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A RANDOMISED, PROSPECTIVE PARALLEL AND OPEN LABEL STUDY TO COMPARE EFFICACY AND SAFTY OF SYNBIOTICES WITH TOCOTRIENOL IN NON ALCHOLIC FATTY LIVER DISEASE

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ABSTRACT

Background: Nonalcoholic fatty liver disease (NAFLD) covers spectrum of clinical entities ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) to cirrhosis. Its incidence is rising worldwide, seriously endangering human health, and its pathogenesis is still unclear. Increasing evidence has shown that intestinal flora plays an important role in the occurrence and development of NAFLD.

Aims and objectives: To compare the effectiveness of synbiotics and tocotrienol in NAFLD patients and to evaluate the safety and compliance of synbiotic and tocotrienol.

Materials & Methods: 60 patients of NAFLD attending the OPD and IPD of a tertiary care hospital. Group A was prescribed: Tocotrienol: 200 mg Twice a day and Group B was prescribed: Synbiotic - 5 Billion Colony Forming Units (BCFU) Twice a day. Liver Function Test and Ultrasound was done and compared on day 0,30,60 and 90.

Results: The mean age of NAFLD patient was 43.030±10.561 years in group A and 45.567±11.312 years in group B. Mean change in ALP (p= 0.930), GGT (p=0.132), direct bilirubin (p= 0.103) and total proteins (p= 0.564) remained statistically non-significant in both groups over 90 days of treatment. In both groups, mean change (decrease) in ALT from baseline became statistically significant (p=0.001) at 30 days and highly significant (p=0.0001) at 90 days 33% change is observed in USG scoring in Group B, 20% in Group A over 90 days of treatment.

Conclusion: Group B is more efficacious than group A which is shown by improvement in ultrasound grading, with statistical significant decrease in AST in group B and non significant changes in ALP, GGT between groups A and group B. However Group A drug is better tolerated than Group B.

Keywords: Non-alcoholic fatty liver disease, Synbiotics, Tocotrienol, LFT, Ultrasonography.

INTRODUCTION

NAFLD is a pathologic condition defined by the deposition of triglyceride (TG) in the liver greater than 5% of the total liver weight [1-3]. The term NAFLD encompasses a spectrum of pathologic conditions where the first stage is characterized by simple steatosis with liver fat accumulation in the hepatocytes [4]. The second stage is non-alcoholic steatohepatitis (NASH) characterized by hepatocyte injury due to inflammation, ballooning and possible collagen deposition. NASH is a progressive form of fatty liver that can worsen over time and may lead to cirrhosis and liver failure [2].

NAFLD is also an independent risk factor for extra-hepatic diseases, such as type 2 diabetes and cardiovascular disease [5,6]. There is recent growing interest in the role of gut microbiota in NAFLD pathogenesis, and there are several metaorganismal pathways linking altered gut microbiota (termed dysbiosis) and NAFLD [7-10]. Recent literature shows that the faecal microbial composition of patients with NAFLD differs from that of healthy individuals. Some studies showed a preponderance of Gram-*negative* bacteria, such as *Proteobacteria*, *Enterobacteriaceae* and *Escherichia* [11,12].

Currently, the only treatments for NAFLD recommended in the guidelines are lifestyle modifications which includes diet control, exercise, and weight reduction. All other pharmacological treatments are reserved for patients with biopsy-proven NASH and liver fibrosis although there is no approved drug for its treatment. According to previous studies, numerous pathophysiologic mechanisms relating the gut microbiome and NAFLD have been indicated, including the dysbiosis-induced dysregulation of the gut endothelial barrier function that allows for the translocation of bacterial components, leading to the accumulation fat and hepatic inflammation. Thus, using microbial therapy, including synbiotics, probiotics, and prebiotics, may help to restore the unbalanced microbiomes.[13,14]

AIMS & OBJECTIVES

This study was conducted to compare the effectiveness of synbiotics and tocotrienol in NAFLD patients and to evaluate the safety and compliance of synbiotic and tocotrienol.

Material and Methods

A total of 60 patients (after excluding the dropouts) of Non-alcoholic fatty liver disease (NAFLD) attending the OPD and IPD of a tertiary care hospital, after taking their written Informed Consent and approval from Institutional Ethics Committee were included in the study.

STUDY DESIGN: The present study was an open label, interventional, randomized, prospective, and parallel. Randomisation was carried out with the help of random numbers generated by computer software programmer (Random number generator).

The sample size was calculated by applying a formula using mean and standard deviation values from previous data. The sample size came out to be 24 in each group. So, in this study, we are taking a sample size of 30 in each group.

INCLUSION CRITERIA:

- NAFLD diagnosed by suggestive imaging findings (ultrasound) with/ without abnormal aminotransferase levels.
- Subjects between age group 20 – 65 years of either sex.

