Fraxinus excelsior and Vitamin E Combination for NAFLD (FEVEN)

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Abstract

Objective: The combination of Vitamin E 400 IU soft gelatin capsule and *Fraxinus excelsior* 500 mg soft gelatin capsules (Ensules+) were evaluated for safety and efficacy as compared to Vitamin E 400 IU soft gelatin capsules in patients with NAFLD.

Material and Methods: The prospective, open-label, comparative, randomized, single centre clinical trial was conducted by Macleods Pharmaceuticals in collaboration with Muktai Hospital, Nashik. Total of 24 NAFLD patients were divided into 2 groups and received either Vitamin E (400 IU) soft gelatin capsule or Ensules+ (combination of Vitamin E 400 IU and *Fraxinus excelsior* 500mg) soft gelatin capsules BID for 8 weeks. The Grades of Liver steatosis after 8 weeks, liver function test (AST, ALT, ALP, bilirubin levels) and serum lipid profile (Tg, Tc, HDL, LDL) was evaluated at the end of the study.

Result: Nonalcoholic fatty liver disease (NAFLD) patients with Vitamin E and Ensules+ therapy BID for 8 weeks revealed no significant difference in the physical parameters like mean body temperature, pulse rate and blood pressure from baseline to end of the study.

Significant improvement in lipid profile and liver function parameters were observed in both the groups at the end of the study as compared to the baseline. The % improvement in Tc and LDL (3.27 % and 4.93% respectively) from baseline were significant in Ensules+ group (B) as compared to Vitamin E group (A) (1.10% and 1.13 % respectively), also HDL levels were significantly elevated (1.34 %) in Ensules+ group.

Percentage improvement in liver function parameters in group A patients treated with Ensules+ Capsule had higher AST (53.64%), ALT (59.53%) ALP (38.43%) and Bilirubin levels (56.54%) as compared to group B patients treated with vitamin E capsules.

5 Patients treated with Ensules+ (combination of Vitamin E 400IU and *Fraxinus excelsior* 500mg) soft gelatin capsules) showed significant regression with the fatty liver disease at the end of the study.

Conclusion: Combination of Vitamin E and *Fraxinus excelsior* (Ensules+) capsule were found to be better in improving the lipid profile and liver function parameters in patients with NAFLD. To the best of our knowledge, this is the first study which compares the combination with Vitamin E capsule and found to be more effective in treating patients with NAFLD.

Keywords: NAFLD, NASH, Vitamin E, *Fraxinus excelsior*, Ensules+

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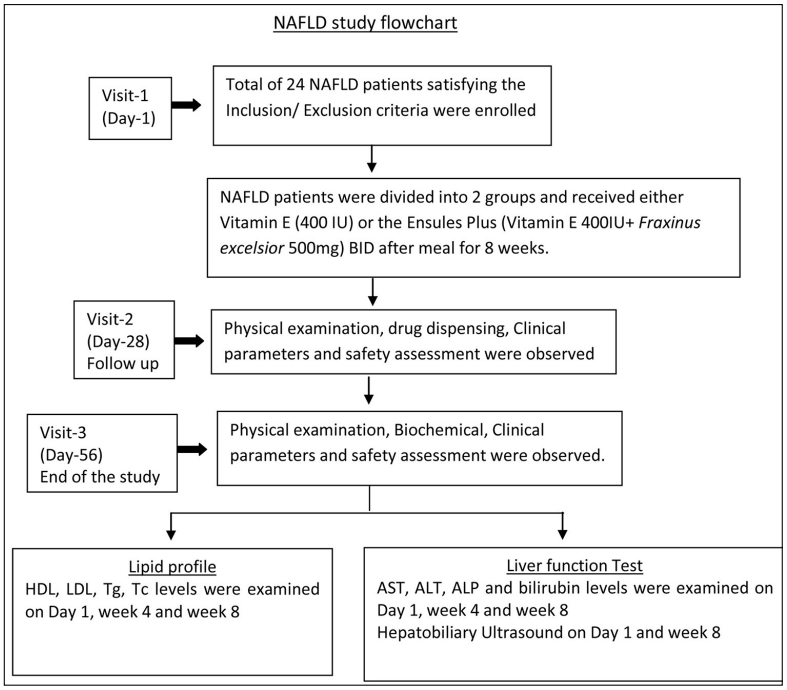
Summary

In the present study conducted by Macleods Pharmaceuticals Ltd, the combination of Vitamin E (400IU) and *Fraxinus excelsior* (500mg) [Ensules+] soft gelatin capsule was found to be effective in the treatment of patients with NAFLD.



