

# CLINICAL AND BIOCHEMICAL EFFECTS OF LIFESTYLE MODIFICATION WITH PENTOXIFYLLINE, VITAMIN E IN PATIENTS WITH NON-ALCOHOLIC STEATOHEPATITIS IN SULAIMANI



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## ABSTRACT

### *Background*

The burden of Non-Alcoholic Steatohepatitis is growing and current pharmacologic treatments are limited by adverse effects and inconsistent efficacy. Pilot studies suggest that Pentoxifylline and vitamin E can reduce degree and pattern of fatty liver, liver enzyme and inflammatory marker in patients with NASH.

### *Objectives*

Evaluation of the clinical, biochemical and ultrasonographic effects of pentoxifylline, and vitamin E in patients with non-alcoholic steatohepatitis.

### *Methods*

A total of 110 participants with NASH which diagnosed by ultrasonography fatty infiltration and raised aminotransferase level were included, only 90 participants completed the study. Demographic data, anthropometric measures such as Body Mass Index (BMI), waist/hip ratio were taken, liver function test, lipid profile, blood glucose, serum creatinine and High sensitivity C-reactive protein (Hs-CRP). The patients were divided into three comparable groups in age, gender and BMI. All enrolled participants were treated by lifestyle modification but pharmacologically they were randomly divided into three groups and followed for 6 months. Group 1; treated with placebo as a control group (starch prepared by capsule 400mg twice daily). Group 2; treated with vitamin E soft gelatin capsule 400 IU twice daily. Group 3; treated with pentoxifylline caplet 400mg twice daily.

### *Results*

Out of 110 participants, only 90 participants (48 male, 42 female) completed this study, with age range of 23-65 years with age mean was  $42.1 \pm 11.2$ . All the three groups showed decreased steatosis by ultrasonography and a significant reduction in aminotransferase levels, Hs-CRP, lipid profile, blood glucose, blood pressure and waist circumference compared to baseline. In the pentoxifylline group, side effects such as nausea, heartburn and dizziness were noted while in vitamin E group; abdominal pain and blurred vision were recorded after the second visit.

### *Conclusions*

Pentoxifylline or Vitamin E with dietary changes and exercise led to significant improvement in hepatic steatosis indicated by ultrasonography, reduction in aminotransferase levels and improved inflammatory marker (Hs-CRP).

**Keywords:** *NASH, Lifestyle, Pentoxifylline, Vitamin E, KCGH, Sulaimani.*

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## INTRODUCTION

Non-Alcoholic Steatohepatitis is defined as a presence of hepatic steatosis and inflammation with hepatocyte damage (ballooning) with or without fibrosis. This has the ability to progress to cirrhosis, liver failure and rarely liver cancer <sup>(1)</sup>.

The most common chronic liver diseases in the western countries are the non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH). They are closely associated with obesity, type2 diabetes mellitus, and metabolic syndrome. The epidemics of diabetes and obesity have also fueled an increasing prevalence of fatty liver disease <sup>(2)</sup>.

Both NAFLD and NASH are associated with an often asymptomatic elevation of serum alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT). Ultrasound monitoring can suggest the presence of a fatty infiltration of the liver; differentiation between NAFLD and NASH, however, often requires a liver biopsy. Such differentiation is important because NASH is associated with a much higher risk of liver fibrosis and cirrhosis than NAFLD <sup>(3)</sup>.

Maintaining a healthy weight through dietary changes and exercise the use of pharmacologic agents such as anti-oxidants, anti-tumor necrosis factor (TNF) and lipid lowering agents have been the most widely recommended treatments.

## PATIENTS AND METHODS

This is a prospective follow up study. It was conducted at Kurdistan Center for Gastroenterology and Hepatology (KCGH) and Central Laboratory in Sulaimani Governorate during 14 months period from April 2015 to June 2016.

The study started after it has been approved by the Ethical committee of the faculty of the Medical Sciences. Informed consent was taken from all participants.

A total of 110 participants were included, only 90 participants completed the study. All participants were asked to attend the hospital after an overnight fasting, a standard questionnaire was designed which include demographic data (name, age, gender, and ethnicity... etc) clinical data and relevant behavior (duration of hypertension, smoking history, drinking consumption, and any chronic medication). Anthropometric measurements were taken including; weight, height, BMI, waist and hip circumference.

