocotrienol, α-tocotrienol, and δ-tocopherol, γ-tocopherol, β-tocopherol, α-tocopherol were appeared in the plasma after 2 h (Tables 2A-2D) [14]. Moreover, δ-tocotrienol treatment resulted in down-regulation of eight cytokines and up-regulation of adiponectin, TGF-β1, and leptin (Table 2). The gene expression of miR-34a (increased in bipolar disorder) was down-regulated, but expression of miR-107, miR-122a, and miR-132 (decreased in Alzheimer’s disease) was up-regulated by δ-tocotrienol treatment (Table 3) [14]. These were the first results, which have described the effect of δ-tocotrienol on pharmacokinetics and bioavailability of all eight isomers of tocol. When tocotrienols are supplemented in absence of tocopherols, δ-tocotrienol has better bioavailability and δ-tocotrienol is converted stepwise to other
tocotrienols/tocopherols as shown in Figure 4 [14]. These results have supported that tocotrienol, particularly δ-tocotrienol, as a dietary supplement might be useful in the prevention of age-related and chronic ailments [14]. Tocotrienols lowered serum lipid parameters below 500 mg/d while increased lipid parameters at higher dose of 750 mg/d compared to 250 mg/d [9]. These results were further supported by our earlier findings of inhibition of chymotrypsin-like activity of 20S rabbit muscle proteasome with 40 μM of δ-tocotrienol and activation with 80 μM [15]. Thus δ-tocotrienol has a novel property of concentration-dependent inhibition and activation. Recently, the bioavailability of various doses of δ-tocotrienol in healthy fed participants plasma has been reported, which showed dose-dependent increases in Area Under the Curve (AUC), maximum Concentration (C\text{max}), and time to achieve maximum peak (T\text{max}) which varies between 3 h to 4 h for isomers of tocotrienols and 3 h to 6 h for isomers of tocopherols at 125, 250, 500 mg doses [14]. The results were also reported about the safety and impact of δ-tocotrienol after administering higher doses (750 mg/d and 1000 mg/d) to healthy subjects on various pharmacokinetic parameters [16]. All subjects (3/dose) were randomly assigned to one of each dose 750 mg/d or 1000 mg/d. Blood samples were collected, and tocols (tocopherols and tocotrienols) were quantified by HPLC of plasma collected at 0, 1, 2, 4, 6, 8 h intervals [16]. The plasma samples of doses 750 mg and 1000 mg resulted in the elution of all isomers of (α-, β-, γ-, δ-) tocotrienols and tocopherols for each time intervals (0 h to 8 h). The tocotrienols (ng/ml) present in 750 mg dose were β-tocotrienol (7838) > γ-tocotrienol (5055) > δ-tocotrienol (4045) α-tocotrienol (1389) (Table 4A). Whereas, for tocopherols were δ-tocopherol (13117) > γ-tocopherol (5544) > (β-tocopherol (3269) α-Tocopherol (1389) (Table 4A). Similar results were obtained with 1000 mg/d of δ-tocotrienol treatment (Table 4B) [16].

The consumption of 750 and 1000 mg/d of tocotrienols resulted in dose-dependent increases of plasma AUC\text{0–8} (ng/ml) 6621, 7450; AUC\text{0–∞}, 8688, 9633; AUMC\text{0–∞}, 52497, 57199; MRT, 6.04, 5.93; C\text{max}, (ng/ml) 1444, 1592; T\text{max}, 3.33 h to 4 h; Elimination of half-life (t\text{1/2}, h) 2.74, 2.68; Time of Clearance (Cl-T, 1/h) 0.086, 0.078; Volume of Distribution (Vd/f, mg/h) 0.34, 0.30; and elimination rate constant (ke; h\text{−1}) 0.25, 0.17 of δ-tocotrienol isomer as observed in (Table 5A, 5B) [16]. Similar results of these parameters were reported for δ-tocopherol, γ-tocopherol, (β-tocopherol except T\text{max} for α-Tocopherol was 6h [16]. These results indicated pharmacokinetics of higher doses of 750 mg and 1000 mg of δ-tocotrienol and confirmed that T\text{max} was 3 h to 4 h for all isomers tocol except α-Tocopherol (6 h). These higher doses of tocotrienols were found to be safe and might be useful for the treatments of various types of cancer, diabetes, and Alzheimer’s disease [16].

Inflammation has been implicated in cancer, diabetes and cardiovascular disease [17-19]. The important role played by lipopolysaccharides (LPS) in up-regulating inflammation is well-established [20]. LPS is expressed on the outer membrane of gram-negative bacteria, and induces several pro-inflammatory cytokines, such as Tumor Necrosis Factor-α (TNF-α), Interleukin-1β (IL-1β), IL-6, IL-8 and production of nitric oxide [20]. The 32 compounds of different categories of organic chemistry as shown in Table 6 were selected to find out potent inflammatory biomarkers. The Peroxisome Proliferator-Activated Receptor-α (PPAR-α) knockout female mice were selected for the study due to their different effects in LPS-induced macrophages of δ-tocotrienol, riboflavin, quercetin on secretion of TNF-α.
(activation) compared to corresponding wild type (C57BL/6) control (inhibition) group [21], and also due to the prolonged response to inflammatory stimuli [22]. Moreover, the PPARs mice contain nuclear receptors, which bind to fatty acid-derived ligands and activate the transcription of genes that govern lipid metabolism. The primary sites of activation of PPAR-α, which recognizes monounsaturated and polyunsaturated fatty acids and eicosanoids, are present in liver, heart, muscle, and kidney [23]. According to its role in regulating fatty acid metabolism, PPAR-α activates gene expression involved in fatty acid uptake (fatty acid binding protein), β-oxidation (medium chain acyl-CoA dehydrogenase, carnitine palmitoyl transferase I, and acyl-CoA oxidase), transport into peroxisomes (ATP-binding cassette transporters D2 and D3), and omega-oxidation of unsaturated fatty acids (cytochrome P-450, 4A1 and 4A3) [23]. Moreover, PPAR-α knockout mice also induce fatty acid catabolism and prevent hypertriglyceridemia, and its activation decreases glucose uptake, and causes a shift glucose use to fatty acid oxidation in cardiac muscle. Therefore, selective PPAR-α agonists that increase fatty acid catabolism without using lipid accumulation in the heart might be effective treatment for dyslipidemia [23]. The hypothesis was that compounds with those anti-inflammatory properties will be useful for treatment of diabetes, cardiovascular disease, and other diseases based on inflammation [23].

The study has reported the effects of 32 compounds of different chemical structures (Table 6) on TNF-α secretion, nitric oxide production, and gene expression of TNF-α, IL-1β, IL-6 and iNOS activity in lipopolysaccharide-induced thioglycolate-elicited peritoneal macrophages obtained from Peroxisome Proliferator-Activated Receptor-α (PPAR-α) knockout mice (that have prolonged response to inflammatory stimuli as mentioned earlier) [23]. There were decreases in chymotrypsin-like activity of 20S rabbit muscle proteasomes with thiostrepton, rifampicin, 2-hydroxyestradiol, 2-methoxyestradiol, 25-hydroxycholesterol, nicotinic acid, vitamin D₃, trans-resveratrol (35% to 68%), and decreases with (−) Corey lactone, ouabain, ampicillin, ascorbic acid, codeine, amiloride-HCL (138% to 168%) in 20S proteasomes (Figure 5, 6A) [23]. All these compounds inhibited TNF-α secretion (33% to 76%) in lipopolysaccharide-induced macrophages of C57BL/6 mice Wild Type; (Figure 6B). However, these compounds activated (127% to 190%), or inhibited secretion of TNF-α (48% to 78%), and production of nitric oxide (37% to 77%) in lipopolysaccharide-induced macrophages from PPAR-α knockout mice (Figure 6C, 6D) [23]. The gene expression of TNF-α, IL-1β, IL-6, and iNOS activity were consistent with results obtained for TNF-α protein and NO production as observed with macrophages of PPAR-α knockout mice (Figures 7A-7C). The possible mechanism for inhibition might be due to decreased proteolytic degradation of P-IκB protein, followed by decreased translocation of activated NF-κB, and depressed transcription of gene expression of TNF-α, and iNOS activity (Figures 7A-7C) [23]. These results have provided two sets of compounds, anti-inflammatory (control of diabetes and cardiovascular disease), and pro-inflammatory for the treatment of cancer and other diseases [23].

Cancer is second most common cause of death in the United State. There are over 100 different types of cancer associated with different human organs, predominantly breast, liver, pancreas, prostate, colon, rectum, lung, and stomach. The properties of pro-inflammatory (for treatment of various types of cancers), and anti-inflammatory (for cardiovascular disease and diabetes) compounds have been reported [17,18]. The major problem associated with
development of anticancer drugs is their lack of solubility in aqueous solutions and severe side effects in cancer patients. Therefore, the anticancer properties, anti-proliferative, and pro-apoptotic activity of novel naturally occurring, or FDA approved, nontoxic, proteasome inhibitors/activators, mostly aqueous soluble (Figure 5) were reported in cancer cell lines obtained from various organs [24]. The results of treatments of several compounds in cancer cell lines were found to be very effective in inducing apoptosis of cancer cells. Thiostrepton, dexamethasone, 2-methoxyestradiol, δ-tocotrienol, quercetin, amiloride, and quinine sulfate have significant anti-proliferation properties in Hela cells (44% to 87%) with doses of 2.5 μM to 20 μM, compared to respective controls (Table 7 and Figures 8(1-4) [24]. However, thiostrepton, dexamethasone, 2-methoxyestradiol, δ-tocotrienol, quercetin, and quinine sulphate were effective in pancreatic, prostate, breast, lungs, melanoma, B-lymphocytes, and T-cells (Jurkat: 40% to 95%) compared to respective controls (Table 7). In lung cancer cells, these compounds were effective between 5 μM to 40 μM (Table 7) [24]. The results of thiostrepton, 2-methoxyestradiol, δ-tocotrienol, and quercetin were very effective and induced apoptosis in the range of (70% to 92%) in Hela and liver cells. All these results were translated into possible IC₅₀ values of anticancer activities and IC₅₀ values of anti-proliferation properties of thiostrepton in most of these cell lines were between doses of 2.5 μM to 5 μM, dexamethasone 2.5 μM to 20 μM, lactone 40 μM to 80 M (Table 8) [24]. These results have demonstrated effectiveness of several natural-occurring compounds with anti-proliferative properties against cancer cells of several organs of humans. Thiostrepton, dexamethasone, 2-methoxyestradiol, δ-tocotrienol and quercetin are very effective for apoptosis of cancer cells in liver, pancreas, prostate, breast, lung, melanoma, B-lymphocytes, and T-cells. The results have provided an opportunity to test these compounds either individually or in combination as dietary supplements in humans for treatment of various types of cancers [24]. As mentioned earlier that δ-tocotrienol is a naturally occurring proteasome inhibitor, which has the capacity to inhibit proliferation and induce apoptosis in several cancer cells obtained from several organs of humans, and other cancer cell lines [24]. Moreover, results of plasma total mRNAs after δ-tocotrienol feeding to hepatitis C patients revealed significant inhibition in the expression of pro-inflammatory cytokines (TNF-α, VCAM1, proteasome subunits) and induction in the expression of ICAM1 and IFN-γ after post-treatment [25]. This down-regulation of proteasome subunits leads to autophagy, apoptosis of immune cells and several genes. The results reported RNA-sequence analysis of plasma total mRNAs obtained from δ-tocotrienol treatment of hepatitis C patients of pre-dose vs. post-dose on gene expression regulated by proteasome [25]. The data based on >1 and 8-fold expression changes of 2136 genes were fed into “Ingenuity Pathway Analyses (IPA)” for core analysis, which describes possible canonical pathways, upstream regulators diseases and functional metabolic networks [25]. The IPA of “molecules” indicated fold change in gene expression of 953 molecules, which covered several categories of biological biomarkers. Out of these, gene expression of 220 related to this study, 12 were up-regulated, and 208 down-regulated after δ-tocotrienol treatment (Table 9A, 9B). The gene expression of transcription regulators (ceramide synthase 3 and Mohawk homeobox) was up-regulated, and gene expression of 208 molecules was down-regulated, involved in several biological functions (HSP90AB1, PSMC3, CYB5R4, NDUFB1, CYP2R1, TNFRF1B, VEGFA, GPRR65, PIAS1, SFPQ, GPS2, EIF3F, GTPBP8, EIF4A1, HSPA14, TLR8, TUSSC2) [25]. IPA of “causal
network" indicated gene regulators (676), in which 76 down-regulated (26s proteasomes, interleukin cytokines, and PPAR-ligand-PPA-Retinoic acid-RXRα, PPARγ-ligand-PPARγ-Retinoic acid-RARα, IL-21, IL-23) with significant P-values (Table 9B) [25]. The IPA of “diseases and functions” regulators (85) were involved with cAMP, STAT2, 26S proteasome, CSF1, IFNγ, LDL, TGFA, and microRNA-155-5p, miR-223, miR-21-5p, and “upstream analysis” (934) showed 57 up-regulated (mainly 38 microRNAs) and 64 gene regulators were down-regulated (IL-2, IL-5, IL-6, IL-12, IL-13, IL-15, IL-17, IL-18, IL-21, IL-24, IL-27, IL-32), interferon β-1α, interferon γ, TNF-α, STAT2, NOX1, prostaglandin J2, NF-κB, IκB, TCF3, and also miRNA-15, miRNA-124, miRNA-218-5P with significant activation of Z-Score (P<0.05) [25]. The effect of δ-tocotrienol treatment to hepatitis C on “canonical pathways (360)” also described of only 33 in (Table 10) [25].