Introduction

Nonalcoholic fatty liver disease (NAFLD) refers to the abnormal fat accumulation in the liver (hepatic steatosis) in the absence of excessive alcohol consumption.[1] NAFLD is the most common cause of chronic liver disease which refers to the spectrum of liver disease ranging from simple steatosis (fat accumulation in hepatocytes) to non-alcoholic steatohepatitis (NASH) which further progresses to cirrhosis and liver cancer.[1]

Worldwide prevalence of NAFLD ranges from 6.3% to 33% in the general population. In Asian countries, the prevalence (12-24%) of NAFLD varies in different areas, and is related to the age, gender, locality and ethnicity.[1] Prevalence of the NAFLD is estimated to be around 9-32% in the general Indian population, with a higher incidence rate amongst obese and diabetic patients. Around 56.5% of Type 2 diabetic individuals have NAFLD as per the study.[2] The prevalence of NAFLD increases to 57.5% - 74% in obese persons.[3]

NAFLD is the hepatic manifestation of the insulin resistance and the underlying risk factors in association with NAFLD includes obesity, type 2 diabetes mellitus, dyslipidaemia, hypertriglyceridemia, and hypertension.[3]

Currently, there is no definitive treatment for NAFLD. Although, the precise mechanism underlying disease progression from steatosis alone to NASH remains poorly understood, it is believed that oxidative stress plays a central role contributing to the hepatocellular injury. The effective therapeutic strategy is to target a reduction in oxidative stress in patients with this disease. Vitamin E (800 IU/day) is effective and recommended for NASH.[4]

The progression of the disease from simple steatosis to cirrhosis and further liver cancer is governed by 'Two- Hit Hypothesis'. The 'first hit' causes hepatic triglyceride accumulation (steatosis) which increases the susceptibility of the liver to injury mediated by 'second hits', such as inflammatory cytokines/ adipokines. This phenomenon accelerates the disease from simple steatosis to NASH (non-alcoholic steatohepatitis) and further non reversible cirrhosis and liver cancer. There are as yet few proven therapies available for patients with NASH, and current strategies are directed towards improving aspects of the metabolic syndrome. [5]

Vitamin E alone is not sufficient for the multifacet disease like NALFD. Vitamin E, being an antioxidant targets on NASH (Hit 2 stage); there is a need of drug which works on steatosis (Hit 1 stage) and other associated comorbidities (insulin resistance, hypertriglyceridemia).

Fraxinus excelsior Linné, Common European ash is a native to most of Europe and found in southwestern Asia. Historically, the trees' seeds and fruit have been used for food, condiments, and medicine for ailments ranging from arthritis, to carpal tunnel syndrome, to obesity. The leaves and bark of *Fraxinus excelsior* extracts contain considerable amounts of calcium malate, tannin, some free malic acid, mannit, dextrose, inosit, gum, quercitrin, and a very aromatic volatile oil, of the composition $C_{10}H_{20}O_2$. Oral administration of *Fraxinus excelsior L.* extract was found to inhibit renal glucose reabsorption with hypoglycaemic activity in normal and diabetic rats, and the hypoglycaemic effect in mice with type 1 diabetes mellitus has been reported to be independent of insulin secretion. *Fraxinus excelsior L.* extract were found to activate peroxisomeproliferator-activated receptor alpha and thereby helps in improv-

ing the lipids parameters.[6,7]

Evidence suggests that extracts of *Fraxinus excelsior L*. promotes insulin sensitivity and increases adiponectin-leptin ratio thereby reducing fat mass and body weight. *Fraxinus excelsior L* also exhibited beneficial effects in improving dyslipidemia (abnormal amounts of lipids in the blood).[8]

Since both *Fraxinus excelsior* and Vitamin E works on Hit 1 / steatosis stage (Triglyceride accumulation/, IR) and Hit 2 / Non-achoholic Steatohepatitis (oxidative stress and inflammation); the combination of Vitamin E and *Fraxinus excelsior* (Enslues$^+$) capsule were evaluated for safety and efficacy compared to Vitamin E capsule in patients with NAFLD.

Material and Methods

The study design:

The study was designed as a prospective, open-label, comparative, randomized, single centre clinical trial and conducted by Macleods Pharmaceuticals in collaboration with Muktai Hospital, Nashik. The Protocol of the study, CT-179-FVAC(F)-2015, Version No.: 01, Dated: 15th October 2015 and ICF (Informed consent form) was sent to Institutional Ethics Committee, Muktai Hospital, Nashik and the study was approved.