Participants were sent for the following investigations: Transabdominal ultrasonography (U/S) using (ACUSON X 300); all U.S evaluations were performed by the same expert radiologist.

On U/S, the radiologist concentrated on the degree of fatty liver (mild, moderate and severe) and whether it is homogeneous (diffuse) or non- homogeneous (patchy).

From each participant 10 ml of blood taken for laboratory investigations, these parameters (ALT, AST, GGT, T.Cholesterol, TG, LDL, HDL, S.cr and B.glucose), by (KENZA 240TX), Hs-CRP was measured by (Cobas c 311/501 analyzer).

NASH was diagnosed based on abdominal U/S and elevated liver transaminases (ALT, AST) <sup>(4)</sup>.

Inclusion criteria included participants Age above 18 years with fatty liver approved by U/S and serum level ALT > 45 IU/L for male, > 30 IU/L for female. Exclusion Criteria were frequently alcohol intake, diabetic patients, pregnant, congestive heart failure, renal failure and severe lung disease. Participants on drugs that may cause fatty liver disease (e.g. methotrexate, Amiodarone...etc). History of hypersensitivity to PTX or the Methylxanthines or vitamin E, history of cerebral or retinal hemorrhage.

All enrolled participants in the study were treated by Life Style Modification. Pharmacologically the participants were randomly divided into three comparable groups (age and gender matched), each group contained 30 participants and followed for 6 months.

Group 1; treated with placebo as a control group (starch prepared by capsule 400mg BID). Group 2; treated with vitamin E soft gelatin capsule 400 IU BID. Group3; treated with Pentoxifylline (PTX) caplet 400 mg BID. All the above parameters were recorded at baseline, after 12 weeks, and after 24 weeks.

All participants were asked to record any side effects during the study and specifically asked about the most commonly reported side effects (nausea, headache, heartburn, abdominal pain, blurred vision .etc)

During the study period, the patients were contacted regularly (every 2 weeks) to check up their adherence to lifestyle modification and the medications.

### Statistical analysis

Data were entered in to excel sheet, then transferred to Statistical Package for the Social Sciences-version 21.

Descriptive analysis (numbers, percentages, means and standard deviation) was performed for all variables. The analysis was conducted to find association and differences between compared variables by using One-Way ANOVA, t- test, chi- square and Fisher exact test. The P value  $\leq 0.05$  was regarded as a significant level.

**RESULTS**

A total of 90 cases were enrolled in the study. 48 participants (53 %) were males and 42 (47 %) were females, (Figure 1). The age of participants ranged between 23-65 years. The mean age among different study groups were comparable (P value: 0.183), Table 1.

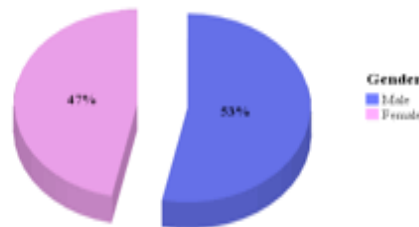
Table 2 shows the waist circumference, but not BMI,

was significantly reduced in all three groups.

The degree of fatty liver was decreased significantly ( $P \leq 0.001$ ) among the study groups, Table 3. Reduced ALT, AST and GGT level were highly significant ( $P \leq 0.001$ ) among the 3 groups, Table 4.

Hs-CRP level was significantly reduced in the placebo group ( $P=0.025$ ) and was highly significantly reduced in vitamin E and pentoxifylline groups ( $P<0.001$ ), Table 5.

The adverse effects were recorded during the study periods, Table 6.



**Figure 1. Gender distribution among the study population.**

**Table 1. Mean age (year) among study groups.**

<b>Groups</b>	<b>Mean Age <math>\pm</math> SD.</b>	<b>P value</b>
<b>Placebo</b>	42.13 $\pm$ 10.61	0.183
<b>Vitamin E</b>	44.73 $\pm$ 11.62	
<b>Pentoxifylline</b>	39.40 $\pm$ 11.05	
<b>Average</b>	42.08 $\pm$ 11.19	

Table 2. BMI and waist circumference at the baseline, after 12 and 24 weeks.