The important signaling pathway modulated by tocotrienols is “Eukaryotic translation Initiation Factors” (EIF2) signaling pathway at the top of the list (Table 10). This is involved in protein synthesis and requires many polypeptides. EIF2 is a GTP-binding protein, which initiates specific forms of met-tRNA onto the ribosome. Its important function is to deliver charged initiator met-tRNA to the ribosome, it also identifies the translational starting site [16,25]. Autophagy is a basic catabolic mechanism that involves cellular degradation of unnecessary or dysfunctional cellular components through the actions of liposome (Figure 9A) [26,27]. Autophagy is generally activated by condition of nutrient deprivation but has also been associated with physiological as well as pathological processes such as development, differentiation, neurodegenerative diseases, stress, infection, and cancer [27-29]. The mammalian Target of Rapamycin (mTOR) kinase is a critical regulator of autophagy induction, with activated mTOR (AKT and MAPK signaling) suppressing autophagy, and negative regulation of mTOR (AMPK and p53 signaling) promoting it [28]. The autophagy pathway (Figure 9A) highlights the key molecular events involved in triggering autophagy. Inhibiting the proteasome activity also causes the onset of autophagy, as observed with tocotrienol treatment. Apoptosis is a coordinated energy-dependent process that involves the activation of a group of cysteine proteases called caspases and a cascade of events that link the initiating stimuli to programmed cell death [29]. The two main pathways of apoptosis are the intrinsic and extrinsic pathways. Each pathway requires specific triggers to initiate a cascade of molecular events that converge at the stage of caspase-3 activation (Figure 9B) [30]. The activation of caspase-3 in turn triggers an execution pathway resulting in characteristic cytomorphological features including cell shrinkage, membrane blebbing, chromatin condensation and DNA fragmentation [30]. Further details of intrinsic and extrinsic pathways were found in the attached Ingenuity Apoptosis Signaling Pathway (Figure 9B), which highlights the key molecular events involved in triggering apoptosis. These are followed by protein ubiquitination, Toll-Like Receptor signaling (TLRs), Signal Transducers and Activators of Transcription (STATs), nuclear factor kappa B (NF-κB) transcription factors pathways play major roles in a variety of cellular processes, such as cell cycle, cell proliferation, apoptosis, DNA repair, transcriptional regulation, cell surface receptors, ion channels regulation have been discussed in several publications [31,32]. These results are consistent with these conclusions and δ-tocotrienol treatment of hepatitis C patients, acts by increasing cell death, and necrosis of malignant tumors, and by decreasing viral infection, cellular growth, and proliferation,
decreasing endocrine system disorders such as diabetes mellitus, and mobilization of
calcium. Therefore, tocotrienols can safely be used for hepatitis C patients, without any side
effects. This is first report describing RNA-sequence analysis of δ-tocotrienol treated plasma
total mRNAs obtained from chronic hepatitis C patients that acts via multiple-signaling
pathways without any side-effect. These studies may lead to development of novel classes of
drugs for the treatment of chronic hepatitis C patients [25]. Diabetes mellitus is a metabolic
disorder identified by hyperglycemia due to insulin resistance. Impaired serum/plasma
fasting glucose, HbA1c, hs-CRP are biomarkers, normally used to determine onset of
diabetes. δ-Tocotrienol, vitamin D3 and resveratrol (nutritional supplement-NS-3) are potent
anti-cholesterolemic, anti-oxidative and anti-inflammatory agents. It was hypothesized that
a mixture of δ-tocotrienol, vitamin D3 resveratrol (NS-3; Figure 5) will be more effective
treatment for reducing diabetes biomarkers as compared to its individual components in
people with type 2 Diabetes Mellitus (T2DM) [33]. Therefore, estimations of NS-3 mixture
and its individual components were carried out to test the hypothesis, on diabetes and
inflammatory biomarkers, using Peripheral Blood Mononuclear Cells (PBMC) obtained
from healthy, normal and people with T2DM. A randomized placebo controlled double-
blinded prospective trial of individual components (n=30/component), and NS-3 trial of
people with T2DM (n=56/group), were given two capsules/d of cellulose/olive oil as
placebo, individual components, or NS-3 mixture for 24-weeks [34]. There was significant
down-regulation (15 to 74) of gene expression with individual components and NS-3
mixture on diabetes biomarkers (IRS-1, SOD-2, GCKR, ICAM-1, VCAM-1, IL-6, IL-8)
in PBMCs of T2DM (Figure 10), and in serum values of fasting glucose (11%), HbA1c
(10%), hs-CRP (23%), fasting insulin (9%), HOMA-IR (20%), MDA (20%) of NS-3 treated
people with T2DM after 24-weeks (Table 11) [34]. Treatment with individual components
showed significant decreases but were less effective than the mixture (Table 12) [34].
The mixture and its components did not induce autophagy in these PBMC (Figure 10). RT-
PCR analysis of blood RNA obtained from NS-3 treated people with T2DM for 24-weeks
resulted down-regulation of gene expression in diabetes biomarkers (IRS-1, SOD-2, GCKR,
IGFPB-2) compared to pre-dose values [34]. Present results of in vitro and in vivo studies
have supported our hypothesis that NS-3 mixture is more effective in lowering serum levels
of several diabetes and inflammatory biomarkers including gene expression biomarkers
compared to its individual components in people with T2DM [34]. The results reported the
effectiveness of NS-3 on gene expression of mRNAs, miRNAs, and paired mRNA-miRNA
in people with T2DM [35], and this was an extension of a randomized placebo controlled
double-blinded clinical trial of T2DM (n=56/group) given two capsules/d of cellulose/olive
oil (placebo), or NS-3 for 24 weeks [34]. Pure mRNAs and miRNAs of plasma of pre-dose
versus post-dose of NS-3 treated samples were analyzed by Next Generation Sequencing
(NGS), and was analyzed by “Ingenuity Pathways Analyses (IPA)” [35]. A total of 4000
genes of miRNAs are considered significant, based on >2-fold gene expression changes. Out
of which 1373 genes are significantly differentially expressed in pre-dose vs. post-dose
samples, 20 are up-regulated and 27 are down-regulated of NS-3 treated miRNAs of
T2DM (Table 13A, 13B) [35]. Gene expression of up-regulated miR-29b-3p modulates
(GLUT4, insulin resistance), miR-624-5p (nephropathy biomarker), miR-361-5p (chronic
inflammation), miR-130a-3p (glucose metabolism, insulin secretion), miR-3912-3p (lipid
metabolism), and miR-11401 (cellular transcription). The miR-374c-5p (insulin resistance),
miR-4326 (HbA1c level), miR-874-3p (β-cell function) are down-regulated of NS-3 treated people with T2DM (Table 13A, 13B) [35]. Whereas gene expression of molecular functions of messengerRNAs (mRNAs), 42 are up-regulated, out of which mainly associated with ML-1621513 (oxidative/stress), mR-CTD-2349P217 (insulin-mediated glucose-uptake) are up-regulated and mR-CTC-246B1810 (β-cell/biology) (Table 14A). The 17 down-regulated gene expression of HBB functions as theranostic molecule, also as a hemoglobin glycation in people with T2DM, CTC-246B1810 is involved with several cytokines and β-cell biology in T2DM (Table 14B) [35]. The other important gene AGBL5-IT1 is associated CRISPR-clones for T2DM. The RN7SL698P gene expression plays role in many inflammatory T2DM cytokines and its complication in diabetes, and COX5BP7 modulate proper glycemic control in T2DM after NS-3 treatment (Table 14B) [35].

The molecules functions of paired mRNAs-miRNA are found fold changes in gene expression of up-regulated (38) with log ratios of 10.2–1.0 and down-regulated (4) with log ratios of −1.1–1.3 out of a total 1000 genes. The summary of paired mRNAs-miRNAs IPA analyses is described in 54 categories associated with diabetes (Table 15A, 15B). The functions of first ten genes are up-regulated (ZNF525, ZNF28, GNG10, NDUFB4, ORMDL1, S100B, BCKDHA, OXA1L, SBF1, RSU1) and four down-regulated (SET, RAB31, BRD4, KANK2) of paired mRNAs-miRNAs of molecular functions are also discussed further (Table 15A, 15B) [35]. All the above gene expression results are also described by Gene Oncology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and mRNA, miRNA, and paired mRNA-miRNA databases of pre-treatment vs. post-treatment groups [35]. Furthermore, all these results are supported by their heat map of miRNAs, in which up-regulated gene expression of pre-treatment were down-regulated after post-treatment as shown in Figure 11, whereas summary of various genomic functions of mRNAs of pre-treatment vs. post-treatment were up-regulated two-fold to three-fold of people with T2DM [35]. These results collectively identified 92 mRNAs that are up-regulated with negative correlation of 14 miRNAs (miR-3074-5p, miR-5481, miR-125a-5p, miR-374c-3p, miR-548-3p, miR-576-3p, miR-1292-5p, miR-296-5p, miR-1304-3p, miR-374-3p, miR-4326, miR-6513-3p, miR-5695, miR-4646-3p), which are down-regulated of post-treatment group of T2DM (Figure 12). It is clear from this Figure 12 that a single miRNA can regulate multiple targets of mRNAs, for example miRNA-5481 targets several mRNAs associated with T2DM (Figure 12) [35]. The interaction network of miR-29b-3p is generated using genes/molecules/pathways based on experimentally observed evidence of directly interacting with miR-29b-3p in people with T2DM. The molecules are organized according to their subcellular locations such as extracellular space, plasma membrane, cytoplasm, nucleus, or “other” category (Figure 13) [35]. The transcriptome expression data of network indicates red shades denote intensities of up-regulation, whereas green shades denote intensities of down-regulation of genes, and gray denote no change in post-treatment group compared with pre-treatment group. For example, LOXL2 enzyme coding gene has log2FC of −2.38 and has a darker green shade as compared to LAMC1 which has log2FC of only −0.19 and hence has lighter shade of green. Whereas the location of TUG1 is specified in “other” category (Figure 13). There were eighteen (18) red up-regulated genes (FAM3C, AGO2, PPM1D, FAM3C, SPARC, ANKHD1/ANKD1/EIF4, EBP3, TP53, MLF1, PURA, CNOT8, DNMT3A, PP1C, 2FP36L, HMGN3, MYBL2, TUBB2A, ZFP36L), four
(4) green down-regulated genes (LOXL2, COL1A2, LAMC1, GPR37) and seven (7) gray no change genes (COL5A2, SHFOOM2, TRIM9, DCP2, RERE, NAV5, HDAC4) [35]. In summary, all results of experimental design with respect to mRNA, miRNA, and paired mRNA-miRNA of IPA analyses data of gene expression profile of post-treatment has been described by Venn diagrams, incorporating network images and canonical pathways. The network images indicate 9 mRNA, 10 miRNA and overlap of 29 paired mRNA-miRNA (Figures 14A, 14B), indicating their functions in Tables 16A-16C [35]. The most specific network images relevant to present study are from mRNA category (RNA-trafficking, cell-mediated-immune-response, indfisease, lipid metabolism) and from miRNA (immunological disease, immune-cell-trafficking, and hematological-disease) as reported in Tables 16A-16C. Similarly, the Venn diagram of canonical pathways indicating 74 mRNAs, 23 miRNAs, and 174 paired mRNA-miRNA (Figure 14A, 14B), and list of all the pathways are listed in Tables 16A-16C [35]. The list of these pathways of mRNAs, miRNAs and paired mRNA-miRNA have confirmed earlier above reported results. In short, Venn diagrams have established genetic regulatory network images and canonical signaling pathways for mRNA, miRNA, and paired mRNA-miRNA of gene expression profiles of pre-dose vs. post-dose of NS-3 treatment group [35]. The NS-3 treatment of people with T2DM indicates up- or down-regulation of several new miRNAs (miR-29b-3p, miR-624-5p, miR-361-5p, miR-130a-3p, miR-3912-3p, miR-374c-5p, miR-1247-3p, miR-874-5p) which may be used to identify onset of T2DM. Overexpression of mRNA-AL1621513 indicates oxidative stress in people with T2DM, resulting in complications of diabetes (neuropathy, retinopathy, and stroke) [35].

Conclusions

These results confirm that consumption of δ-tocotrienol plus AHA Step-1 diet causes significant reduction in serum lipid parameters and several cytokines (TNF-α, IL-2, IL-4, IL-6, IL-8, IL-10) at a lower optimum dose of 250 mg/d. The capacity of δ-tocotrienol to modulate inflammation is partly attributable to dose-dependent properties of inhibition/activation, which may play a major role in future treatment of cardiovascular diseases. The effect of δ-tocotrienol on pharmacokinetics and bioavailability of all eight isomers of tocol indicated that when tocotrienols are supplemented in absence of tocopherols, δ-tocotrienol has better bioavailability, and δ-tocotrienol is converted stepwise to other tocotrienols/tocopherols. These results also support that tocotrienol, particularly δ-tocotrienol, as a dietary supplement might be useful in the prevention of age-related and chronic ailments. The pharmacokinetics of higher doses of 750 mg and 1000 mg of δ-tocotrienol have confirmed that T_max was 3 h to 4 h for all tocol isomers except α-tocopherol (6 h), and these higher doses of tocotrienols are found to be safe and might be useful for the treatments of various types of cancer, diabetes, and Alzheimer’s disease. The present results have provided two sets of compounds, anti-inflammatory (for the control of diabetes and cardiovascular disease), and pro-inflammatory for the treatment of cancer and other diseases. These results also demonstrate effectiveness of several natural-occurring compounds with anti-proliferative properties against cancer cells of several organs of humans. Thiostrepton, dexamethasone, 2-methoxyestradiol, δ-tocotrienol and quercetin are very effective for apoptosis of cancer cells in liver, pancreas, prostate, breast, lung, melanoma, B-lymphocytes,
and T-cells. The results have provided an opportunity to test these compounds either individually or in combination as dietary supplements in humans for treatment of various types of cancers. The results of fold-change expression data analyzed by “Ingenuity Pathway Analysis” describe the effect of δ-tocotrienol in chronic hepatitis C patients on biological mechanisms at molecular level. It also revealed an insight of correlation of signaling pathways and transcriptional factors. The collective results indicated that tocotrienols inhibit cancer cell proliferation, promotes cell cycle arrest, decreases angiogenesis and acts via multiple signaling pathways. These results clearly indicates that δ-tocotrienol treatment of hepatitis C patients, acts by increasing cell death, and necrosis of malignant tumors, and by decreasing viral infection, cellular growth, and proliferation, decreasing endocrine system disorders such as diabetes mellitus, and mobilization of calcium. Therefore, tocotrienols can safely be used for hepatitis C patients, without any side effects. These results of in vitro and in vivo studies support our hypothesis that NS-3 mixture is more effective in lowering serum levels of several diabetes and inflammatory biomarkers including gene expression markers compared to its individual components in people with T2DM. Moreover, the NS-3 treatment of people with T2DM indicates up- or down-regulation of several new miRNAs (miR-29b-3p, miR-624-5p, miR-361-5p, miR-130a-3p, miR-3912-3p, miR-374c-5p, miR-4326 [HbA1c], miR-1247-3p, miR-874-5p) which may be used to identify onset of T2DM. Overexpression of mRNA-AT1621513 indicates oxidative stress in people with T2DM, resulting in complications of diabetes (neuropathy, retinopathy, and stroke).

Acknowledgement

I thank my wife (of more than fifty years, Dr. Nilofer Qureshi) of tremendous support, encouragement, helpful discussion, motivation and editing the several manuscripts throughout my research. Also thanks to Arif A. Qureshi, Ari A. Qureshi, and Zoe N. Qureshi for preparing figures and editing the manuscripts. I thank Dr. Ronald Howard Lane (Ex CEO of Bioutrics, Inc. Phoenix Arizona; present Chairman and CEO of ImmunoRes Partners, LLC, Phoenix, AZ) for helpful discussion and financial support to set up state of the art laboratories at 8251 Raymond Road, Madison, Wisconsin. I also thank Dr. Winston A. Salser (Professor at UCLA, & founding President of Amgen), Dr. JJ Kim Wright (Director of Cardiovascular Division at Bristol Myer) and B.C. Pearce for very helpful discussion, collaborative research and for providing generous funds to carry out research in my laboratory for several years. I sincerely thanks to Dr. W.C. Burger, N. Prentice, D.M. Peterson (Barley and Mart Laboratory, Madison Wis), Vinod K. Chaudhary, F.E. Weber (Milwaukee Brewing Company, Milwaukee, Wis), Lesar Packer (Professor at University of California, CA), Prof. Dr. C.E. Elson (department of Nutrition, University of Wisconsin, Madison, Wisconsin) for helpful discussion and collaborative research. Dr. Betty Derees (Dean Emerita), and Christopher Papasian for appointment as Research Professor and salary at the Medical School, University of Kansas City, Missouri. I greatly value my association with Professor Dr. Dilshad A. Khan and his team (Drs. Wajiha Mahjabeen, Shahid Saleem, Shahida Mushfaq), Dr. Farooq A. Khan, and Saeed A. Sami to carry out most of human studies at Department of Chemical Pathology & Endocrinology Armed Forces Institute of Pathology & National University of Medical Science, Rawalpindi, Pakistan, and Dr. Basil A. Bradlow (Department of Pathology University of Illinois, Chicago, IL, 60612). Most of my research was possible due to the gift of large number of Annatto-tocotrienols capsules and 70% Annatto-tocotrienol mixture, also helpful suggestions of Dr. Barry Tan and Anne M. Trias of American River Nutrition Inc. Hadley, MA, USA. My sincere thanks to my colleagues, Drs. Suzanne G. Yu, David Morrison, Saira Khalid, Neerupma Silswal, Adeela Z. Siddiqui, Afnan M. Aladdad, Burale Suban, Julia Reis and Ghulam Nabi Kazi. I am also thankful to Dr. Paula Monaghan Nichols (Associate Dean of Research Administration, chairperson) for providing laboratory space at UMKC, School of Medicine, University of Missouri Kansas City, MO. 64108. I thank personals of Mayo Clinic, Rochester, MN., Mr. Chris P. Kolbert (Manager Research Operations), Mr. Robert A. Vierkant (Supervisor, section of Computational Genomics-Division of Biomedical Statistics and Informatic), Ms. Jane Kahl (Project Manager) for making arrangement of generating Venn diagrams and particularly, Ms. Mrunal K. Dehankar (Informatics Specialist) who has generated Venn diagrams for the manuscript.
Funding

The studies were funded in part by NIH funds 3452, 5RO1GM 102631, 3RO1 GN 631S1, RO1 GM 50870 (N. Qureshi), and by Advanced Medical Research (AMR), Madison, Wisconsin, 53719.