All the patients gave their written informed consent to take part in the trial; the procedures in the study have been performed according to Good Clinical Practice, following Standard Operating Procedures.

Inclusion/Exclusion Criteria

Male or female subjects who are 18 to 80 years of age; who were diagnosed with NAFLD using ultrasound and who were able to follow all the study directions and committed to come at all follow-up visits were included in the study. Ongoing oral medications not expected to interfere with study assessments were allowed if the subject was on a stable regimen. Individuals with >20 gr/day alcohol consumption or with infection (Hepatitis B/C or HIV) or other liver/ metabolic disease and known allergies, or sensitivity to any of the treatments given in the study were excluded.

Clinical Trial

A total of 24 patients who were diagnosed with non-alcoholic fatty liver disease (NAFLD) using ultrasoundwere considered eligible and accordingly randomized to take part in the study. The results of the clinical examination, the elevated level of liver enzymes, an ultrasonographic study of the liver were the basis for the diagnosis of NAFLD.

The patients with NAFLD were divided into 2 groups, each received either Vitamin E 400 IU soft gel capsuleor combination of Vitamin E 400 IU + *Fraxinus excelsior* 500mg soft gel capsule (Ensules$^+$), BID after the meal for 8 weeks and were asked to come for follow up visit on week 4 and 8 week/ end of the study. Patients were advised to bring all remaining capsules at each visit and accountability of each capsule was recorded. The compliance was found to be good, with the calculated compliance rates of >80 per cent for both studygroups. Clinical and biochemical parameters like serum lipid profile and liver function tests were performed on Visit 1 (Day 1), Visit 2 (week 4) and Visit 3 (week 8)/ end of the study and ultrasonographic measurement of the liver and the spleen were performed at Visit 1 (Day 1) and Visit 3 (8 weeks)/ end of the study in NAFLD patients. The grade of steatosis and the size of the spleen were measured by the radiologist, blinded to the treatment method of the patients. The efficacy and safety of Ensules$^+$ Capsules and Vitamin E capsules were evaluated in NAFLD patients.

Clinical and Biochemical parameters

Blood samples were collected during each visit 1, 2 (Week 4) and 3 (Week 8) end of the study by phlebotomist from NAFLD patients.

The serum sample of the patients were analyzed for Tc (normal 140-250 mg/dl), Tg (normal Up to190 mg/dl), LDL (normal 75-165 mg/dl), HDL (normal 30-70 mg/dl), bilirubin (normal 0.2–1.1 mg/dL), ALT/ SGPT (normal 8-40 IU/L), AST/SGOT (normal 5-50 IU/L), ALP (normal 37–147 U/L) were estimated using Bio-chem Analyser, Aspan star 21 plus, RT -1904.

Statistical analysis

Data were presented as mean ± SD, median. Statistical analysis of the data was carried out using SAS® version 9.4 software. The value of $p < 0.05$ was considered as statistically significant.

Result

Demographic data:

Total 24 patients with NAFLD were divided into 2 groups; Group A- Vitamin E (400IU) and Group B: Ensules$^+$ soft gelatin capsule [combination of Vitamin E (400 IU) and *Fraxinus excelsior* (500mg)]. The demographic data of the patients were documented during enrollment for the study.

The mean age of the patients in Group A was 41 years and the mean weight of patients was 67.7 kg for patients treated with Vitamin E 400 IU Soft Gelatin Capsules, respectively. Out of 12 patients, 01 was male

and 11 were females. The mean age of the patients in Group B was 45.8 years and the mean weight of patients was 70.3 kg for patients treated with a combination of Vitamin E and *Fraxinus excelsior* Soft Gelatin Capsule, respectively. Out of 12 patients 05 were males and 07 were females [Table 1].

Parameters	Group A	Group B
No. of patients	12	12
Age (yrs) Mean/SD range	23-59 yrs, 41.0 ± 13.11	33-60 yrs, 45.8 ± 10.66
Weight (kg) Mean/SD range	67.7kg ± 7.75	70.3kg ± 8.39
Height (cm)	160.8 cm ± 5.89	162 cm ± 8.01
BMI (kg/cm^2)	25.6 kg/cm^2 ± 3.09	26.4kg/cm^2 ± 2.71
Sex	Males=01; females= 11	Males=05; females=07

Table1: Demographic data of the patients with NAFLD, enrolled in the study conducted for 8 weeks. Group A- Vitamin E (400IU); Group B: Ensules$^+$ (Combination of Vitamin E 400 IU and *Fraxinus excelsior* 500mg soft gelatin capsules).