Variables	Placebo	Vitamin E	Pentoxifylline	
	Mean ± SD	Mean ± SD	Mean ± SD	
<b>BMI kg/m<sup>2</sup></b>	<b>Baseline</b>	32.14±3.73	33.60±4.71	35.21±3.67
	<b>After 12 wks</b>	31.48±3.41	32.64±4.75	34.38±3.63
	<b>After 24 wks</b>	31.25±3.45	32.27±4.9	33.77±3.65
	<b>Average</b>	31.62±3.51	32.84±4.77	34.46±3.66
	<b>P values</b>	0.60	0.539	0.313
<b>Waist C. cm</b>	<b>Baseline</b>	107.36±10.54	111.00±11.06	113.26±9.08
	<b>After 12 wks</b>	103.00±9.98	106.53±9.8	109.06±8.99
	<b>After 24 wks</b>	100.00±8.44	102.92±6.64	104.66±7.76
	<b>Average</b>	103.45±10.05	106.90±9.87	109.00±9.24
	<b>P values</b>	0.016	0.006	0.001

Table 3. Degree of fatty liver, at the baseline, after 12 and 24 weeks.

U/S	Degree of Fatty Liver				P values	
	Normal N (%)	Mild N (%)	Moderate N (%)	Severe N (%)		
<b>Placebo</b>	<b>Baseline</b>	0(0.0%)	10(31.3%)	20(43.5%)	0(0.0%)	0.001
	<b>After 12 wks</b>	2(16.7%)	10(31.3%)	18(39.1%)	0(0.0%)	
	<b>After 24 wks</b>	10(83.3%)	12(37.5%)	8(17.4%)	0(0.0%)	
	<b>Average</b>	12(100%)	32(100%)	46(100%)	0(0.0%)	
<b>Vitamin E</b>	<b>Baseline</b>	0(0.0%)	6(13.0%)	22(61.1%)	2(100%)	<0.001
	<b>After 12 wks</b>	2(33.3%)	16(34.8%)	12(33.3%)	0(0.0%)	
	<b>After 24 wks</b>	4(66.7%)	24(52.2%)	2(5.6%)	0(0.0%)	
	<b>Total</b>	6(100%)	46(100%)	36(100%)	2(100%)	
<b>PTX</b>	<b>Baseline</b>	0(0.0%)	4(12.5%)	22(50.0%)	4(100%)	<0.001
	<b>After 12wks</b>	0(0.0%)	14(43.8%)	16(36.4%)	0(0.0%)	
	<b>After 24wks</b>	10(100%)	14(43.8%)	6(13.6%)	0(0.0%)	
	<b>Average</b>	10(100%)	32(100%)	44(100%)	4(100%)	

Table 4. Liver enzymes at the baseline, after 12 and 24 weeks of treatment.

Variables		Placebo	Vitamin E	Pentoxifylline
		Mean ± SD	Mean ± SD.	Mean ± SD
ALT U/L	Baseline	62.43±19.19	56.53±14.26	59.46±16.69
	After 12 wks	44.33±19.05	36.66±14.68	41.60±15.9
	After 24 wks	35.31±13.55	29.13±13.07	30.00±12.69
	Average	47.49±20.68	40.77±18.09	43.68±19.34
P values		<0.001	<0.001	<0.001
AST U/L	Baseline	46.80±33.30	37.33±15.39	41.20±10.74
	After 12 wks	32.40±12.38	31.20±13.84	34.00±7.77
	After 24 wks	27.20±10.87	24.80±10.84	24.13±6.88
	Average	35.56±22.87	31.11±14.3	33.11±11.05
P values		0.002	0.002	<0.001
GGT U/L	Baseline	42.66±24.66	35.33±21.19	52.53±32.48
	After 12 wks	28.60±19.79	23.80±12.54	37.46±19.94
	After 24 wks	24.31±15.76	17.13±6.11	25.93±15.14
	Average	31.94±21.7	25.42±16.34	38.64±25.84
P values		0.002	<0.001	<0.001

Table 5. Hs-CRP level at the baseline, after 12 and 24 weeks of treatment.

Variables		Placebo Mean ± SD.	Vitamin E Mean ± SD.	Pentoxifylline Mean ± SD
Hs-CRP mg/dl	Baseline	7.39±2.53	6.72±1.82	9.66±3.94
	After 24 wks	5.74±2.99	3.81±1.48	4.02±2.26
	Average	6.56±2.87	5.26±2.20	6.84±4.27
P values		0.025	<0.001	<0.001

Table 6. Recorded Adverse effects among different groups.