Abbreviations

| Abbreviation | Definition |
|--------------|------------|
| Tocol        | Mixture of Isomers of Tocotrienol and Tocopherol |
| T2DM         | Type 2 Diabetes Mellitus |
| CRP          | C-Reactive Protein |
| γ-GT         | γ- Glutamyltransferase |
| LPS          | Lipopolysaccharide |
| TNF-α        | Tumor Necrosis Factor-α |
| NO           | Nitric Oxide |
| NF-κB        | Nuclear Factor-kappa B |
| IFN-κB       | Interferon kappa B |
| iNOS         | Inducible Nitric Oxide |
| IL-4         | Interleukin-4 |
| IL-6         | Interleukin-6 |
| PBMC         | Peripheral Blood Mononuclear Cells |
| IRS-1        | Insulin Receptor Substrate-1 |
| SOD-2        | Superoxide Dismutase-2 |
| GCKR         | Glucokinase Regulators |
| IGFBP-2      | Insulin Like Factor Binding Protein-2 |
| IL-4         | Interleukin-4 |
| IL-6         | Interleukin-6 |
| iNOS         | Inducible Nitric Oxide |
| IPA          | Ingenuity Pathway Analysis |

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Figure 1:
Chemical structures of various isomers of tocotrienol and tocopherol.
Figures 2 A-D: Inhibitory effects of various doses of δ-tocotrienol plus AHA Step-1 diet on serum levels of lipid parameters in hypercholesterolemic subjects:
The treatments 1-8 correspond to six phases, and each phase lasted for 4-weeks: 1. baseline (n=31); 2, AHA Step-1 diet; 3, δ-tocotrienol-125 mg/d + AHA Step-1 diet; 4, δ-tocotrienol-250 mg/d + AHA Step-1 diet; 5, δ-tocotrienol-500 mg/d + AHA Step-1 diet; 6, δ-tocotrienol-750 mg/d + AHA Step-1 diet. Data are means ± SE (standard error). Values in a column not sharing a common symbol are significantly different at P<0.05.
Figures 3 A-D: Estimation of plasma peak concentration ($C_{\text{max}}$, ng/ml) of $\delta$-, $\gamma$-, $\beta$-, $\alpha$-tocotrienol of various doses:

The single dose of 125 mg, 250 mg, or 500 mg of $\delta$-tocotrienol was administered in one day to well-fed healthy subject (11/dose). The blood samples were collected in Ethylene Diamine Tetra Acetic acid (EDTA) glazed tubes at pre-dose (0 h) to post-dose 1, 2, 3, 4, 6, 8, 10 h intervals of each subject. The plasma samples were harvested and processed to carry out normal phase HPLC analyses of each subject as described in [14]. Values are means ± standard deviation ($n=11$/dose). Values are significantly different at $P<0.001$ from each other.
Figure 4: Stepwise conversion of δ-tocotrienol to α-tocopherol:
The δ-tocotrienol 125 mg was administered to well-fed subjects for pharmacokinetic study. After 2 h of consumption, δ-tocotrienol was appeared, which gives rise to γ-tocotrienol, β-tocotrienol, α-tocotrienol by successive C-methylation, and further leads to successive reduction to give rise to δ-tocopherol, γ-tocopherol, β-tocopherol, and α-tocopherol. The end-product is α-tocopherol (vitamin E).
Figure 5:
Chemical structures of various compounds used in the studies.
Figure 6A: Effects of selected compounds on chymotrypsin-like activity of 20S rabbit muscle proteasome:
The 20S rabbit muscle proteasome was treated with various compounds (100 mL) dissolved in 0.5% DMSO of 2. Lactacystin (5 μM); 3. (−) corey lactone (20 μM); 4. ouabain (20 μM); 5. thiostrepton (5 μM); 6. rifampicin (20 μM); 7. ampicillin (40 μM); 8. 2-hydroxyestradiol (40 μM); 9. 2-methoxyestradiol (80 μM); 10. 25-hydroxycholesterol (20 μM); 11. Acetylsalicylic acid (160 μM); 12. Ascorbic acid (10 μM); 13. Nicotinic acid (20 μM); 14. Vitamin D (40 μM); 15. trans-resveratrol (20 M) for 30 min. The proteolytic activity was measured by adding succinyl-Leu-Leul-Val-Tyr-amino methyl coumarin as substrate and fluorescence (absorption at 360 nm and emission at 460 nm) was measured by using Flx 800 microplate fluorescence reader. Data are average of triplicate analyses of each sample as ± SD (standard deviation). Percentage values of each treatment compared to control are at the top of the column. Values in a column not sharing a common asterisk are significantly different at \(*P<0.01; **P<0.001.\)
Figures 6B-D: Effects of selected compounds on the secretion of TNF-α or production of nitric oxide (NO) in LPS-induced thioglycolate-elicited peritoneal macrophages of 8-week-old C57BL/6, and PPAR-α knockout mice.

Thioglycolate-elicited peritoneal macrophages were prepared from 8-week-old female C57BL/6 (Wild Type), and PPAR-α knockout mice as described previously [23]. The macrophages of each mouse were treated with same 14 compounds as in Figure 6A. The TNF-α was assayed by using ELISA assay kit or assayed for production of nitric oxide by measuring the amount of nitrite using Griess reagent. Data are average of triplicate analyses of each sample as ± SD (standard deviation). Percentage values of each treatment compared to control are at the top of the column. Values in a column not sharing a common asterisk are significantly different at *P < 0.05; **P < 0.01; ***P < 0.001 [19].
Figures 7A - C: Effect of on gene expression of IL-1β, IL-6, or iNOS enzyme in LPS-induced thioglycolate-elicited peritoneal macrophages from 8-week-old PPAR-α knockout mice:
The procedures to quantitate gene expression of IL-1β, IL-6 or iNOS enzyme were exactly same as described in experiment section [23].
Figures 8 (1–4): Dose-dependent response for anti-proliferative properties of various compounds in cancer cells of Hela, liver, pancreas, and prostate.

The cancer cell lines of Hela, liver, pancreas, and prostate were maintained in DMEM supplemented with 10% heat inactivated FBS and 10 mg/mL gentamicin at 37°C in a humidified atmosphere with 5% carbon dioxide (CO2) and 95% oxygen (O2) as described previously [24]. Cancer cells (1 x 105) of various organs were seeded in 48 well tissue culture plate with 900 ml of medium containing 0.2% dimethyl sulfoxide of different types of cancer cell lines (Hela cell, liver, pancreas, and prostate), and incubated at 37 °C for 2 h. After 2 h, different concentrations (100 μl of 2.5, 5, 10, 20, 40, or 80 μM) of thiostrepton, ampicillin, dexamethasone, 2-methoxyestradiol, δ-tocotrienol, (−) riboflavin, ascorbic acid, quercetin, amiloride, and quinine sulphate in triplicate were added to each well, incubated for 48 h at 37 °C in a humidified atmosphere of 5% CO2. The anticancer properties and dose-dependence for eleven compounds are presented for Hela, liver, pancreas, and prostate cancer cell lines. Values in a column not sharing a common symbol are significantly different at $P < 0.001-0.05$ [24].
Figure 9A: Effect on autophagy in plasma of total mRNAs obtained from δ-tocotrienol treatment of hepatitis C patients:

The autophagy modulated by δ-tocotrienol treatment of hepatitis C patients: Autophagy is a general term for the basic catabolic mechanism that involves cellular degradation of unnecessary or dysfunctional cellular components through the actions of lysosome. Autophagy is generally activated by conditions of nutrient deprivation, but it has also been associated with physiological as well as pathological processes such as development, differentiation, neurodegenerative diseases, stress, infection, and cancer. The mammalian target of rapamycin (mTOR) kinase is a critical regulator of autophagy induction [25].
Figure 9B: Effect on apoptosis in plasma of total mRNAs obtained from δ-tocotrienol treatment of hepatitis C patients:

Apoptosis modulated by δ-tocotrienol treatment of hepatitis C patients. Apoptosis is a coordinated energy-dependent process that involves the activation of a group of cysteine proteases called caspases and a cascade of events that link the initiating stimuli to programmed cell death. There are two main pathways of apoptosis are the intrinsic and extrinsic pathways as shown here [25].
Figures 10 (1-6): Effect of a mixture of NS-3 and its components in vitro on diabetes biomarkers in PBMCs obtained from people with T2DM:

The peripheral blood mononuclear cells (PBMCs) obtained from people with T2DM, were added in each well (500,000 cells/well) in 96-well tissue culture plates and treated individually each compound and NS-3 mixture (10 μM of each; triplicate of each compound and mixture) as outlined in [34]. Data are means ± SD. Values in a column sharing a common symbol are significantly different at compared to †control, ‡P < 0.05, §P < 0.02, ¶P < 0.01, §§P <0.001 [34].
Figure 11: Effect on total RNAs of a mixture of NS-3 treated people with T2DM on heat map:
The molecular functions of miRNAs heatmap has provided very limited information, except those miRNAs which are up-regulated in pre-treatment are down-regulated significantly after post-treatment, such as (miRNA-548aq-3p, miR-1292-5p, miR-83, miR-54, miR-50, miR-48, miR-35, miR-33, miR-30, miR-25). Whereas miRNA-3912-3p, miR-548au-5p, miR-301a-3p were up-regulates after post-treatment of NS-3 of people with T2DM [35].
Figure 12: Effect of a mixture of NS-3 on interaction of biological functions of paired mRNA-miRNA in people with T2DM:
The interaction of paired mRNA-miRNA indicating gene expression of 92 mRNAs up-regulated have negative correlation with 14 miRNAs down-regulated and a single miRNA can regulate multiple target mRNAs after post-treatment of a mixture of NS-3 to people with T2DM [35].
Figure 13: The interaction network of miR-29b-3p and other miRNAs w/seed AGCACCA: The interaction network of miR-29b-3p was generated using genes/molecules/pathways based on experimentally observed evidence of directly interacting with miR-29b-3p in people with T2DM. There were eighteen (18) red up-regulated genes (FAM3C, AGO2, PPM1D, FAM3C, SPARC, ANKHD1/ANKD1/EIF4, EBP3, TP53, MLF1, PURA, CNOT8, DNMT3A, PP1C, 2FP36L, HMGN3, MYBL2, TUBB2A, ZFP36L), four (4) green down-regulated genes (LOXL2, COL1A2, LAMC1, GPR37) and seven (7) gray no change genes (COL5A2, SHFOOM2, TRIM9, DCP2, RERE, NAV5, HDAC4) [35].
Figure 14A: Summary of results of “network images” of experimental design with respect to mRNA, miRNA and paired mRNA-miRNA gene expression analysis based on Venn diagram: The network images showed mRNA (9), miRNA (10) and 29 overlap of paired mRNA-miRNA (29). The most specific network relevant to present study were from mRNA category (RNA-trafficking, cell-mediated-immune-response, inflammatory-disease, lipid metabolism) and from miRNA (immunological disease, immune-cell-trafficking, hematological-disease) as reported in Table 16B, C [35].
Figure 14B: Summary of results of “canonical pathways” of experimental design with respect to mRNA, miRNA and paired mRNA-miRNA gene expression analysis based on Venn diagram: Venn diagram of canonical pathways indicated mRNAs (74), 23 miRNAs (23), paired mRNA-miRNA (174), and list of all the pathways of mRNAs, miRNAs and paired mRNA-miRNA (Table 16A, B, C) has confirmed all the results have been described [35].
Table 1A:
Effects of δ-tocotrienol (250 mg/d) + AHA Step-1 diet on various cytokines in hypercholesterolemic subjects.

| #  | Cytokines | Baseline | AHA Step-1 diet | AHA Step-1 diet plus δ-Tocotrienol | Description | Functions |
|----|-----------|----------|----------------|----------------------------------|-------------|-----------|
| 1  | TNF-α     | 100      | 91.0 ± 1.41    | 48.5 ± 0.7 **                     | Tumor Necrosis | Produced during inflammation. |
| 2  | IL-2      | 100      | 94.0 ± 1.41    | 55.5 ± 0.71 ++                   | Interleukin-2 | for the growth, proliferation, |
| 3  | IL-4      | 100      | 93.0 ± 1.41    | 49.0 ± 1.41 ++                   | Interleukin-4 | Stimulation of activated B-cell |
| 4  | IL-6      | 100      | 98.0 ± 1.41    | 38.5 ± 2.21 ++                   | Interleukin-6 | Regulates immune response,    |
| 5  | IL-8      | 100      | 85.5 ± 2.12    | 43.5 ± 0.71 ++                   | Interleukin-8 | Potent angiogenic factor       |
| 6  | IL-10     | 100      | 92.5 ± 2.02    | 63.5 ± 2.12 ++                   | Interleukin-10| Immunoregulation & inflammation |

Table 1B: Effects of δ-tocotrienol (250 mg/d) + AHA Step-1 diet on various gene expressions in hypercholesterolemic subjects.

| Gene Expression | Baseline | AHA Step-1 diet | AHA Step-1 diet plus δ-Tocotrienol | Description | Functions |
|----------------|----------|----------------|----------------------------------|-------------|-----------|
| #  | Cytokines |           |                                  |             |           |
| 1  | TNF-α     | 100      | 84.5 ± 0.71 **                | Tumor Necrosis Factor-α | Inflammation |
| 2  | IL-2      | 100      | 91.5 ± 3.54 *                | Interleukin-2 | Cytokine (Proliferation, & differentiation). |
| 3  | IL-4      | 100      | 77.5 ± 2.12 **               | Interleukin-4 | Stimulation of activated B-cell & T-cell proliferation |
| 4  | IL-6      | 100      | 73.5 ± 0.71 **               | Interleukin-6 | NF-κB and IL-6 signaling. |
| 5  | IL-8      | 100      | 92.0 ± 2.83 *                | Interleukin-8 | Chemokine (involved in angiogenesis). |
| 6  | IL-10     | 100      | 89.0 ± 1.41 *                | Interleukin-10| Immunoregulation and inflammation |

Table 1C: The effect of δ-tocotrienol (250 mg/d) + AHA Step-1 diet on plasma miRNAs of cardiovascular disease in hypercholesterolemic subjects.

| #  | MicroRNA = miRNA | Baseline | AHA Step-1 diet | AHA Step-1 diet plus δ-Tocotrienol |
|----|------------------|----------|----------------|----------------------------------|
| 1  | miRNA-7a         | 100      | 103.5 ± 2.12 * | 168.0 ± 1.41 **                  |
| 2  | miRNA-15a        | 100      | 107.6 ± 0.71 * | 179.0 ± 1.41 **                  |
| 3  | miRNA-20a        | 100      | 102.5 ± 0.71   | 168.00 ± 2.24 **                 |
| 4  | miRNA-21         | 100      | 108.0 ± 2.83 * | 143.0 ± 2.83 **                  |
Table 1C: The effect of δ-tocotrienol (250 mg/d) + AHA Step-1 diet on plasma miRNAs of cardiovascular disease in hypercholesterolemic subjects.