Physical Parameters

Physical parameters like body temperature, pulse rate and blood pressure were assessed in group A and group B patients enrolled during the start of the study, follow-up and end of the study.

When compared with Group A and Group B individuals; No significant difference was observed in the mean body temperature, pulse rate and blood pressure in NAFLD patients from baseline toend of the study [Table 2].

Changes in the Lipid Profile parameter:

The serum samples were collected at the baseline, before the start of the study (Day 1) and after the end of the study (week 8), from Group A and Group B patients treated with Vitamin E 400 IU soft gelatin capsules and Ensules$^+$ (Combination of Vitamin E 400 IU and *Fraxinus excelsior* 500mg soft gelatin capsules) respectively and the serum lipid profile parameters [Tg (mg/dl), Tc (mg/dl), LDL(mg/dl), HDL(mg/dl)] were evaluated.

Total Cholesterol (Tc) (mg/dl)

The Mean Tg and Tc (mg/dl) in group A (Vitamin E (400IU) soft gelatin capsules) and group B (Ensules$^+$ (Combination of Vitamin E 400 IU and *Fraxinus excelsior* 500mg soft gelatin capsules)) patients were evaluated. At the end of the study (Week 8), thepercent improvement in Tc was 3.27% significantly higher (*P*-Value: 0.0129 respectively) in group B patients with Ensules$^+$ than group A patients (1.10%) with Vitamin E capsules [Table 3 and Figure 1].

Group	Tg (mg/dl)			Tc (mg/dl)		
	Day 1: Baseline	Week 8:End of the study	% Improvement in Triglyceride from Baseline	Day 1: Baseline	Week 8: End of the study	% Improvement in Total Cholesterol from Baseline
Group A	104.64 ±11.16	100.33 ±7.23	4.12 %	171.6 ± 14.02	169.72 ±8.02	1.10 %
Group B	116.05 ±17.89	112.22 ±12.69	3.30 %	185.83 ± 19.86	179.75 ± 11.95	3.27%

Table 3: Tg (mg/dl) and Tc (mg/dl) levels were evaluated from the serum sample collected at day 1 (baseline) and week 8 (end of the study) of patients and Data were presented as mean ± SD. Group A: Vitamin E (400IU) soft gelatin capsules; Group B: Ensules$^+$ (Combination of Vitamin E 400 IU and *Fraxinus excelsior* 500mg soft gelatin capsules).

Low density lipoprotein (LDL) and High density lipoprotein (HDL)

The Mean LDL and HDL (mg/dl) in group A (Vitamin E (400 IU) soft gelatin capsules) and group B (Ensules$^+$ (Combination of Vitamin E 400 IU and *Fraxinus excelsior* 500mg soft gelatin capsules)) pa-

Groups	Mean Temperature (Mean ± SD) ^{0}F			Mean Pulse Rate (Mean ± SD) (beats/Per minute)			Blood pressure in mm Hg (SBP/DBP)		
	Day-1	Week 4	Week 8	Day-1	Week 4	Week 8	Day-1	Week 4	Week 8
A	98.29± 0.254	98.03 ± 0.379	97.98± 0.314	71± 1.28	74.3± 3.58	77.8± 1.99	129.3± 5.14/ 83.7± 2.67	137.3± 6.29/ 80.2± 3.95	138.7± 3.55/ 81.5± 3.83
B	98.4± 0.357	98.21± 0.454	98.23± 0.339	71.3± 1.36	75.1± 3.48	74.9± 3.23	129.8± 4.71/ 83.3±1.56	135.3±5.68/ 81.2± 5.49	135.2± 6.35/ 81.2± 5.01

Table 2- Mean temperature, pulse rate and blood pressure of NAFLD patients were observed during the enrollment, follow-up and end of the study period. Group A: Vitamin E (400IU) soft gelatin capsules; Group B: Ensules+ (Combination of Vitamin E 400 IU and *Fraxinus* excelsior 500mg soft gelatin capsules).