EXCLUSION CRITERIA:

- Patients with age group < 20 years and > 65 years.
- Pregnancy and lactation.
- Patients with hepatitis B & C and significantly deranged enzymes (ALT> 3 times normal values).
- Patients with end stage liver disease- i.e. Hepatocellular carcinoma.

- Chronic drug or alcohol abuse (daily intake > 20g (2.5 units) in females and 30g (3.5 units) in males.
- Patients on drugs causing fatty liver changes like – Amiodarone, Antiretroviral drugs, Acetaminophen, Corticosteroids, Methotrexate, Tamoxifen, and Tetracycline.
- Patients chronically on antibiotics.
- Patients refusing to give written informed consent.

Experimental Groups:
Patients underwent Randomization by using Graph Pad Quick Calcs and divided into 2 groups- Group A and Group B consisting of 30 patients each.

Group A was prescribed: Tocotrienol: 200 mg Twice a day

Group B was prescribed: Synbiotic - 5 Billion Colony Forming Units (BCFU) Twice a day containing Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus rhamnosus, Streptococcus thermophiles, Saccharomyces boulardii and Fructo Oligosccharide 100mg.
Assessment of Liver Function tests: was done by comparing the AST, ALT, ALP,GGT,Total Bilirubin,Direct Bilirubin,Total protein and albumin levels on day 0,30,60 and 90.
Ultrasound grading was done on day 0 and day 90.
Safety was assessed through ADR monitoring.

Results
65 patients were recruited in the present research work for a treatment of 90 days. There were 5 dropouts, who were lost to follow up.
Group A: Tocotrienol 200mg BD
Group B: Synbiotic 5BCFU BD

TABLE 1: COMPARISON OF AGE DISTRIBUTION IN GROUP 'A' AND 'B'

Age group (Years)	Group A		Group B	
	N	%	n	%
20-30	5	22.23	3	10
31-40	10	77.76	8	26.66
41-50	8	66.66	10	33.33
51-60	7	44.43	9	30
Total	30		30	
Mean % change	43.030±10.561		45.567±11.312	
p-value	0.374			

p> 0.05: Not significant *p<0.05: Significant **p<0.001: highly significant (p-value: chi square)
Table 1 Shows age variation between the two groups. Mean age in years was 43.030±10.561 in group A and 45.567±11.312 in group B. Both the groups were comparable with no statistical significance (p-value=0.374).

TABLE 2: COMPARISON OF GENDER DISTRIBUTION IN GROUP A AND B

Gender	Group A		Group B	
	n	%	n	%
Female	19	63.33	23	76.67
Male	11	36.67	7	23.33
Total	30	100.00	30	100.00
p-value	0.260			

p> 0.05: Not significant *p<0.05: Significant **p<0.001: highly significant (p-value: chi square) Gender distribution in both the groups is comparable (p=0.260).

TABLE 3: INTRAGROUP COMPARISON OF AST, ALT, other parameters OF PATIENTS IN GROUP A AND B OVER 90 DAYS OF TREATMENT

AST (IU/L)	Group A			Group B		
	Mean	±SD	p-value	Mean	±SD	p-value
Day 0	42.460±16.85			41.960±14.15		
Day 30	32.733±14.203		0.018	34.767±11.477		0.035
Day 60	28.467±10.470		0.001	22.120±8.920		0.001
Day 90	28.600±9.370		0.001	23.210±8.755		0.001
ALT (IU/L)						
Day 0	44.940±22.007			46.800±21.204		
Day 30	34.100±11.784		0.020	37.933±11.237		0.074
Day 60	31.533±11.518		0.001	32.733±10.346		0.001
Day 90	31.467±12.317		0.001	31.733±10.272		0.000
Alkaline Phosphatase (IU/L)						
Day 0	95.747±22.869			103.333±28.238		
Day 30	92.037±16.804		0.869	98.733±29.202		0.922
Day 60	88.160±16.417		0.399	94.000±27.809		0.578
Day 90	93.067±18.015		0.945	92.533±27.754		0.453
GGT (IU/L)						
Day 0	33.253±15.371			30.483±9.926		
Day 30	33.067±15.090		0.998	30.133±9.670		0.999
Day 60	33.467±16.051		0.997	29.500±9.836		0.980
Day 90	34.433±17.749		0.992	28.800±9.582		0.909
Total Bilirubin (mg/dl)						
Day 0	0.617±0.242			0.642±0.198		
Day 30	0.623±0.250		0.998	0.632±0.216		0.999
Day 60	0.671±0.258		0.890	0.611±0.223		0.890
Day 90	0.661±0.385		0.166	0.562±0.245		0.166

Mean change (decrease) in AST from baseline became statistically significant from 30 days onwards in both groups A and B over 90days of treatment. Mean change (decrease) in ALT from baseline became statistically significant (p=0.001) at 30 days and highly significant 60 day onwards. Similarly mean change (decrease) in ALT in group B became highly significant (p=0.0001) from 60 days onwards. Mean change in ALP from the baseline remained statistically non significant in both the groups over 90 days of treatment.Mean change in GGT from the baseline remained statistically non significant (p=0.132) in both the groups A and B over 90 days of treatment.Mean change in total bilirubin from the baseline remained statistically non significant in both the groups over 90 days of treatment.Mean change in direct bilirubin from the baseline remained statistically non significant in both the groups over 90 days of treatment.