| #  | MicroRNA = miRNA | Baseline | AHA Step-1 diet | AHA Step-1 diet plus δ-Tocotrienol |
|----|-------------------|----------|-----------------|----------------------------------|
| 5  | miRNA-29a         | 100      | 102.5 ± 0.71    | 142.0 ± 2.83 **                  |
| 6  | miRNA-92a         | 100      | 106.5 ± 2.12 *  | 153.5 ± 2.12 **                  |
| 7  | miRNA-200         | 100      | 104.0 ± 1.41    | 146.0 ± 1.41 **                  |
| 8  | miRNA-206         | 100      | 109.0 ± 2.83 *  | 150 ± 2.83 **                   |

* X ± SD (mean ± standard deviation)

**Values in a row sharing a common symbol are significantly different

* P<0.05

** P<0.01
Table 2:
Pharmacokinetic parameters after feeding single dose of various concentrations of δ-tocotrienol (125 or 250 or 500 mg) in one day.

| Table 2a: | δ-Tocotrienol |  | γ-Tocotrienol |  |
| --- | --- | --- | --- | --- |
| # | A: 125 mg | 250 mg | 500 mg | B: 125 mg | 250 mg | 500 mg |
| 1 | Area Under Curve-0 - 10 (AUC\textsubscript{0-10}; ng/mL) | 2463.91 ± 191.62\textsuperscript{a} | 5412.50 ± 274.04\textsuperscript{b} | 14985.73 ± 362.63\textsuperscript{c} | 1258.18 ± 126.26\textsuperscript{a} | 5412.50 ± 274.04\textsuperscript{b} | 6895.96 ± 159.49\textsuperscript{c} |
| 1 | Area Under Curve-0 - ∞ (AUC\textsubscript{0-∞}; ng/mL) | 2886.41 ± 201.01\textsuperscript{a} | 5514.75 ± 287.01\textsuperscript{b} | 17111.94 ± 444.71\textsuperscript{c} | 1647.95 ± 270.72\textsuperscript{a} | 5514.75 ± 287.01\textsuperscript{b} | 7818.82 ± 397.38\textsuperscript{c} |
| 2 | Plasma Peak Concentration (C\textsubscript{max}; ng/mL) | 828.82 ± 24.28\textsuperscript{a} | 1920.36 ± 57.99\textsuperscript{b} | 3278.00 ± 124.13\textsuperscript{c} | 281.34 ± 21.22\textsuperscript{a} | 833.73 ± 28.22\textsuperscript{b} | 1224.64 ± 61.28\textsuperscript{c} |
| 3 | Time to achieve plasma peak (T\textsubscript{max}; h) | 3.00 | 3.00 | 4.00 | 3.00 | 3.00 | 3.00 |
| 4 | Elimination of Half-life time (t1/2; h) | 1.74 ± 0.36\textsuperscript{a} | 1.39 ± 0.22\textsuperscript{a} | 2.54 ± 0.05\textsuperscript{b} | 3.82 ± 0.99\textsuperscript{a} | 1.39 ± 0.28\textsuperscript{a} | 2.25 ± 0.32\textsuperscript{c} |
| 5 | Time of clearance (Cl-T; L/h) | 0.049 ± 0.004\textsuperscript{a} | 0.045 ± 0.002\textsuperscript{a} | 0.030 ± 0.001\textsuperscript{b} | 0.078 ± 0.012\textsuperscript{a} | 0.045 ± 0.002\textsuperscript{a} | 0.061 ± 0.010\textsuperscript{b} |
| 6 | Apparent volume of distribution (Vd/f) | 0.179 ± 0.035\textsuperscript{a} | 0.114 ± 0.011\textsuperscript{b} | 0.613 ± 0.102\textsuperscript{c} | 0.553 ± 0.084\textsuperscript{a} | 0.461 ± 0.114\textsuperscript{a} | 0.635 ± 0.066\textsuperscript{b} |
| 7 | Elimination rate constant (ke; h\textsuperscript{-1}) | 0.381 ± 0.059\textsuperscript{a} | 0.401 ± 0.039\textsuperscript{b} | 0.050 ± 0.008\textsuperscript{c} | 0.113 ± 0.026\textsuperscript{a} | 0.133 ± 0.037\textsuperscript{a} | 0.097 ± 0.021\textsuperscript{b} |

Table 2b:

| Table 2b: | β-Tocotrienol | α-Tocotrienol |  |
| --- | --- | --- | --- |
| # | C: 125 mg | 250 mg | 500 mg | D: 125 mg | 250 mg | 500 mg |
| 1 | Area Under Curve-0 - 10 (AUC\textsubscript{0-10}; ng/mL) | 6933.73 ± 129.58\textsuperscript{a} | 7080.36 ± 206.62\textsuperscript{a} | 7680.41 ± 272.59\textsuperscript{b} | 869.96 ± 43.95\textsuperscript{a} | 1369.91 ± 26.30\textsuperscript{b} | 1900.68 ± 46.29\textsuperscript{c} |
| 1 | Area Under Curve-0 - ∞ (AUC\textsubscript{0-∞}; ng/mL) | 8839.28 ± 656.29\textsuperscript{a} | 9184.14 ± 674.76\textsuperscript{a} | 10391.37 ± 621.69\textsuperscript{b} | 1041.77 ± 108.82\textsuperscript{a} | 1558.09 ± 77.13\textsuperscript{b} | 2290.14 ± 65.53\textsuperscript{c} |
| 2 | Plasma Peak Concentration (C\textsubscript{max}; ng/mL) | 979.00 ± 79.45\textsuperscript{a} | 1083.73 ± 82.26\textsuperscript{b} | 1279.00 ± 116.44\textsuperscript{c} | 139.91 ± 11.03\textsuperscript{a} | 204.91 ± 9.47\textsuperscript{b} | 290.09 ± 9.84\textsuperscript{c} |
| 3 | Time to achieve plasma peak (T\textsubscript{max}; h) | 4.00 | 3.64 ± 0.41 | 3.00 | 2.73 ± 0.65 | 3.00 | 3.00 |
| 4 | Elimination of Half-life time (t1/2; h) | 3.92 ± 0.86\textsuperscript{a} | 3.90 ± 0.79\textsuperscript{a} | 4.39 ± 0.63\textsuperscript{a} | 2.62 ± 0.81\textsuperscript{a} | 2.89 ± 0.84\textsuperscript{a} | 3.71 ± 0.29\textsuperscript{b} |
| 5 | Time of clearance (Cl-T; L/h) | 0.094 ± 0.007\textsuperscript{a} | 0.090 ± 0.007\textsuperscript{a} | 0.080 ± 0.005\textsuperscript{b} | 0.303 ± 0.135\textsuperscript{a} | 0.161 ± 0.008\textsuperscript{b} | 0.109 ± 0.003\textsuperscript{c} |
| 6 | Apparent volume of distribution (Vd/f) | 0.174 ± 0.011\textsuperscript{a} | 0.223 ± 0.013\textsuperscript{b} | 0.415 ± 0.019\textsuperscript{c} | 0.590 ± 0.061\textsuperscript{a} | 0.922 ± 0.067\textsuperscript{b} | 1.365 ± 0.045\textsuperscript{c} |
### Table 2c:

| #     | A: 125 mg | 250 mg | 500 mg | B: 125 mg | 250 mg | 500 mg |
|-------|-----------|--------|--------|-----------|--------|--------|
| 1     | n/a       | n/a    | n/a    | n/a       | n/a    | n/a    |
| 2     | n/a       | n/a    | n/a    | n/a       | n/a    | n/a    |
| 3     | n/a       | n/a    | n/a    | n/a       | n/a    | n/a    |
| 4     | n/a       | n/a    | n/a    | n/a       | n/a    | n/a    |
| 5     | n/a       | n/a    | n/a    | n/a       | n/a    | n/a    |
| 6     | n/a       | n/a    | n/a    | n/a       | n/a    | n/a    |
| 7     | n/a       | n/a    | n/a    | n/a       | n/a    | n/a    |
| 8     | n/a       | n/a    | n/a    | n/a       | n/a    | n/a    |

### Table 2d:

| #     | C: 125 mg | 250 mg | 500 mg | D: 125 mg | 250 mg | 500 mg |
|-------|-----------|--------|--------|-----------|--------|--------|
| 1     | n/a       | n/a    | n/a    | n/a       | n/a    | n/a    |
| 2     | n/a       | n/a    | n/a    | n/a       | n/a    | n/a    |
| 3     | n/a       | n/a    | n/a    | n/a       | n/a    | n/a    |
| 4     | n/a       | n/a    | n/a    | n/a       | n/a    | n/a    |
| 5     | n/a       | n/a    | n/a    | n/a       | n/a    | n/a    |
| 6     | n/a       | n/a    | n/a    | n/a       | n/a    | n/a    |
| 7     | n/a       | n/a    | n/a    | n/a       | n/a    | n/a    |
| 8     | n/a       | n/a    | n/a    | n/a       | n/a    | n/a    |

### Notes:

- Values in a row not sharing a common letter are significantly different at P<0.001 - 0.01.
Table 3:
Plasma miRNAs of δ-tocotrienol at 0 h to 3 h (125 mg) and 0 h to 6 h (500 mg) of pharmacokinetic study in humans.

| miRNA   | 0-h (125 mg) | 3-h (125 mg) | 0-h (500 mg) | 6-h (500 mg) |
|---------|--------------|--------------|--------------|--------------|
|         | Percentages  | Percentages  | Percentages  | Percentages  |
| A       | Inflammation |              |              |              |
| 1       | miR-9        | 100          | 88           | 100          | 44           |
| 2       | miR-34a      | 100          | 72           | 100          | 59           |
| 3       | miR-107      | 100          | 156          | 100          | 173          |
| 4       | miR-122a     | 100          | 166          | 100          | 196          |
| 5       | miR-132      | 100          | 199          | 100          | 145          |
| 6       | miR-148a     | 100          | 208          | 100          | 233          |
| 7       | miR-181a     | 100          | 48           | 100          | 21           |
| B       | Cardiovascular |            |              |              |
| 8       | miR-24       | 100          | 77           | 100          | 45           |
| 9       | miR-19b      | 100          | 91           | 100          | 70           |
| C       | Cancer       |              |              |              |
| 10      | miR-1        | 100          | 78           | 100          | 63           |
| 11      | miR-7        | 100          | 94           | 100          | 85           |
| 12      | miR-15b      | 100          | 110          | 100          | 132          |
| 13      | miR-17-5p    | 100          | 106          | 100          | 168          |
| 14      | miR-19a      | 100          | 95           | 100          | 36           |
| 15      | miR-26a      | 100          | 80           | 100          | 62           |
| 16      | miR-106a     | 100          | 74           | 100          | 56           |
| 17      | miR-143      | 100          | 63           | 100          | 36           |
| 18      | miR-145      | 100          | 54           | 100          | 44           |
| 19      | miR-182      | 100          | 76           | 100          | 64           |
| 20      | miR-192      | 100          | 28           | 100          | 21           |
| 21      | miR-194      | 100          | 50           | 100          | 21           |
| 22      | miR-196a     | 100          | 65           | 100          | 43           |
| 23      | miR-199a     | 100          | 81           | 100          | 65           |
| miRNA  | 0-h (125 mg) | 3-h (125 mg) | 0-h (500 mg) | 6-h (500 mg) |
|--------|-------------|-------------|-------------|-------------|
| miR-204| 100         | 45          | 100         | 41          |
| miR-205| 100         | 39          | 100         | 45          |
| miR-222| 100         | 55          | 100         | 51          |
| miR-342| 100         | 70          | 100         | 52          |
Table 4A:

Estimation of plasma tocols by normal phase HPLC of pharmacokinetic human study after feeding single dose of 750 mg of δ-tocotrienol in one day.

| Normal Phase-Silica column. | Tocols ----> | δ-Tocotrienol | γ-Tocotrienol | β-Tocotrienol | α-Tocotrienol | δ-Tocopherol | γ-Tocopherol | β-Tocopherol | α-Tocopherol |
|-----------------------------|-------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Hour | ng/mL | ng/mL | ng/mL | ng/mL | ng/mL | ng/mL | ng/mL | ng/mL | ng/mL |
| 0 Hr. | 132 ± 12 | 795 ± 19 | 1145 ± 61 | 241 ± 16 | 894 ± 69 | 319 ± 30 | 542 ± 11 | 1644 ± 59 * |
| 1 hr. | 310 ± 28 | 1015 ± 10 | 1565 ± 62 | 268 ± 13 | 949 ± 11 | 404 ± 44 | 525 ± 54 | 2080 ± 68 |
| 2 hr. | 877 ± 24 | 1183 ± 16 | 1568 ± 77 | 278 ± 7 | 1195 ± 69 | 433 ± 26 | 599 ± 14 | 2002 ± 91 |
| 4 hr. | 1444 ± 53 | 1352 ± 23 | 1885 ± 91 | 293 ± 11 | 1348 ± 93 | 547 ± 12 | 704 ± 29 | 2231 ± 35 |
| 6 hr. | 759 ± 30 | 361 ± 74 | 1538 ± 22 | 223 ± 5 | 726 ± 51 | 273 ± 67 | 621 ± 28 | 2754 ± 84 |
| 8 hr. | 523 ± 13 | 329 ± 17 | 137 ± 102 | 86 ± 16 | 432 ± 45 | 212 ± 16 | 278 ± 16 | 2406 ± 51 |
| Total tocols (ng/ml) | 4045 | 5055 | 7838 | 1389 | 5544 | 2188 | 3269 | 13117 |

Table 4B: Estimation of plasma tocols by normal phase HPLC of pharmacokinetic human study after feeding single dose of 1000 mg of δ-tocotrienol in one day.

| Normal Phase-Silica column. | Tocols ----> | δ-Tocotrienol | γ-Tocotrienol | β-Tocotrienol | α-Tocotrienol | δ-Tocopherol | γ-Tocopherol | β-Tocopherol | α-Tocopherol |
|-----------------------------|-------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Hour | ng/mL | ng/mL | ng/mL | ng/mL | ng/mL | ng/mL | ng/mL | ng/mL | ng/mL |
| 0 Hr. | 360 ± 20 | 843 ± 13 | 1220 ± 93 | 292 ± 39 | 888 ± 22 | 330 ± 34 | 639 ± 38 | 1817 ± 80 * |
| 1 hr. | 993 ± 33 | 1065 ± 7 | 1516 ± 21 | 653 ± 42 | 986 ± 18 | 412 ± 17 | 763 ± 69 | 2025 ± 66 |
| 2 hr. | 1953 ± 44 | 1251 ± 32 | 1830 ± 91 | 807 ± 49 | 1266 ± 25 | 457 ± 33 | 1025 ± 58 | 2115 ± 92 |
| 4 hr. | 884 ± 45 | 1387 ± 10 | 1937 ± 72 | 1125 ± 35 | 1473 ± 71 | 589 ± 39 | 1535 ± 32 | 2228 ± 22 |
| 6 hr. | 562 ± 47 | 484 ± 17 | 1315 ± 48 | 375 ± 49 | 765 ± 44 | 241 ± 28 | 717 ± 56 | 2915 ± 35 |
| 8 hr. | 4565 | 374 ± 27 | 519 ± 20 | 309 ± 17 | 500 ± 25 | 183 ± 14 | 516 ± 51 | 2139 ± 61 |
| Total tocols (ng/ml) | 5404 | 8337 | 3561 | 5878 | 2212 | 5185 | 13239 |

*Values represent ± Standard Deviation (± SD).
Table 5A: Pharmacokinetic parameters after feeding single dose of 750 mg of δ-tocotrienol in one day.