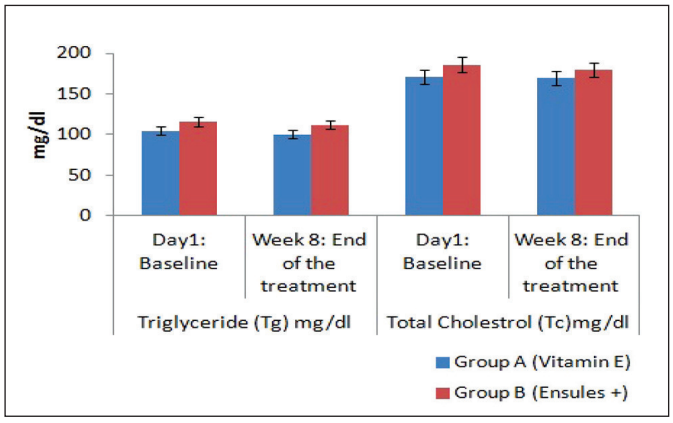


Figure 1: For Triglyceride (Tg) (mg/ dl) and total cholesterol (Tc) (mg/dl) parameter, Group A vs Group B shown significant *P* values (*P*-Value: 0.0058 and *P*-Value: 0.0129 respectively) by using Student's t-test between the Treatment Groups. Group A: Vitamin E (400IU); Group B: Ensules⁺ (Combination of Vitamin E 400 IU and *Fraxinus excelsior* 500mg soft gelatin capsules).

tients were evaluated. At the end of the study (Week 8), the reduction in LDL was found to be significantly higher (*P*-Value: 0.0273) in group B patients (4.93%) with Ensules⁺ than group A patients (1.13%) with Vitamin E capsules. Whereas the HDL level was found to be significantly elevated (*P*-Value: 0.0399) in group B patients (1.34%) with Ensules+ than group A patients (0.45%) with Vitamin E capsules [Table 4 and Figure 2].

Changes in the liver function parameter:

The serum samples were collected at the baseline, before the start of the study (Day 1) and after the end of the study (week 8), from group A and group B patients treated with Vitamin E and Ensules⁺ (Combination of Vitamin E 400 IU and *Fraxinus excelsior* 500mg soft gelatin capsules) respectively and the liver function parameters [AST/ SGOT (U/L), ALT/SGPT (U/L), ALP (U/L) and bilirubin (mg/dl)] were evaluated.

Group	LDL (mg/dl)			HDL (mg/dl)		
	Day 1: Baseline	Week 8:End of the study	% Improvement in LDL(mg/dL) from Baseline	Day 1: Baseline	Week 8:End of the study	% Improvement in HDL (mg/dL) from Baseline
Group A	108.41±10.46	107.18 ±7.38	1.13 %	42.31 ±3.83	42.5 ±2.28	0.45%
Group B	119.06 ±16.97	113.19 ±8.58	4.93 %	43.58 ±2.75	44.17 ±1.68	1.34 %

Table 4: LDL (mg/dl) and HDL (mg/dl) levels were evaluated from the serum sample collected at day 1 (baseline) and week 8 (end of the study) of patients and Data were presented as mean ± SD. Group A- Vitamin E (400IU) soft gelatin capsules; Group B: Combination of Vitamin E (400 IU) and *Fraxinus* excelsior (500mg) soft gelatin capsules (Ensules⁺).

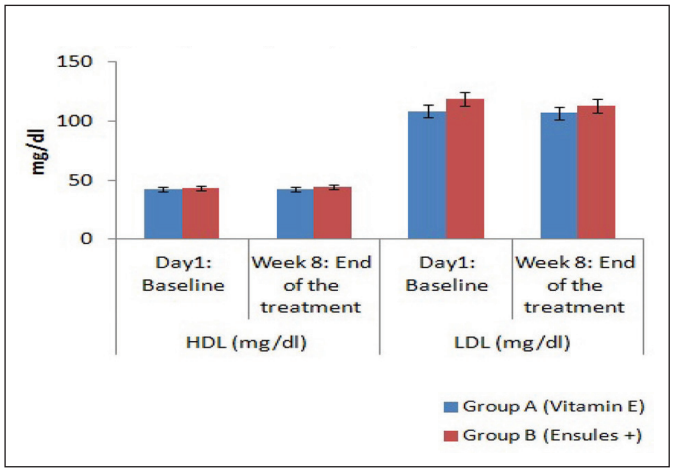


Figure 2: For HDL and LDL (mg/dl) parameters, Group A vs Group B shown significant *P* values (*P*-Value: 0.0273 and *P*-Value: 0.0399 respectively) by using Student's t Test between the Treatment Groups. Group A: Vitamin E (400 IU); Group B: Ensules⁺ (Combination of Vitamin E 400 IU and *Fraxinus excelsior* 500mg soft gelatin capsules).