TABLE 4: INTERGROUP COMPARISON OF LIPID LEVELS OF GROUP A AND GROUP B

Cholesterol (mg/dl)	Group A	Group B	p-value
	Mean±SD	Mean±SD	
Day 0	149.000±50.165	165.467±50.727	0.211
Day 90	137.967±36.513	150.767±47.964	0.250
Triglyceride (mg/dl)			
Day 0	148.300±85.581	165.867±75.160	0.402
Day 90	139.133±82.225	145.033±56.736	0.747
HDL (mg/dl)			

Day 0	43.467±4.967	42.400±5.876	0.451
Day 90	40.967±4.279	40.233±4.240	0.508
LDL (mg/dl)			
Day 0	85.167±31.521	107.400±23.949	0.003
Day 90	80.533±33.659	105.300±20.772	0.001

p> 0.05: Not significant *p<0.05: Significant **p<0.001: highly significant (p-value: paired t-test)
Mean change in lipid level (cholesterol, Triglyceride, and HDL) in both group A and B remained statistically non significant over 90 days. Mean change in LDL was statistically highly significant over 90 days between group A and B.

TABLE 5: SAFETY PROFILE GROUP A (TOCOTRIENOL) AND GROUP B (SYNBIOTIC)

Group A	ADR	Group B	ADR
Nausea	0	Gastritis	8 (26%)
Fatigue	0	Chills	0
Headache	1 (3%)	Bloating	1 (3%)
Bleeding(haemorrhoids)	1 (3%)	Others (itching on legs)	1 (3%)
Others (leg pain)	1 (3%)		
Total	3		10

10 ADRs were observed in group B and 3 ADRs were observed group A over 90 days of treatment.

DISCUSSION

The mean age in years of Group A is 43.030±10.56 and 45.567±11.312 in group B which is non significant (p< 0.37).This is on the lines of a metanalysis done by Balakrishnan et al in 2021 which included 54 studies. He found that, the mean age of NAFLD patients is 48 years.[15] Our study shows that the disease is more prevalent among women than men but not significant. However most of the studies show high prevalence among males. Majumdar conducted a study in Haryana in 2016 showing 33.3% prevalence among men than women (30.1%) had NAFLD.[16] Mean change in AST (42.460 to 28.60, -14IU/L) was significant (p=0.001) over 90 days of treatment in Group A. Present study is in agreement with the study (n=71) by Parvez et al (2018), Islamabad, Pakistan showing reduction in AST levels (61.06 to 52.16 -8.77IU/L) (p<0.001) at 12 weeks of treatment with δ-tocotrienol (600mg/day) for 12 weeks.[55] However Magasso (2013) (n=53) found a reduction in AST (37 to 32.2 -4.8IU/L) which was non significant (p=0.41) after Tocotrienol 200mg BD for 1year.[54]

The present study in group B found the reduction in AST levels (41.960 to 23.21 -18IU/L) to be significant (p=0.001) over 90 days of treatment. Present study is in agreement with study (n=2460) conducted in Tehran by Eslamprast et al(2014), who used 2BCFU of synbiotic Protexin for 7 weeks and observed a reduction (66.38 to 31.5IU/L -31.38IU/L) in AST levels which was significant (p=0.001).[64]

We did not observe a significant reduction (p = 0.992) in GGT levels in Group A (Table 9). A study by (n=71) Parvez et al (2018) conducted in Islamabad found significant (p=0.001) reduction in GGT (51.45 to 42.25,-9.2IU/L) by tocotrienol supplementation of 300 mg BD for 12weeks.[17]

20% change was seen in Group A over 90 days. Effect of tocotrienol was studied(2013) by Mogasso (n=34) who observed 50% reduction from grade 1 to 0 and 33% reduction from grade 2 to1 after 200mg BD tocotrienol supplementation for 1 year. 33.33% change was seen in Group B over 90 days. A study (n=80) by Ashgarian (2016) compared the synbiotic (500mg/d – Familact) with placebo for 8 weeks and showed that 50% and 25% of patients with mild (grade 1) and moderate (grade 2) NAFLD became normal, respectively.[20]

Number of ADRs reported in Group A (3) were less than Group B (10) over 90 days of treatment. In a study (2023) conducted by KN Owen used Vitamin E in the dose range of 100-1000 mg/day for 1 year and reported bleeding as a side effect.[21]

CONCLUSION

Group B is more efficacious than group A which is shown by improvement in ultrasound grading, with statistical significant decrease in AST in group B and non significant changes in ALT, ALP, GGT between groups A and group B.

Conflict of Interest: NIL

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