| #  | A: | δ-Tocotrienol | γ-Tocotrienol | β-Tocotrienol | α-Tocotrienol |
|----|----|---------------|---------------|---------------|---------------|
| 1  | Area Under the Curve-$t_0$-$t_8$ (AUC$_0$-$t_8$; ng/ml) | 6620.87 ± 49.67$^a$ | 6961.92 ± 97.55$^b$ | 11473.96 ± 316.15$^c$ | 197.89 ± 1.02$^d$ |
| 2  | Area Under the Curve-$t_0$-$∞$ (AUC$_0$-$∞$; ng/ml) | 8687.69 ± 201.01$^a$ | 7895.14 ± 73.43$^b$ | 11709.23 ± 459.66$^c$ | 225.50 ± 6.79$^d$ |
| 3  | Cumulative Area Under the Curve-$t_0$-$∞$ (AUMC$_0$-$∞$; ng/ml) | 52496.47 ± 2095.81$^a$ | 32479.70 ± 606.11$^b$ | 43200.35 ± 3122.43$^c$ | 1009.47 ± 88.31$^d$ |
| 4  | Mean Residence Time (h) | 6.04 ± 0.139$^a$ | 4.11 ± 0.049$^b$ | 3.69 ± 0.16$^b$ | 4.47 ± 0.26$^b$ |
| 5  | Peak Plasma Concentration ($C_{max}$; ng/ml) | 1444.23 ± 53.07$^a$ | 1352.41 ± 28.14$^b$ | 1885.20 ± 90.95$^c$ | 30.25 ± 1.06$^d$ |
| 6  | Time to achieve plasma peak ($T_{max}$; h) | 4.00$^a$ | 4.00$^a$ | 4.00$^a$ | 3.33 ± 1.16$^a$ |
| 7  | Elimination of Half-life time ($t_{1/2}$; h) | 2.74 ± 0.13$^a$ | 1.96 ± 0.06$^b$ | 1.02 ± 0.34$^b$ | 2.21 ± 0.18$^a$ |
| 8  | Time of clearance (Cl-T; L/h) | 0.086 ± 0.002$^a$ | 0.095 ± 0.001$^a$ | 0.064 ± 0.003$^b$ | 3.33 ± 0.102$^c$ |
| 9  | Apparent volume of distribution (Vd/f; ml) | 0.341 ± 0.012$^a$ | 0.269 ± 0.008$^b$ | 0.094 ± 0.029$^b$ | 10.583 ± 0.543$^d$ |
| 10 | Elimination rate constant (ke; h$^{-1}$) | 0.253 ± 0.167$^a$ | 0.353 ± 0.125$^a$ | 0.681 ± 0.103$^b$ | 0.315 ± 0.188$^a$ |

Table 5B: Pharmacokinetic parameters after feeding single dose of 1000 mg of δ-tocotrienol in one day.

| #  | B: | δ-Tocotrienol | γ-Tocotrienol | β-Tocotrienol | α-Tocotrienol |
|----|----|---------------|---------------|---------------|---------------|
| 1  | Area Under the Curve-$t_0$-$t_8$ (AUC$_0$-$t_8$; ng/ml) | 7450.10 ± 89.01$^a$ | 57198.99 ± 5006.46$^a$ | 4444.23 ± 53.09$^a$ | 1444.23 ± 53.07$^a$ |
| 2  | Area Under the Curve-$t_0$-$∞$ (AUC$_0$-$∞$; ng/ml) | 9633.18 ± 382.98$^a$ | 7479.89 ± 129.37$^a$ | 1352.41 ± 28.14$^b$ | 1386.99 ± 12.49$^b$ |
| 3  | Cumulative Area Under the Curve-$t_0$-$∞$ (AUMC$_0$-$∞$; ng/ml) | 11950.22 ± 231.01$^b$ | 11895.22 ± 231.01$^b$ | 1885.20 ± 90.95$^c$ | 1948.13 ± 66.43$^b$ |
| 4  | Mean Residence Time (h) | 5.93 ± 0.364$^a$ | 5.93 ± 0.364$^a$ | 4.33 ± 0.159$^b$ | 4.00$^a$ |
| 5  | Peak Plasma Concentration ($C_{max}$; ng/ml) | 1591.89 ± 43.97$^a$ | 1591.89 ± 43.97$^a$ | 1985.20 ± 90.95$^c$ | 1948.13 ± 66.43$^b$ |
| 6  | Time to achieve plasma peak ($T_{max}$; h) | 4.00$^a$ | 4.00$^a$ | 4.33 ± 0.159$^b$ | 4.00$^a$ |
| 7  | Elimination of Half-life time ($t_{1/2}$; h) | 2.74 ± 0.13$^a$ | 2.74 ± 0.13$^a$ | 2.12 ± 0.14$^b$ | 2.12 ± 0.14$^b$ |
| 8  | Time of clearance (Cl-T; L/h) | 0.086 ± 0.002$^a$ | 0.086 ± 0.002$^a$ | 0.44 ± 0.076$^b$ | 2.12 ± 0.14$^b$ |
| 9  | Apparent volume of distribution (Vd/f; ml) | 0.341 ± 0.012$^a$ | 0.341 ± 0.012$^a$ | 0.681 ± 0.103$^b$ | 0.681 ± 0.103$^b$ |
|   | Elimination rate constant (ke; h⁻¹) |
|---|-----------------------------------|
| 10 | 0.260 ± 0.143<sup>a</sup>         |
| 5  | 0.328 ± 0.286<sup>a</sup>         |
| 7  | 0.327 ± 0.167<sup>a</sup>         |
| 0.694 ± 0.443<sup>a</sup>          |

<sup>a</sup>-d</sup> Values in a row not sharing a common letter are significantly different at P<0.01-0.001.

* Values represent Standard Deviation (SD)
Table 6:
Evaluation of following compounds on several inflammatory biomarkers in PPAR-α knockout mice.

| # | Section I: Known Proteasome Inhibitors | # | Section VII: Vitamins |
|---|----------------------------------------|---|-----------------------|
|   | Water Insolubles                       |   |                       |
| 1 | Lactacystin                             | 19| Vitamin D₃            |
| 2 | Dexamethasone                           | 20| Vitamin E             |
| 3 | (−) Corey Lactone                      | 19| α-, β-, γ-, δ-Tocopherols |
| 4 | Ouabain                                | 20| α-, β-, γ-, δ-Tocotrienols |
| 5 | Thiostrepton                            | 21| Quercetin Sulphate    |
| 6 | Rifampicin                              | 22| trans-Resveratrol     |
| 7 | Ampicillin                              |   |                       |
| 8 | Mevinolin (Lovastatin)                 | 23| trans-Petrostilbene   |
| 9 | 2-Hydroxyestradiol                     | 24| Morin Hydrate         |
| 10| 2-Methoxyestradiol                     |   |                       |
| 11| 25-Hydroxycholesterol                  | 25| Vincaleukoblastine Sulphate |
| 12| Acetylsalicylic Acid (Aspirin)          | 26| Codeine               |
| 13| α-Tocopherol                            | 27| Dopamine-HCL          |
| 14| γ-Tocotrienol                          | 28|                       |
| 15| δ-Tocotrienol                          | 29|                       |
| 16| Section II: Known Proteasome stimulators | 30|                       |
| 17| α-, β-, γ-, δ-Tocopherols              | 31|                       |
| 18| α-, β-, γ-, δ-Tocotrienols             | 32|                       |
| 19| Section III: Antibiotics               |   |                       |
| 20| Section IV: Cholesterol Inhibitors     |   |                       |
| 21| Section V: Antioxidants               |   |                       |
| 22| Section VI: Vitamins                  |   |                       |
| 23| Section VII: Polyphenols              |   |                       |
| 24| Section VIII: Polyphenols             |   |                       |
| 25| Section IX: Alkaloids + Narcotics     |   |                       |
| 26| Section X: Neurotransmitter           |   |                       |
| 27| Section XI: Miscellaneous Useful Pharmaceutical Products |   |                       |
| 28| Quinine Sulphate                      |   |                       |
| 29| Amiloride                              |   |                       |
| 30| α- Lipoic Acid                         |   |                       |
| 31| Coenzyme Q10                           |   |                       |
| 32| Hydrochlorothiazide-HCL                |   |                       |
| Water Solubles |   |   |
|---------------|---|---|
| 16            | Ascorbic Acid (Vitamin C) |   |
| 17            | Riboflavin (Vitamin B2)   |   |
| 18            | Niacin (Vitamin B3)       |   |
Table 7:
Impact of effective dose of various compounds in different cancer cell lines.

| #  | Hela cells | Liver cancer cells | Pancreas cancer cells | Prostate cancer cells | Breast cancer cells | Lung cancer cells | Melanoma cells | B-Lymphocyte cells | T-cells (Jurkat) |
|----|------------|--------------------|-----------------------|-----------------------|---------------------|-------------------|---------------|--------------------|-----------------|
| 1  | Thiostrepton | 40; 8.7 ± 1.5 (16) | 10; 6.7 ± 2.2 (8) | 10; 7.7 ± 0.6 (16) | 5; 7.0 ± 1.7 (19) | 10; 6.7 ± 0.6 (29) | 5; 7.0 ± 2.0 (28) | 5; 2.3 ± 1.5 (13) | 2.5; 15.7 ± 4.0 (23) |
| 2  | Ampicillin | 80; 47.7 ± 5.5 (88) | 40; 70.3 ± 4.2 (88) | 80; 35.3 ± 5.0 (74) | 20; 15.0 ± 1.0 (41) | 40; 16.7 ± 6.1 (74) | 80; 21.3 ± 1.5 (85) | 20; 12.3 ± 1.2 (70) | 10; 44.7 ± 5.1 (66) |
| 3  | Dexamethasone | 80; 18.0 ± 4.0 (53) | 80; 67.3 ± 4.0 (93) | 20; 34.7 ± 4.0 (73) | 20; 16.7 ± 1.5 (32) | 80; 15.7 ± 3.1 (85) | 20; 16.7 ± 2.1 (78) | 40; 13.0 ± 2.7 (74) | 10; 61.0 ± 3.6 (41) |
| 4  | 2-Methoxyestradiol | 20; 4.3 ± 1.5 (13) | 10; 19.7 ± 2.1 (27) | 40; 20.0 ± 2.0 (42) | 10; 1.7 ± 1.5 (30) | 20; 8.0 ± 2.0 (44) | 10; 2.3 ± 1.5 (11) | 10; 2.0 ± 1.7 (11) | 5; 4.0 ± 2.0 (33) |
| 5  | δ-Tocotrienol | 20; 8.3 ± 2.1 (18) | 20; 17.7 ± 2.1 (24) | 20; 5.7 ± 1.5 (5) | 20; 12.3 ± 1.5 (35) | 20; 10.7 ± 0.6 (56) | 20; 8.3 ± 2.1 (11) | 20; 7.0 ± 3.6 (37) | 20; 2.7 ± 1.5 (21) |
| 6  | (−) Riboflavin | 40; 21.0 ± 1.6 (64) | 80; 74.0 ± 2.0 (91) | 40; 40.0 ± 2.0 (67) | 40; 20.0 ± 2.0 (61) | 80; 12.7 ± 1.5 (61) | 10; 7.7 ± 2.1 (23) | 40; 26.3 ± 3.1 (91) | 20; 19.3 ± 3.1 (69) |
| 7  | Ascorbic Acid | 80; 21.0 ± 1.6 (64) | 80; 74.3 ± 2.5 (90) | 80; 42.3 ± 2.5 (71) | 80; 29.3 ± 2.1 (81) | 40; 15.0 ± 1.7 (81) | 20; 15.0 ± 4.4 (46) | 80; 23.7 ± 4.7 (81) | 20; 17.3 ± 2.5 (62) |
| 8  | Quercetin | 40; 21.3 ± 1.5 (38) | 40; 24.0 ± 2.0 (29) | 40; 11.7 ± 0.6 (26) | 20; 7.3 ± 1.2 (17) | 20; 5.7 ± 0.6 (27) | 20; 11.7 ± 1.5 (36) | 40; 8.3 ± 0.6 (21) | 10; 8.0 ± 3.0 (29) |
| 9  | Amiloride-HCL | 80; 19.7 ± 1.5 (36) | 40; 36.7 ± 2.5 (65) | 80; 30.7 ± 1.2 (69) | 80; 27.0 ± 4.6 (70) | 20; 14.0 ± 5.3 (61) | 40; 23.3 ± 2.5 (86) | 40; 33.0 ± 5.6 (90) | 20; 5.0 ± 1.0 (56) |
| 10 | (−) Corey Lactone | 80; 36.0 ± 5.6 (66) | 80; 31.7 ± 2.1 (56) | 40; 30.7 ± 1.2 (58) | 40; 23.2 ± 2.1 (60) | 40; 17.7 ± 3.2 (77) | 40; 17.3 ± 3.8 (64) | 40; 18.3 ± 3.5 (62) | 20; 5.3 ± 1.5 (59) |
| 11 | Quinine Sulphate | 20; 10.7 ± 1.5 (19) | 80; 47.7 ± 2.1 (58) | 40; 35.0 ± 3.0 (78) | 20; 15.7 ± 2.1 (36) | 80; 12.3 ± 2.3 (60) | 40; 21.3 ± 1.5 (66) | 20; 22.7 ± 2.9 (56) | 80; 20.3 ± 3.1 (19) | 40; 267.7 ± 29.7 (77) |
Table 8:
The IC\textsubscript{50} values of various compounds in different cancer cell lines.

| # | Compounds       | Hela cells | Liver cancer cells | Pancreas cancer cells | Prostate cancer cells | Breast cancer cells | Lung cancer cells | Melanoma cells | B-Lymphocyte cells | T-cells (Jurkat) |
|---|-----------------|------------|--------------------|-----------------------|-----------------------|---------------------|-------------------|----------------|---------------------|------------------|
|   | μM; value (%)   | μM; value (%) | μM; value (%) | μM; value (%) | μM; value (%) | μM; value (%) | μM; value (%) | μM; value (%) | μM; value (%) | μM; value (%) |
| 1 | Thiostrepton    | 10; 13.3 ± 0.6 (24) | 2.5; 42.3 ± 3.5 (53) | 5; 14.0 ± 1.0 (29) | 2.5; 19.7 ± 0.6 (53) | 20; 3.7 ± 1.5 (16) | 5; 7.0 ± 2.0 (48) | 2.5; 6.0 ± 2.0 (34) | 2.5; 15.7 ± 4.0 (23) | 2.5; 21.3 ± 2.1 (5) |
| 2 | Ampicillin      | 5; 18.3 ± 1.5 (50)    | 2.5; 23.3 ± 2.0 (45) | 2.5; 10.3 ± 2.1 (47) | 5; 66.0 ± 5.3 (45) |
| 3 | Dexamethasone   | 2.5; 16.3 ± 1.5 (48) | 5; 32.7 ± 2.5 (45) | 20; 0.0 ± 2.0 (42) | 10; 8.3 ± 2.9 (45) | 2.5; 3.7 ± 1.2 (17) | 2.5; 4.3 ± 0.6 (25) | 2.5; 8.0 ± 2.0 (5) | 2.5; 32.7 ± 5.0 (8) |
| 4 | 2-Methoxyestradiol | 2.5; 16.3 ± 1.5 (48) | 5; 32.7 ± 2.5 (45) | 40; 20.0 ± 2.0 (39) | 2.5; 20.0 ± 2.0 (39) | 10; 8.3 ± 2.9 (45) | 2.5; 3.7 ± 1.2 (17) | 2.5; 4.3 ± 0.6 (25) | 2.5; 8.0 ± 2.0 (5) | 2.5; 32.7 ± 5.0 (8) |
| 5 | δ-Tocotrienol   | 5; 20.7 ± 1.2 (45) | 20; 38.3 ± 5.9 (53) | 10; 18.0 ± 2.0 (36) | 10; 14.0 ± 2.0 (39) | 10; 9.3 ± 2.1 (49) | 20; 2.3 ± 2.1 (11) | 10; 9.3 ± 3.1 (50) | 10; 7.0 ± 2.1 (55) | 20; 142.0 ± 4.4 (32) |
| 6 | (-) Riboflavin  | 80; 18.0 ± 2.0 (55) | 2.5; 9.3 ± 2.5 (28) | 80; 15.3 ± 3.5 (55) |
| 7 | Ascorbic Acid   | 10; 16.0 ± 4.6 (49) | 10; 16.0 ± 4.6 (49) | 10; 16.0 ± 4.6 (49) | 10; 16.0 ± 4.6 (49) | 10; 16.0 ± 4.6 (49) | 10; 16.0 ± 4.6 (49) | 10; 16.0 ± 4.6 (49) | 10; 16.0 ± 4.6 (49) | 10; 16.0 ± 4.6 (49) |
| 8 | Quercetin       | 10; 28.3 ± 8.1 (51) | 20; 26.3 ± 3.5 (32) | 40; 20.3 ± 2.1 (45) | 10; 21.0 ± 3.6 (48) | 10; 8.0 ± 1.7 (39) | 10; 14.7 ± 2.5 (43) | 10; 21.7 ± 7.1 (54) | 10; 8.0 ± 3.0 (29) | 10; 157.7 ± 20.5 (45) |
| 9 | Amiloride-HCL   | 10; 27.7 ± 2.1 (51) | 80; 31.7 ± 2.1 (56) | 40; 12.0 ± 2.0 (32) | 40; 12.0 ± 2.0 (32) | 40; 4.3 ± 1.5 (48) |
| 10 | (−) Corey Lactone | 80; 31.7 ± 2.1 (56) | 80; 28.7 ± 3.1 (54) | 80; 19.7 ± 1.5 (51) | 80; 12.7 ± 1.5 (55) | 80; 12.0 ± 2.7 (44) | 80; 13.7 ± 2.3 (46) | 40; 3.3 ± 1.5 (37) | 80; 124.3 ± 17.4 (29) |
| 11 | Quinine Sulphate | 10; 15.3 ± 2.5 (24) | 80; 47.7 ± 2.1 (58) | 20; 15.7 ± 2.1 (36) | 80; 20.3 ± 5.0 (50) | 80; 174.7 ± 8.1 (50) |
Table 9A:
Effect of δ-tocotrienol on up-regulation of fold change gene expression of "Molecules" (953) section of IPA analysis in hepatitis C patients.