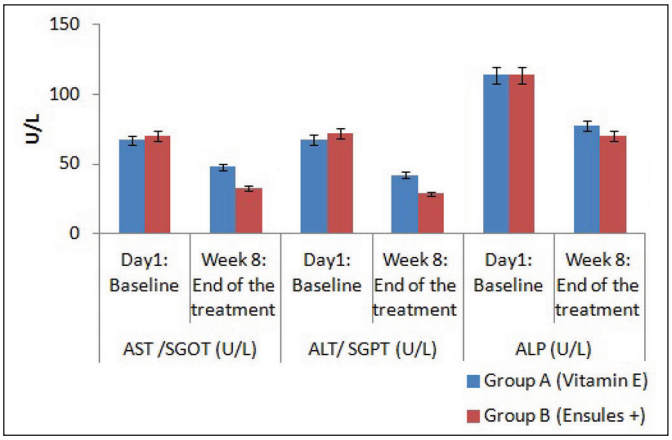


Figure 3: The statistical analysis of ASTS/GOT, ALT/SGPT and ALP (UL) parameters between Group A and Group B exhibited significant difference in *P*- values (*P*-Value: 0.0002, *P*-Value: 0.0007 and *P*-Value: 0.0044 respectively) by using Student's t-Test between the treatment groups. Group A: Vitamin E (400IU); Group B: Ensules⁺ (Combination of Vitamin E 400 IU and *Fraxinus excelsior* 500mg soft gelatin capsules).

Group	AST /SGOT (U/L)			ALT/ SGPT (U/L)			ALP (U/L)		
	Day 1	Week 8	AST/ SGOT Improvementi n percent	Day 1	Week 8	ALT/ SGPT improvement in percent	Day 1	Week 8	ALPimprovem ent in percent
Group A	67.05± 13.76	47.98 ± 10.76	28.45 %	67.50 ± 13.07	42.30 ± 10.80	37.33 %	113.53 ± 13.16	77.53 ± 7.68	31.71 %
Group B	70.04± 13.52	32.47 ± 6.18	53.64 %	71.58 ± 13.78	28.97 ± 5.26	59.53 %	113.66 ± 15.74	69.98± 4.42	38.43 %

Table 5: AST/ SGOT (Normal Range: 5-50 IU/L), ALT/SGPT (Normal Range: 8-40 IU/L), ALP (Normal Range: 37-147 IU/L) were evaluated from the serum sample collected at day 1 (baseline) and week 8 (end of the study) of patients and data were presented as mean ± SD. Group A: Vitamin E (400IU) soft gelatin capsules; Group B: Combination of Vitamin E (400 IU) and *Fraxinus excelsior* (500mg) soft gelatin capsules (Ensules⁺).

AST/ SGOT, ALT/SGPT and ALP(U/) parameters:

The mean serum AST/ SGOT, ALT/SGPT and ALP(U/) levels were evaluated ingroup A (vitamin E capsule) and group B (Ensules⁺) patients at the end of the study (Week 8). The percent improvement in AST/ SGOT, ALT/SGPT, ALP levels were higher (P-Value: 0.0002, *P*-Value: 0.0007 and *P*-Value: 0.0044 respectively) in group B patients (53.64%, 59.53%, 38.43% respectively) with Ensules⁺ than group A patients (28.45 %, 37.33%, 31.71% respectively) with Vitamin E capsules [Table 5 and Figure 3].

Bilirubin levels (mg/dl):

The mean serum Bilirubin (mg/dl) levels were evaluated ingroup A (Vitamin E capsule) and group B (Ensules⁺) patients at the end of the study (Week 8). The percent improvement in Bilirubin levels were found to be higher (*P*-Value: 0.0000) in group B patients (56.54%) with Ensules⁺ than Group A patients (34.16%) with Vitamin E capsules [Table 6 and Figure 4].

The global assessment by an investigator for "Safety" and "Efficacy" of patients was observed by end of the treatment after physical, clinical and biochemical examination. The Group B patients treated with Ensules⁺ (Combination of Vitamin E 400 IU and *Fraxinus excelsior* 500mg soft gelatin capsules) had excellent improvement in Safety (8.33%) and Efficacy (33.33%) and good improvement in Safety (58.33%) and Efficacy (58.33%) respectively as compared to Group A patients who received vitamin E capsules.