| # | Symbol       | Entrez Gene Name                        | Expr. Fold Change | Type(s)     |
|---|--------------|-----------------------------------------|-------------------|-------------|
| 1 | HIST1H2AD    | histone cluster 1 H2A family member D   | 1804955.068       | other       |
| 2 | HHIL2        | HHIP like 2                             | 28.710            | other       |
| 3 | RPP38        | ribonuclease P/MRP subunit p38          | 24.946            | enzyme      |
| 4 | CERS3        | ceramide synthase 3                     | 19.082            | transcription regulator |
| 5 | HBG1         | hemoglobin subunit gamma 1              | 17.945            | other       |
| 6 | MT-TQ        | tRNA                                    | 14.252            | other       |
| 7 | AKR1D1       | aldo-keto reductase family 1 member D1  | 14.056            | enzyme      |
| 8 | TSPAN15      | tetraspanin 15                          | 11.523            | other       |
| 9 | HBG2         | hemoglobin subunit gamma 2              | 11.413            | other       |
| 10 | MKX          | mohawk homeobox                         | 9.573             | transcription regulator |
| 12 | P4HA3        | prolyl 4-hydroxylase subunit alpha 3    | 8.686             | enzyme      |

Table 9B: Effect of δ-tocotrienol on down-regulation of fold change gene expression of "Molecules" (953) section of IPA analysis in hepatitis C patients.

| #  | Symbol         | Entrez Gene Name                        | Expr. Fold Change | Type(s)     |
|----|----------------|-----------------------------------------|-------------------|-------------|
| 1  | ATP1A1         | ATPase Na+/K+ transporting subunit alpha 1 | -8.014           | transporter |
| 2  | HSP90AB1       | heat shock protein 90 alpha family class B member 1 | -8.049 | enzyme     |
| 3  | APOBEC3A       | adipoprotein B mRNA editing enzyme catalytic subunit 3A | -8.163 | enzyme     |
| 4  | CXCR2          | C-X-C motif chemokine receptor 2        | -8.208            | G-protein coupled receptor |
| 5  | IL16           | interleukin 16                           | -8.239            | cytokine    |
| 6  | PSMC3          | proteasome 26S subunit, ATPase 3        | -8.346            | transcription regulator |
| 7  | NDUFB9         | NADH: ubiquinone oxidoreductase subunit B9 | -8.354           | enzyme      |
| 8  | CYB5R4         | cytochrome b5 reductase 4               | -8.367            | enzyme      |
| 9  | ATG3           | autophagy related 3                     | -8.376            | enzyme      |
| 10 | CREB1          | cAMP responsive element binding protein 1 | -8.452           | transcription regulator |
| 12 | NDUFB1         | NADH: ubiquinone oxidoreductase subunit B1 | -8.566           | enzyme      |
| 13 | PDE3B          | phosphodiesterase 3B                    | -8.568            | enzyme      |
|   | Gene   | Description                                              | Log2FoldChange | Class     |
|---|--------|----------------------------------------------------------|----------------|-----------|
|14 | IGF2R  | insulin like growth factor 2 receptor                   | −8.68         | transmembrane receptor |
|15 | CYP2R1 | cytochrome P450 family 2 subfamily R member 1           | −8.682        | enzyme    |
|16 | NDUFA11| NADH:ubiquinone oxidoreductase subunit A11              | −8.686        | enzyme    |
|17 | IGSF6  | immunoglobulin super family member 6                    | −8.712        | transmembrane receptor |
|18 | TNFRSF1B|TNF receptor super family member 1B                      | −8.746        | transmembrane receptor |
|19 | PRPF18 | pre-mRNA processing factor 18                           | −8.777        | transporter |
|20 | SERP1  | stress associated endoplasmic reticulum protein 1       | −8.872        | other     |
|21 | UBE2J1 | ubiquitin conjugating enzyme E2 J1                       | −8.874        | enzyme    |
|22 | VEGFA  | vascular endothelial growth factor A                     | −8.933        | growth factor |
|23 | GYS1   | glycogen synthase 1                                      | −9.027        | enzyme    |
|24 | GPR65  | G protein-coupled receptor 65                            | −9.054        | G-protein coupled receptor |
|25 | ILF2   | interleukin enhancer binding factor 2                    | −9.105        | transcription regulator |
|26 | OSBPL11| oxysterol binding protein like 11                        | −9.201        | other     |
|27 | PSMA5  | proteasome subunit alpha 5                               | −9.31         | peptidase |
|28 | PIAS1  | protein inhibitor of activated STAT 1                    | −9.326        | transcription regulator |
|29 | TRAF7  | TNF receptor associated factor 7                         | −9.341        | enzyme    |
|30 | COX14  | COX14, cytochrome c oxidase assembly factor              | −9.447        | other     |
|31 | RPS26  | ribosomal protein S26                                    | −9.456        | other     |
|32 | SFPQ   | splicing factor proline and glutamine rich               | −9.469        | other     |
|33 | ATF4   | activating transcription factor 4                        | −9.515        | transcription regulator |
|34 | PECAM1 | platelet and endothelial cell adhesion molecule 1        | −9.552        | other     |
|35 | GPS2   | G protein pathway suppressor 2                           | −9.56         | transcription regulator |
|36 | NFIL3  | nuclear factor, interleukin 3 regulated                  | −9.568        | transcription regulator |
|37 | PSMB8  | proteasome subunit beta 8                                | −9.709        | peptidase |
|38 | UBP1   | upstream binding protein 1 (LBP-1a)                      | −9.718        | transcription regulator |
|39 | RAP2C  | RAP2C, member of RAS oncogene family                     | −9.792        | enzyme    |
|40 | PIBF1  | progesterone immunomodulatory binding factor 1           | −9.876        | other     |
|41 | USP25  | ubiquitin specific peptidase 25                          | −9.911        | peptidase |
|42 | FRS2   | fibroblast growth factor receptor substrate 2            | −9.962        | kinase    |
|43 | PSMB4  | proteasome subunit beta 4                                | −10.119       | peptidase |
|    | Gene Symbol | Protein Name                                      | Score | Category         |
|----|-------------|---------------------------------------------------|-------|------------------|
| 44 | USP15       | ubiquitin specific peptidase 15                  | -10.16| peptidase        |
| 45 | UBA52       | ubiquitin A-52 residue ribosomal protein fusion product 1 | -10.176| enzyme          |
| 46 | UBE4A       | ubiquitination factor E4A                         | -10.189| enzyme         |
| 47 | GTPBP8      | GTP binding protein 8 (putative)                | -10.19 | other            |
| 48 | USP19       | ubiquitin specific peptidase 19                 | -10.713| peptidase        |
| 49 | TNFAIP8     | TNF alpha induced protein 8                     | -10.974| other            |
| 50 | HSPA14      | heat shock protein family A (Hsp70) member 14    | -10.978| peptidase        |
| 51 | TLR8        | toll like receptor 8                             | -11.975| transmembrane receptor |
| 52 | IL27RA      | interleukin 27 receptor subunit alpha            | -12.004| transmembrane receptor |
| 53 | SCP2        | sterol carrier protein 2                        | -13.672| transporter      |
| 54 | IFNGR2      | interferon gamma receptor 2                     | -13.844| transmembrane receptor |
| 55 | ID2         | inhibitor of DNA binding 2, HLH protein          | -14.133| transcription regulator |
| 56 | TUSC2       | tumor suppressor candidate 2                    | -15.922| other            |
| 57 | IL2RG       | interleukin 2 receptor subunit gamma             | -16.787| transmembrane receptor |
| 58 | IL1R2       | interleukin 1 receptor type 2                   | -19.547| transmembrane receptor |
| 59 | IRF2        | interferon regulatory factor 2                  | -22.655| transcription regulator |
| 60 | PTGS2       | prostaglandin-endoperoxide synthase 2           | -25.841| enzyme           |
| 61 | mir-877     | microRNA 877                                    | -4497.07| microRNA         |
| 62 | mir-1250    | microRNA 1250                                   | -4755.79| microRNA         |
| 63 | mir-140     | microRNA 140                                    | -5668.259| microRNA         |
| 64 | KLRC4-KLRK1/KLRK1 | killer cell lectin like receptor K1 | -1565687.642| transmembrane receptor |
Table 10: Effect of δ-tocotrienol on canonical pathways (33) of IPA Ingenuity canonical pathways analysis (360) in hepatitis C patients.

| Ingenuity Canonical Pathways | Fold Change | Expression | Z-Score | Molecules |
|-----------------------------|-------------|------------|---------|-----------|
| EIF2 Signaling; Eukaryotic translation initiation factors (221) | 36.900 | 0.303 | −5.692 | RPL7A, EIF3G, RPL13A, RPL32, RPS24, RPL37A, RPL23, RPL26, RPS13, FRS2, RPS11, RPL29, RPL30, RPS29, RPL39, RPS18, VEGFA, RPL11, RPL35, EIF3L, AGO4, EIF1AY, RPS2, RPL12, RPL5, EIF2S2, RPL28, RPL38, RPS15A, RPL37, RPL22L1, RPS4Y1, EIF4A1, RPL31, RPS8, EIF4A3, EIF3J, RPL18A, RPS25, RPL17, RPL26L1, RPS2, RPL10A |
| Regulation of eIF4 and p70S6K signalig (157) | 13.300 | 0.210 | 0.000 | PPP2R5E, EIF3G, RPS26 |
| Protein ubiquitination pathway (265) | 3.130 | 0.091 | 0.000 | UBE2J1, USP19, UBA52 |
| mTOR signaling; Mammalian target of rapamycin (201) | 12.900 | 0.184 | −2.138 | PPP2R5E, EIF3G, RPS26 |
| Type I Diabetes Mellitus Signaling (111) | 5.760 | 0.162 | −2.496 | NFKB1, MAP3K5, JAK2, HLA-DQB1, IFNGR2, TNFRSF1B, PIAS1, TRADD, HLA-DRA, IFNGR1, HLA-A, HLA-DMA, CD3D, HLA-DMB, MAPK1, CASP3, RIPK1, TRAF6, JAK2, NOTCH1, HLA-DQB1, IFNGR2, PIK3R1, HLA-DRA, NOTCH2, IL2RG, IKZF1, IL10RA, IFNGR1, N CSTN, CXCR4, TGFBR2, HLA-A, HLA-DMA, FRS2, CD3D, HLA-DPA1, HLA-DMA, NFI3, IL27RA, S1PR1 |
| Th1 and Th2 Activation Pathway (185) | 5.640 | 0.130 | 0.000 | NFKB1, JAK2, NOTCH1, HLA-DQB1, IFNGR2, PIK3R1, HLA-DRA, NOTCH2, IL2RG, IKZF1, IL10RA, IFNGR1, NCSTN, CXCR4, TGFBR2, HLA-A, HLA-DMA, FRS2, CD3D, HLA-DPA1, HLA-DMA, NFI3, IL27RA, S1PR1 |
| Interferon Signaling (36) | 4.700 | 0.250 | −2.333 | IFNGR1, OAS1, IFIT1, JAK2, IFITM1, IFNGR2, IFITM2, PIAS1, PSMB8 |
| Role of IL-17F (44) | 3.960 | 0.205 | −3.000 | NFKB1, ATF4, CREB1, RPS6KA3, CXCL1, MAPK1, CXCL8, RPS6KA4, TRAF6 |
| IL-8 Signaling (197) | 3.320 | 0.102 | −4.123 | NFKB1, GNA13, GNB4, RACK1, VEGFA, MYL12B, PIK3R1, ARRB2, NCF2, CXCL8, FRS2, PTGS2, CXCR2, CXCL1, MAPK1, RHOT1, CYBB, EIF4EBP1, FKBP1, TRAF6 |
| Fold Change Expression | Z-Score | Molecules |
|------------------------|---------|-----------|
| 181                    | -4.243  | NF-κB Signaling |
| 35                     | 0.000   | IL-17A Signaling in Fibroblasts |
| 128                    | -3.051  | IL-6 Signaling |
| 61                     | -2.828  | Induction of Apoptosis by HIV1 |
| 133                    | -3.606  | HMGB1 Signaling |
| 95                     | 1.897   | PPAR Signaling |
| 69                     | 0.000   | IL-10 Signaling |
| 45                     | -2.449  | iNOS Signaling |
| 141                    | -1.508  | Insulin Receptor Signaling |
| 111                    | 0.000   | p53 Signaling |
| 69                     | 0.000   | Role of IL-17A in Arthritis |
| 76                     | -1.000  | Toll-like Receptor Signaling |
| 92                     | -2.449  | IL-1 Signaling |
| 90                     | -0.378  | Apoptosis Signaling |
| 32                     | -2.449  | FKIR,TLR8,TGFBR2,BCL10,MAP3K1,FRS2,RIPK1,MAP3K3,TRAF6 |
| Molecules | Fold Change | Expression | Z-Score | Signaling Pathway |
|-----------|-------------|------------|---------|------------------|
| PDGF Signaling (90) | 0.987 | 0.078 | −2.646 | ABL1, JAK2, CSNK2B, MAP3K1, FRS2, MAPK1, PIK3R1 |
| Type II Diabetes Mellitus Signaling (128) | 0.944 | 0.070 | −2.333 | NFKB1, MAP3K5, TNFRSF1B, MAP3K1, FRS2, CEBPB, MAPK1, PIK3R1, TRADD |
| IL-15 Signaling (76) | 0.904 | 0.107 | 0.000 | NFKB1, JAK2, TXK |
| autophagy (62) | 0.859 | 0.081 | 0.000 | CTSW, ATG3, ATG5, CTSC, LAMP2 |
| IL-2 Signaling (64) | 0.818 | 0.078 | −2.000 | CSNK2B, FRS2, MAPK1, PIK3R1, IL2RG |
| PPARα/RXRα Activation (180) | 0.759 | 0.061 | 3.000 | TGS1, GNAQ, TGFBR2, NFKB1, JAK2, IL18RAP, MAPK1, MED12, IL1R2, HSP90AB1, TRAF6 |
| TNFR1 (32) | 2.210 | 0.140 | −2.646 | NFKB1, MAP4K2, MAP3K1, PAK1, CASP3, TRADD, RIPK1 |
| STAT3 Pathway (74) | 0.641 | 0.068 | −1.342 | TGFBR2, JAK2, MAPK1, PTPN6, IGF2R |
| Nitric Oxide Signaling in the Cardiovascular System (113) | 0.633 | 0.062 | −2.646 | ITPR2, VEGFA, PDE3B, FRS2, MAPK1, PIK3R1, HSP90AB1 |
| Osteoarthritis Pathway (210) | 3.370 | 0.100 | −2.524 | NFKB1, CREB1, NOTCH1, TNFRSF1B, VEGFA, KEF1, IL-1R2, mir-140 |

Ann Clin Case Rep. Author manuscript; available in PMC 2022 December 19.
**Table 11:**

Impact of a Mixture of NS-3 on various biomarkers of diabetes in serum/plasma of people with T2DM ($n=104$).

| #  | Various biomarkers | $^a$Placebo ($n=52$) | Placebo ($n=52$) | $^c$P-values | $^b$Mixture ($n=52$) | Mixture ($n=52$) | $^c$P-values |
|----|--------------------|---------------------|-----------------|-------------|---------------------|-----------------|-------------|
| 1  | Fasting glucose (mmol/L) | 7.65 ± 1.66 | 7.59 ± 1.49 (99)$^d$ | 0.098 | 7.39 ± 1.71 | 6.56 ± 1.66 (89)$^d$ | 0.000 |
| 2  | Fasting HbA1c (%) | 8.53 ± 1.16 | 8.50 ± 1.17 (98) | 0.764 | 8.20 ± 1.30 | 7.40 ± 0.93 (90) | 0.000 |
| 3  | hs-CRP (mg/L) | 3.46 ± 1.51 | 3.42 ± 1.68 (99) | 0.690 | 3.65 ± 1.31 | 2.82 ± 1.07 (77) | 0.000 |
| 4  | Fasting Insulin (mIU/L) | 15.96 ± 4.37 | 15.94 ± 4.26 (100) | 0.601 | 15.90 ± 5.78 | 14.42 ± 5.56 (91) | 0.000 |
| 5  | HOMA-IR | 5.58 ± 2.44 | 5.50 ± 2.25 (99) | 0.060 | 5.44 ± 2.85 | 4.35 ± 2.25 (80) | 0.000 |
| 6  | Malondialdehyde (MDA; μmol/L) | 3.75 ± 0.65 | 3.76 ± 0.63 (100) | 0.960 | 3.81 ± 0.65 | 3.04 ± 0.47 (80) | 0.030 |
| 7  | Microalbuminuria (mg/mmol) | 11.32 ± 0.96 | 11.03 ± 9.45 (97) | 0.345 | 12.56 ± 1.19 | 11.90 ± 1.21 (95) | 0.015 |
| 8  | Creatinine (μmol/L) | 89.79 ± 12.30 | 88.77 ± 12.69 (99) | 0.109 | 89.75 ± 18.10 | 82.40 ± 16.97 (92) | 0.000 |
| 9  | Total cholesterol (mmol/L) | 5.37 ± 0.71 | 5.34 ± 0.99 (99) | 0.844 | 5.36 ± 0.72 | 4.95 ± 0.72 (92) | 0.000 |
| 10 | HDL-C (mmol/L) | 0.92 ± 0.29 | 0.92 ± 0.30 (100.00) | 0.255 | 0.92 ± 0.34 | 0.94 ± 0.28 (102) | 0.498 |
| 11 | LDL-C (mmol/L) | 3.49 ± 0.83 | 3.49 ± 1.21 (100.00) | 0.956 | 3.36 ± 0.79 | 3.02 ± 0.78 (90) | 0.000 |
| 12 | Triglycerides (mmol/L) | 2.12 ± 1.27 | 2.03 ± 0.89 (96) | 0.621 | 2.36 ± 0.00 | 2.18 ± 0.98 (92) | 0.038 |
| 13 | TNF-α (pg/mL) | 8.98 ± 4.37 | 8.77 ± 4.12 (98) | 0.154 | 9.65 ± 5.60 | 7.28 ± 4.41 (75) | 0.000 |
| 14 | IL-6 (pg/mL) | 14.97 ± 7.82 | 14.82 ± 7.22 (99) | 0.591 | 14.86 ± 8.01 | 11.13 ± 6.96 (75) | 0.003 |

$^a$Two capsules of cellulose/olive oil (250 mg/capsule; placebo) were administered to people with T2DM for 24-weeks

$^b$Two capsules of a mixture of NS-3 (250.062 mg/capsule) were administered to people with T2DM for 24-weeks

$^c$The calculation of post treatment variables are based on an analysis of covariance (ANCOVA), adjusted for one covariates: Baseline (pre-treatment) variables

$^d$Percentage of control values are in parentheses

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Ann Clin Case Rep. Author manuscript; available in PMC 2022 December 19.
Table 12:

Summary of impact of placebo supplement or a mixture of (NS-3) or its components after treatment for 24-weeks on various biomarkers of diabetes in serum of people with T2DM.

| Biomarkers | Fasting Glucose | Fasting HbA1c | hs-CRP | HOMA-IR | MDA | MDA |
|------------|----------------|--------------|--------|---------|-----|-----|
|            | Pre-dose mmol/L | Post-dose %  | Pre-dose mg/L | Post-dose mg/L | Pre-dose | Post-dose | Pre-dose | Post-dose | Pre-dose | Post-dose | Pre-dose | Post-dose |
| # Values in --------→ mmol/L | mmol/L | % | % | % | % | μmol/L | μmol/L |
| 1 | Control\(^a\) (placebo) | 7.62 (100)\(^b\) | 7.57 (99) | 8.42 (100) | 8.42 (100) | 3.59 (100) | 3.47 (100) | 5.51 (100) | 5.44 (99) | 3.57 (100) | 3.58 (100) |
| 2 | δ-tocotrienol\(^a\) | 7.35 (100) | 6.85 (93) | 8.44 (100) | 7.79 (92) | 3.53 (100) | 3.10 (88) | 5.23 (100) | 4.51 (86) | 3.63 (100) | 3.22 (89) |
| 3 | Vitamin D\(^a\)\(^d\) | 7.55 (100) | 7.16 (95) | 8.81 (100) | 8.19 (93) | 3.37 (100) | 3.09 (92) | 5.32 (100) | 4.74 (89) | 3.59 (100) | 3.48 (97) |
| 4 | Resveratrol\(^a\) | 7.39 (100) | 6.98 (94) | 8.58 (100) | 7.86 (92) | 3.69 (100) | 3.28 (89) | 5.37 (100) | 4.98 (93) | 3.82 (100) | 3.49 (91) |
| 5 | Mixture (2 + 3 + 4\(^a\)) | 7.39 (100) | 6.56 (89) | 8.20 (100) | 7.40 (90) | 3.65 (100) | 2.82 (77) | 5.44 (100) | 4.35 (80) | 3.81 (100) | 3.04 (80) |

\(^a\) Two capsules of placebo (cellulose/olive oil; 250 mg/capsule) or two capsules of δ-tocotrienol (250 mg/capsule), or vitamin D₃ (5000 IU = 0.062/capsule) or resveratrol (250 mg/capsule) were administered to people with T2DM for 24-weeks.

\(^b\) Percentage of control values are in parentheses.
### Table 13A:

IPA analysis (miRNA) of gene expression of "molecular functions" (up-regulated [20]) after NS-3 treated RNAs of people with T2DM.

| # | Genes ID  | Expr Log Ratio | Symbol |
|---|-----------|----------------|--------|
| 1 | hsa-miR-29c-3p | 10.4 | miR-29b-3p (and other miRNAs w/seed AGCACCA) |
| 2 | hsa-miR-548ad-5p | 8.1 | miR-548h-5p (and other miRNAs w/seed AAGUAAGA) |
| 3 | hsa-miR-624-5p | 7.6 | miR-624-5p (miRNAs w/seed AGUACCA) |
| 4 | hsa-miR-361-5p | 7.5 | miR-361-5p (miRNAs w/seed UAUCAGA) |
| 5 | hsa-miR-301a-3p | 6.0 | miR-130a-3p (and other miRNAs w/seed AGUGCA) |
| 6 | hsa-miR-3912-3p | 5.5 | miR-3912-3p (miRNAs w/seed AACGCA) |
| 7 | hsa-miR-1976 | 4.7 | miR-1976 (and other miRNAs w/seed CUCCUC) |
| 8 | hsa-miR-11401 | 4.0 | miR-11401 (miRNAs w/seed CACGUC) |
| 9 | hsa-miR-1284 | 4.0 | miR-1284 (and other miRNAs w/seed CUAAAC) |
| 10 | hsa-miR-3605-3p | 3.3 | miR-3605-3p (miRNAs w/seed CUCCUG) |
| 11 | hsa-miR-23c | 2.0 | miR-23a-3p (and other miRNAs w/seed UCACAU) |
| 12 | hsa-miR-329-3p | 1.6 | miR-329-3p (miRNAs w/seed ACACCC) |
| 13 | hsa-miR-195-5p | 1.4 | miR-16-5p (and other miRNAs w/seed AGCAGCA) |
| 14 | hsa-miR-133a-3p | 1.0 | miR-133a-3p (and other miRNAs w/seed CUUUGC) |
| 15 | hsa-miR-136-3p | 1.0 | miR-136-3p (miRNAs w/seed AUUAUC) |
| 16 | hsa-miR-153-3p | 1.0 | miR-153-3p (miRNAs w/seed UGAUAG) |
| 17 | hsa-miR-543 | 1.0 | miR-543-3p (and other miRNAs w/seed ACACUA) |
| 18 | hsa-miR-544b | 1.0 | miR-544b (miRNAs w/seed CCUGAG) |
| 19 | hsa-miR-548ad-3p | 1.0 | miR-548ad-3p (miRNAs w/seed AAACUG) |
| 20 | hsa-miR-95-3p | 1.0 | miR-95-3p (miRNAs w/seed UCAACC) |

### Table 13B: IPA analysis (miRNA) of gene expression of "molecular functions" (down-regulated [27]) after NS-3 treated RNAs of people with T2DM.

| # | Genes ID  | Expr Log Ratio | Symbol |
|---|-----------|----------------|--------|
| 21 | hsa-miR-324-3p | -9.1 | miR-324-3p (miRNAs w/seed CCACUUC) |
| 22 | hsa-miR-576-3p | -8.0 | miR-576-3p (miRNAs w/seed AGAUGUG) |
| 23 | hsa-miR-374c-5p | -7.8 | miR-374c-5p (and other miRNAs w/seed UAUAAC) |
| 24 | hsa-miR-4326 | -5.9 | miR-4326 (miRNAs w/seed GUUCCUC) |
| 25 | hsa-miR-548l | -5.6 | miR-548l (miRNAs w/seed AAAGUAU) |
| 26 | hsa-miR-4646-3p | -4.8 | miR-4646-3p (miRNAs w/seed UUGUCCC) |
| 27 | hsa-miR-1292-5p | -4.6 | miR-1247-3p (and other miRNAs w/seed GGGAAACG) |
| 28 | hsa-miR-548aq-3p | -4.6 | miR-548aq-3p (and other miRNAs w/seed AAAAACU) |
| 29 | hsa-miR-5695 | -4.5 | miR-5695 (miRNAs w/seed CUCCCAAG) |
| 30 | hsa-miR-874-3p | -4.3 | miR-874-3p (miRNAs w/seed UGCCCCUG) |
| 31 | hsa-miR-320d | -2.6 | miR-320b (and other miRNAs w/seed AAAGCGUG) |
| 32 | hsa-miR-33b-5p | -2.6 | miR-33-5p (and other miRNAs w/seed UGCACUUG) |
| 33 | hsa-miR-326 | -1.6 | miR-330-5p (and other miRNAs w/seed CUCUOGG) |
| 34 | hsa-miR-636 | -1.4 | miR-636 (miRNAs w/seed GUGCUUG) |
| 35 | hsa-miR-744-5p | -1.4 | miR-744-5p (and other miRNAs w/seed GCCGGGCG) |
| 36 | hsa-miR-589-5p | -1.3 | miR-589-5p (and other miRNAs w/seed GAGAACC) |
| 37 | hsa-miR-618 | -1.3 | miR-618 (and other miRNAs w/seed AACUCUA) |
| 38 | hsa-miR-324-5p | -1.2 | miR-324-5p (miRNAs w/seed GCAUCCC) |
| 39 | hsa-miR-190b-5p | -1.2 | miR-190a-5p (and other miRNAs w/seed GAAUUGU) |
| 40 | hsa-miR-7-5p | -1.2 | miR-7a-5p (and other miRNAs w/seed GGAAGAC) |
| 41 | hsa-miR-223-3p | -1.1 | miR-223-3p (miRNAs w/seed GUCAGUU) |
| 42 | hsa-miR-501-3p | -1.1 | miR-501-3p (and other miRNAs w/seed AUUGCACC) |
| 43 | hsa-miR-197-3p | -1.0 | miR-197-3p (and other miRNAs w/seed UCACCC) |
| 44 | hsa-miR-487a-3p | -1.0 | miR-154-3p (and other miRNAs w/seed AUCAUAC) |
| 45 | hsa-miR-526b-3p | -1.0 | miR-17-5p (and other miRNAs w/seed AAAGUGC) |
| 46 | hsa-miR-184 | -1.0 | miR-184 (and other miRNAs w/seed GGACCGGA) |
| 47 | hsa-miR-9-5p | -1.0 | miR-9-5p (and other miRNAs w/seed CUUUGGU) |

266 Analysis ready miRNA; Up > 2 (95); Down > 2 (171)

*a* Location = cytoplasm

*b* Types = mature micro RNA
Table 14A:

IPA analysis of gene expression of mRNAs of "molecular functions" (up-regulated [42]) after NS-3 treated RNAs of people with T2DM.