The global assessment by patient for "Safety" and "Efficacy" of patients were observed by end of the treatment after physical, clinical and biochemical examination. The Group B patients treated with Ensules⁺ (Combination of Vitamin E 400 IU and *Fraxinus excelsior* 500mg soft gelatin capsules) had excellent improvement in Safety (8.33%) and Efficacy (16.67%) and good improvement in Safety (83.33%) and Efficacy (75%) respectively as compared to Group A patients who received vitamin E capsules.

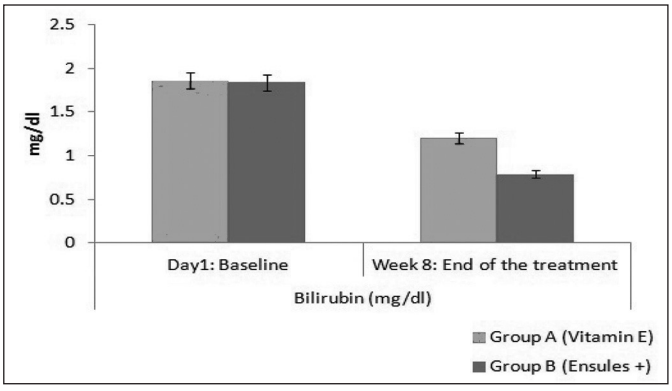


Figure 4: The statistical analysis of serum Bilirubin (mg/dl) levels between Group A and Group B shown a significant difference in *P*- values (*P*-Value: *P*-Value: 0.0000) by using Student's t-Test between the Treatment Groups. Group A: Vitamin E (400IU); Group B: Ensules⁺ (Combination of Vitamin E 400 IU and *Fraxinus excelsior* 500mg soft gelatin capsules).

Group	Bilirubin (mg/dl)		
	Day 1: Baseline	Week 8: End of the study	% Improvement in Bilirubin from Baseline
Group A	1.86 ± 0.31	1.22 ± 0.24	34.16 %
Group B	1.84 ± 0.37	0.80 ± 0.11	56.54 %

Table 6: Bilirubin levels (Normal Range: 0.2-1.1 mg/dl) were evaluated from the serum sample collected at day 1 (baseline) and week 8 (end of the study) of patients and Data were presented as mean ± SD. Group A: Vitamin E (400IU) soft gelatin capsules; Group B: Combination of Vitamin E (400 IU) and *Fraxinus excelsior* (500mg) soft gelatin capsules (Ensules⁺).

Discussion

Non-alcoholic fatty liver disease (NAFLD), which is highly prevalent worldwide, is the most common cause of chronic liver disease. The prevalence of NAFLD both in adults and children is likely to increase over time and continue to become a serious public health burden. [4]

Currently, there is no definitive

Group	Safety of the patient			Efficacy of the treatment		
	Excellent	Good	Poor	Excellent	Good	Poor
Group A patients (%)	0.00%	50.00%	50.00%	0.00%	58.33%	41.67%
Group B patients (%)	8.33%	58.33%	33.33%	33.33%	58.33%	8.33%

Table 7: Global assessment of treatment for safety and efficacy by investigator were evaluated in a tabular format.

Group	Safety of the patient			Efficacy of the treatment		
	Excellent	Good	Poor	Excellent	Good	Poor
Group A patients (%)	8.33%	41.67%	50%	8.33%	25%	66.67%
Group B patients (%)	8.33%	83.33%	8.33%	16.67%	75%	8.33%

Table 8: Global assessment of treatment for safety and efficacy by patient were evaluated in a tabular format.

treatment for this disease. The progression and pathogenesis of NASH is based on '2-hit hypothesis'. The 'first hit' causes hepatic triglyceride accumulation (steatosis) which thereby increases the susceptibility of the liver to injury mediated by 'second hits', such as oxidative stress and inflammatory cytokines which in turn lead to steatohepatitis and/or fibrosis.[4,5] The best treatment option would be targeting both hit 1 and hit 2 pathway simultaneously. Vitamin E is recommended for treatment of NASH (hit 2 pathway) and *Fraxinus excelsior* helps in reducing steatosis (hit 1 pathway).[5] The present study evaluates the safety and efficacy of combination of vitamin E and *Fraxinus excelsior* fixed dose formulation capsule (Ensules⁺ capsule) compared to vitamin E soft gelatin capsule in NAFLD patients.

In the present study, patients with NAFLD received treatment with either Vitamin E 400 IU soft gelatin capsule or Ensules⁺ (Combination of Vitamin E 400 IU + *Fraxinus excelsior* 500mg soft gelatin capsule BID for the period of 8 weeks. Physical, clinical and biochemical parameters were evaluated at the end of the study. Lipid profile (Tg, Tc, LDL, HDL) and liver function parameters (AST, ALT, ALP and Bilirubin) were examined at the end of the study.

A randomized controlled trial was conducted by *Sanyal AJ, et al* on Pioglitazone, Vitamin E, or placebo for Non-alcoholic Steatohepatitis (PIVENS), 2010; on 247 patients with NAFLD for the period of 96 weeks.

Vitamin E was found to be superior to placebo in reducing lipid profile parameters Tg, Tc, HDL, LDL levels (0.6mg/dl, 3.6mg/dl, 0.9mg/dl, 12mg/dl respectively) as compared to placebo and Pioglitazones. [10]

In our study; we also observed similar result in lipid profile parameters (reduction in Tg, Tc, LDL and elevation of HDL) in Group A patients treated with Vitamin E 400 IU BID for 8 weeks (percent improvement were 4.12%, 1.10%, 1.13%, 0.45% respectively); but when compared with Group B patient received Ensules⁺ (combination of Vitamin E 400 IU and *Fraxinus excelsior* 500mg) capsule BID for 8 weeks, there was significant reduction in Tg, Tc, LDL, (3.30%, 3.27%, 4.93 %) and elevation of HDL (1.34%) levels. From the study, it was observed that Ensules⁺ capsule was found to be more effective in improving the lipid profile parameters than vitamin E capsule in patients with NAFLD.

A meta-analysis of randomized controlled trials conducted by *Sato K, et al*; found vitamin E significantly improved liver function and histologic changes in patients with NAFLD/NASH. Vitamin E treatment with NASH (Non-alcoholic Stetatohepatitis) adult patients showed significant reductions in only AST of 13.91 U/L, ALT by 22.44U/L, ALP by 10. 39 U/L and steatosisby 0.67 U/L as compared tothe control treatment.[11]

In our study we observed similar reduction in the group A patients treated with Vitamin E capsules for 8 weeks where the patients showed improvement in AST levels by 28.45% ALT levels by 37.33%, ALP levels by 31.71% but when compared to group B patients treated with Ensules⁺ (combination of Vitamin E 400 IU and *Fraxinus excelsior* 500mg) capsule BID for 8 weeks, there was significant improvement inliver function parameters [AST (53.64%), ALT (59.53%) and ALP (38.43%)] than Group A patients with only vitamin E medication for NAFLD. From the study it was observed that Ensules⁺ capsule was found to be more effective in improving the liver function parameters (AST, ALT, ALP and Bilirubin) than Vitamin E capsule in patients with NAFLD.

A randomized, crossover, double-blind, placebo-controlled study was conducted by Zulet MA *et al* for a period of 7 weeks, to evaluate the beneficial effect of *Fraxinus excelsior L.* seeds/fruits extract in glucose homeostasis and adiposity related markers in elderly overweight/obese patients. The administration of an extract from *Fraxinus excelsior L.* seeds/fruits in combination with a moderate hypocaloric diet may be beneficial in metabolic disturbances linked to impaired glucose tolerance, obesity, insulin resistance and inflammatory status, specifically in older adults.[12]

From the studies; it was found that *Fraxinus excelsior* extract has a beneficial role in improving lipid

profiles and insulin resistance reducing steatosis. The global assessment for Safety and Efficacy of patients were observed after the end of the treatment after physical, clinical and biochemical examination; it was observed that patients treated with Ensules+ capsule BID for 8 weeks had better improvement in safety and efficacy parameters.

Conclusion

This study provides evidence that Ensules+ (combination of Vitamin E 400 IU and *Fraxinus excelsior*-500mg soft gelatin capsule) was found to be a clinically relevant pharmacologic approach and effective medication in treating patients with NAFLD.

Conflict of Interest:

There are no conflicts of interest. Dr. Chetan Patil, Dr. Ashish Mungantiwar and Priyanka Shrivastava, have authored this publication in the capacity of employees of Macleods Pharmaceuticals Ltd.

Financial support and sponsorship:

The study was funded and sponsored by Macleods Pharmaceuticals Ltd.

Acknowledgement:

We acknowledge the efforts of Dr. Sachin Kadoo, Dr. Soumen Roy, Dr. Naveen Parmar in getting this article published.

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