| #  | ID               | Symbol     | Expr Log Ratio | Entrez Gene Name | Location     | Type(s)                  |
|----|------------------|------------|----------------|------------------|--------------|--------------------------|
| 1  | ENSG00000275215  | RNA5-8SN3  | 14.8           | RNA, 5.8S ribosomal N3 | Other        | other                    |
| 2  | ENSG00000201183  | RNVU1-3    | 14.5           | RNA, variant U1 small nuclear 3 | Other        | other                    |
| 3  | ENSG00000241069  | CTD_3141N221 | 12.6         | chondroitin sulfate proteoglycan 4 pseudogene 3 Y-linked | Other        | other                    |
| 4  | ENSG00000234648  | AL1621513  | 12.3           | chondroitin sulfate proteoglycan 4 pseudogene 3 Y-linked | Other        | other                    |
| 5  | ENSG00000273711  | RP5_10211208 | 11.9         | chondroitin sulfate proteoglycan 4 pseudogene 3 Y-linked | Other        | other                    |
| 6  | ENSG00000241588  | RN7SL484P  | 10.6           | chondroitin sulfate proteoglycan 4 pseudogene 3 Y-linked | Other        | other                    |
| 7  | ENSG00000279337  | CTD_2349P217 | 10.4         | chondroitin sulfate proteoglycan 4 pseudogene 3 Y-linked | Other        | other                    |
| 8  | ENSG00000203326  | ZNF525     | 10.2           | zinc finger protein 525 | Nucleus     | transcription regulator   |
| 9  | ENSG00000198538  | ZNF28      | 10.0           | zinc finger protein 28 | Nucleus     | transcription regulator   |
| 10 | ENSG00000211716  | TRBV9      | 10.0           | T cell receptor beta variable 9 | Plasma Membrane | other                    |
| 11 | ENSG00000235576  | LINC01871  | 9.8            | long intergenic non-protein coding RNA 1871 | Other        | other                    |
| 12 | ENSG00000276185  | TP53TG1_1_2 | 9.7           | chondroitin sulfate proteoglycan 4 pseudogene 3 Y-linked | Other        | other                    |
| 13 | ENSG00000282939  | TRBV7-2    | 9.7            | T cell receptor beta variable 7-2 | Other        | other                    |
| 14 | ENSG00000269981  | RP11_34P1316 | 9.6          | chondroitin sulfate proteoglycan 4 pseudogene 3 Y-linked | Other        | other                    |
| 15 | ENSG00000242616  | GNG10      | 9.5            | G protein subunit gamma 10 | Plasma Membrane | other                    |
| 16 | ENSG00000227191  | TRGC2      | 8.9            | T cell receptor gamma constant 2 | Other        | other                    |
| 17 | ENSG00000239951  | IGKV3-20   | 8.6            | immunoglobulin kappa variable 3-20 | Extracellular Space | other                |
| 18 | ENSG00000065518  | NDUFB4     | 8.5            | NADH:ubiquinone oxidoreductase subunit B4 | Cytoplasm   | transporter               |
| 19 | ENSG00000211801  | TRAV21     | 8.2            | T cell receptor alpha variable 21 | Other        | other                    |
| 20 | ENSG00000148484  | RSU1       | 4.6            | Ras suppressor protein 1 | Cytoplasm   | other                    |
| 21 | ENSG00000141232  | TOB1       | 4.1            | transducer of ERBB2, 1 | Nucleus     | transcription regulator   |
| 22 | ENSG00000170989  | SHPR1      | 4.0            | sphenamine-1-phosphate receptor 1 | Plasma Membrane | G-protein coupled receptor |
| 23 | ENSG00000060971  | ACAAI      | 3.9            | acetyl-CoA acyltransferase 1 | Cytoplasm   | enzyme                   |
| 24 | ENSG00000110324  | IL10RA     | 3.6            | interleukin 10 receptor subunit alpha | Plasma Membrane | transmembrane receptor |
| 25 | ENSG00000134539  | KLRD1      | 2.8            | killer cell lectin like receptor D1 | Plasma Membrane | transmembrane receptor |
| 26 | ENSG00000170458  | CD14       | 2.8            | CD14 molecule | Plasma Membrane | transmembrane receptor |
| 27 | ENSG00000172349  | IL16       | 2.5            | interleukin 16 | Extracellular Space | cytokine               |
| #  | ID                | Symbol                   | Expr Log Ratio | Entrez Gene Name                                      | Location       | Type(s)    |
|----|-------------------|--------------------------|----------------|--------------------------------------------------------|----------------|------------|
| 28 | ENSG00000063046   | EIF4B                    | 2.1            | eukaryotic translation initiation factor 4B            | Cytoplasm      | translation regulator |
| 29 | ENSG00000150045   | KLRF1                    | 2.1            | killer cell lectin like receptor F1                   | Plasma Membrane| transmembrane receptor |
| 30 | ENSG00000162011   | G6PD                     | 2.1            | glucose-6-phosphate dehydrogenase                      | Cytoplasm      | enzyme     |
| 31 | ENSG00000136888   | ATP6V1G1                 | 2.1            | ATPase H+ transporting V1 subunit G1                  | Cytoplasm      | transporter |
| 32 | ENSG00000147779   | TNFAIP8                  | 2.1            | TNF alpha induced protein 8                            | Cytoplasm      | other      |
| 33 | ENSG00000159128   | IFNGR2                   | 1.9            | interferon gamma receptor 2                            | Plasma Membrane| transmembrane receptor |
| 34 | ENSG0000027697    | IFNGR1                   | 1.9            | interferon gamma receptor 1                            | Plasma Membrane| transmembrane receptor |
| 35 | ENSG0000077238    | IL4R                     | 1.9            | interleukin 4 receptor                                 | Plasma Membrane| transmembrane receptor |
| 36 | ENSG00000185201   | IFITM2                   | 1.9            | interferon induced transmembrane protein 2            | Cytoplasm      | other      |
| 37 | ENSG00000110801   | PSMD9                    | 1.7            | proteasome 26S subunit, non-ATPase 9                   | Cytoplasm      | transcription regulator |
| 38 | ENSG0000014216    | CAPN1                    | 1.3            | calpain 1                                              | Cytoplasm      | peptidase  |
| 39 | ENSG0000099341    | PSMD8                    | 1.2            | proteasome 26S subunit, non-ATPase 8                   | Cytoplasm      | other      |
| 40 | ENSG000010955     | ATP5F1B                  | 1.0            | ATP synthase F1 subunit beta                           | Cytoplasm      | transporter |
| 41 | ENSG000014925     | ALDOA                    | 1.0            | aldolase, fructose-bisphosphate A                      | Cytoplasm      | enzyme     |
| 42 | ENSG0000105122    | RASAL3                   | 1.0            | RAS protein activator like 3                          | Cytoplasm      | other      |

Table 14B: IPA analysis of gene expression of mRNAs of "molecular functions" (down-regulated [17]) after NS-3 treated RNAs of people with T2DM.
|   | Gene ID       | Description                                           | Location       | Function          |
|---|--------------|-------------------------------------------------------|----------------|-------------------|
| 56| ENSG00000233461 | chondroitin sulfate proteoglycan 4 pseudogene 3 Y-linked | Other          | other             |
| 57| ENSG00000128829 | eukaryotic translation initiation factor 2 alpha kinase 4 | Cytoplasm      | kinase            |
| 58| ENSG00000103342 | G1 to S phase transition 1                            | Cytoplasm      | translation regulator |
| 59| ENSG00000267681 | chondroitin sulfate proteoglycan 4 pseudogene 3 Y-linked | Other          | other             |
Table 15A:

IPA analysis of paired (mRNA-miRNA) gene expression of "molecular functions" (up-regulated [38]) after NS-3 treated RNAs of people with T2DM.

| #  | Gene ID                | Expr Log Ratio | Symbol          | Entrez Gene Name                  | Location       | Type(s)            |
|----|------------------------|----------------|-----------------|-----------------------------------|----------------|------------------|
| 1  | ENSG00000203326        | 10.2           | ZNF525          | zinc finger protein 525           | Nucleus        | transcription regulator |
| 2  | ENSG00000198538        | 10.0           | ZNF28           | zinc finger protein 28            | Nucleus        | transcription regulator |
| 3  | ENSG00000242616        | 9.5            | GNG10           | G protein subunit 10              | Plasma Membrane| other            |
| 4  | ENSG0000065518         | 8.5            | NDUFB4          | NADH:ubiquinone oxidoreductase subunit B4 | Cytoplasm      | transporter       |
| 5  | ENSG00000128699        | 8.5            | ORMDL1          | ORMDL sphingolipid biosynthesis regulator 1 | Cytoplasm      | other            |
| 6  | ENSG00000160307        | 8.3            | S100B           | S100 calcium binding protein B    | Cytoplasm      | other            |
| 7  | ENSG00000248098        | 7.9            | BCKDHA          | branched chain keto acid dehydrogenase E1, alpha polypeptide | Cytoplasm      | enzyme           |
| 8  | ENSG00000155463        | 4.7            | OXAX1L          | OXAX1L mitochondrial inner membrane protein | Cytoplasm      | enzyme           |
| 9  | ENSG00000100241        | 4.6            | SBF1            | SET binding factor 1              | Plasma Membrane| phosphatase      |
| 10 | ENSG00000148484        | 4.6            | RSU1            | Ras suppressor protein 1          | Cytoplasm      | other            |
| 11 | ENSG00000666322        | 4.5            | ELOVL1          | ELOVL fatty acid elongase 1       | Cytoplasm      | enzyme           |
| 12 | ENSG00000114125        | 4.5            | RNF7            | ring finger protein 7             | Nucleus        | enzyme           |
| 13 | ENSG00000113328        | 4.4            | CCNG1           | cyclin G1                       | Nucleus        | other            |
| 14 | ENSG00000154473        | 4.4            | BUB3            | BUB3 mitotic checkpoint protein   | Nucleus        | other            |
| 15 | ENSG00000103254        | 4.2            | FAM173A         | family with sequence similarity 173 member A | Other           | other            |
| 16 | ENSG00000144895        | 4.2            | EIF2A           | eukaryotic translation initiation factor 2A | Cytoplasm      | translation regulator |
| 17 | ENSG00000153563        | 4.1            | CDA8           | CDA8 molecule                    | Plasma Membrane| other            |
| 18 | ENSG00000100796        | 4.0            | PPP4R3A         | protein phosphatase 4 regulatory subunit 3A | Plasma Membrane| other            |
| 19 | ENSG00000110324        | 3.6            | IL10RA          | interleukin 10 receptor subunit alpha | Plasma Membrane| transmembrane receptor |
| 20 | ENSG00000185627        | 2.6            | PSMD13          | proteasome 26S, non-ATPase 13     | Cytoplasm      | peptidase        |
| 21 | ENSG00000172349        | 2.5            | IL16            | interleukin 16                   | Extracellular Space| cytokine          |
| 22 | ENSG00000161921        | 2.5            | CXCL16          | C-X-C motif chemokine ligand 16   | Extracellular Space| cytokine          |
| 23 | ENSG00000275302        | 2.2            | CCL4            | C-X-C motif chemokine ligand 4    | Extracellular Space| cytokine          |
| 24 | ENSG00000128272        | 2.1            | ATF4            | activating transcription factor 4  | Nucleus        | transcription regulator |
| 25 | ENSG00000120129        | 2.0            | DUSP1           | dual specificity phosphatase 1    | Nucleus        | phosphatase      |
| 26 | ENSG00000077238        | 1.9            | IL4R            | interleukin 4 receptor            | Plasma Membrane| transmembrane receptor |
| #  | Gene ID         | Expr Log Ratio | Symbol      | Entrez Gene Name                                      | Location        | Type(s)               |
|----|----------------|----------------|-------------|-------------------------------------------------------|-----------------|-----------------------|
| 27 | ENSG00000070831 | 1.5            | CDC42       | cell division cycle 42                                 | Cytoplasm       | enzyme                |
| 28 | ENSG00000204389 | 1.5            | HSPA1A/HSPA1B | heat shock protein family A (Hsp70) member 1A         | Cytoplasm       | enzyme                |
| 29 | ENSG000000125818| 1.5            | PSMF1       | proteasome inhibitor subunit 1                         | Cytoplasm       | other                 |
| 30 | ENSG00000010278 | 1.4            | CD9         | CD9 molecule                                           | Plasma Membrane | other                 |
| 31 | ENSG00000139318 | 1.3            | DUSP6       | dual specificity phosphatase 6                         | Cytoplasm       | phosphatase           |
| 32 | ENSG00000086061 | 1.3            | DNAJA1      | heat shock protein family (Hsp40) member A1            | Nucleus         | other                 |
| 33 | ENSG0000014216  | 1.3            | CAPN1       | calpain 1                                              | Cytoplasm       | peptidase             |
| 34 |                | 1.2            | COX4I1      | cytochrome c oxidase subunit 4I1                       | Cytoplasm       | enzyme                |
| 35 | ENSG00000163636 | 1.1            | PSMD6       | proteasome 26S subunit, non-ATPase 6                   | Cytoplasm       | enzyme                |
| 36 | ENSG00000130741 | 1.1            | EIF2S3      | eukaryotic translation initiation factor 2 subunit gamma | Cytoplasm       | translation regulator |
| 37 | upilumab,      | 1.0            | CCR7        | C-C motif chemokine receptor 7                         | Plasma Membrane | G-protein coupled receptor |
| 38 | ENSG00000168685 | 1.0            | IL7R        | interleukin 7 receptor                                 | Plasma Membrane | transmembrane receptor |

Table 15B: IPA analysis of paired (mRNA-miRNA) gene expression of "molecular functions" (down-regulated [4]) after NS-3 treated RNAs of people with T2DM.

Drug: Dupilumab, MDNA55

Drug: Recombinant human interleukin-7

Drug: PLX-51107, PLX-2853
Table 16 A-C:

Summary of Network based "Venn diagram" of IPA analysis of NS-3 treated RNAs of people with T2DM.

| #  | Name                                                                 | Total |
|----|-----------------------------------------------------------------------|-------|
| A. Paired mRNA-miRNA                                                                                      |
| 1  | Cell_Death_and_Survival                                               | 29    |
| 2  | Cell-To-Cell_Signaling_and_Interaction                               |       |
| 3  | Cellular_Compromise                                                  |       |
| 4  | Gene_Expression                                                       |       |
| 5  | RNA_Post-Transcriptional_Modification                               |       |
| 6  | Cellular_Growth_and_Proliferation                                    |       |
| 7  | Infectious_Diseases                                                  |       |
| 8  | Cell_Cycle                                                           |       |
| 9  | RNA_Damage_and_Repair                                                |       |
| 10 | DNA_Replication                                                       |       |
| 11 | Cellular_Function_and_Maintenance                                    |       |
| 12 | Cellular_Movement                                                    |       |
| 13 | Cell_Signaling                                                       |       |
| 14 | Cellular_Assembly_and_Organization                                   |       |
| 15 | Cellular_Development                                                 |       |
| 16 | Post-Translational_Modification                                     |       |
| 17 | Protein_Synthesis                                                    |       |
| 18 | Developmental_Disorder                                               |       |
| 19 | Hematological_System_Development_and_Function                        |       |
| 20 | Metabolic_Disease                                                    |       |
| 21 | Cancer                                                                |       |
| 22 | Inflammatory_Response                                                |       |
| 23 | Recombination                                                        |       |
| 24 | Cardiovascular_Disease                                               |       |
| 25 | Energy_Production                                                    |       |
| 26 | Hereditary_Disorder                                                   |       |
| 27 | Small_Molecule_Biochemistry and_Repair                               |       |
| 28 | and_Repair                                                           |       |
| 29 | Embryonic_Development                                                |       |
| B. mRNAs                                                                                               |
| 1  | Dermatological_Diseases_and_Conditions                               | 9     |
| 2  | Organismal_Injury_and_Abnormalities                                  |       |
| 3  | Amino_Acid_Metabolism                                                |       |
| 4  | RNA_Trafficking                                                       |       |
| 5  | Cell-mediated_Immune_Response                                        |       |
| 6  | Molecular_Transport                                                  |       |
| #  | Name                                                                 | Total |
|----|----------------------------------------------------------------------|-------|
| 7  | Inflammatory_Disease                                                |       |
| 8  | Lipid_Metabolism                                                    |       |
| 9  | Reproductive_System_Development_and_Function                        |       |
|    | C. miRNAs                                                           | 10    |
| 1  | Tissue_Morphology                                                   |       |
| 2  | Immunological_Disease                                               |       |
| 3  | Nervous_System_Development_and_Function                             |       |
| 4  | Nucleic_Acid_Metabolism                                            |       |
| 5  | Immune_Cell_Trafficking                                            |       |
| 6  | Cell_Morphology                                                     |       |
| 7  | Connective_Tissue_Disorders                                         |       |
| 8  | Lymphoid_Tissue_Structure_and_Development                           |       |
| 9  | Neurological_Disease                                               |       |
| 10 | Hematological_Disease                                              |       |