Side effects	Placebo	Vitamin E	Pentoxifylline	Total
Nausea	0	0	4	4
Heartburn	0	0	2	2
Dizziness	0	0	1	1
Abdominal pain	0	2	0	2
Blurred Vision	0	1	0	1
Total	0	3	7	10

## DISCUSSION

Prior randomized follow-up studies of PTX and Vit E in NASH demonstrated improvement in waist circumference, degree and pattern of fatty liver by U/S, serum aminotransferase and Hs-CRP with a dose of (PTX 400mg) and (Vit E 400IU) twice daily for 6 months<sup>(5)</sup>.

In this study, NASH was more common (statistically not significant) in male (53%) than female (47%), as Hosseinpanah *et al*, in Iran<sup>(6)</sup>. while in another study done by Akbar and Kawther, in Saudi Arabia found that presence of NASH was significantly associated with female gender<sup>(7)</sup>.

The current study showed that more than half (54%) of NASH participants were among age group (30-50) years which is similar to Vernon *et al*, in the USA who found that the prevalence of NASH and NAFLD-related fibrosis increases with age<sup>(8)</sup>, another study which done by Frith and colleagues, showed that older patients had significantly more NAFLD risk factors due to hypertension, obesity, diabetes and Hyperlipidemia<sup>(9)</sup>, this can be due to lack of physical activity and consumption of high saturated fat in their meals.

In the current study, baseline BMI of the participants was above 30 kg/m<sup>2</sup>, this supports that NASH correlates significantly with BMI<sup>(10)</sup>.

In this study, there was no significant reduction of BMI in both groups (vitamin E, pentoxifylline), as Juan Du *et al*, in China who evaluated changes in BMI after pentoxifylline treatment or placebo and showed no significant difference (P = 0.28)<sup>(11)</sup>, although another study by Lisa. *et al*, in Washington, found that PTX in NASH causes a significant reduction in BMI with a dose of 1600 mg/day for 12 months<sup>(5)</sup>, another study done by Hoofnagle *et al*, in California showed that vitamin E improved steatosis with or without weight loss<sup>(12)</sup>.

We also demonstrated a significant correlation between decreased waist circumference and improvement in aminotransferase, as Rocha *et al*,<sup>(10)</sup>. Obesity is clearly associated with NASH, body fat distribution appears to play an important role in the pathogenesis of NASH, possibly as source of FFAs, accumulation of hepatic fat (increased intraabdominal fat deposition) these include increased hepatic lipogenesis, decreased excretion of hepatic lipid stores and/or diminished oxidation of FFAs in the liver<sup>(13)</sup>. In addition, those patients who

lost weight within the placebo (control) group also demonstrated reduced waist circumference.

In the present study, although PTX led to improvement in degree and pattern of fatty liver by U/S (P ≤ 0.001) as Juan. *et al*, in China, showed that Pentoxifylline in the dose range of 800 mg to 1200 mg/day with a low-calorie diet and exercise significantly improved NASH and lobular inflammation on ultrasound examination<sup>(11)</sup>. Another study was done by Naga Chalasani *et al* (2012), in India which showed vitamin E was administered within 800 IU/day for 96 weeks with low fat and exercise cause improvement in steatosis, inflammation, and ballooning by ultrasound (P < 0.001)<sup>(1)</sup>.

In current study, there is significant reduction of aminotransferase levels (P < 0.001) in pentoxifylline group compared to baseline, as Aung and Zhou in Singapore which showed ALT level (P = 0.065) and AST level (P = 0.038) significantly reduced in pentoxifylline treated (1200 mg daily for 3 months) with diet, lifestyle and exercise<sup>(14)</sup>.

This is similar to another study in China that showed highly significantly reduced aminotransferase when 800-1200 mg of pentoxifylline was taken daily for 12 months plus low fat and daily exercise<sup>(11)</sup>.

Liver function response was assessed by serum activities of ALT, AST and GGT. these activities were evaluated in all included trials, Anti-TNF agents potential components targeting the "second hit" in the pathogenesis of NASH include those agents improving necrosis, inflammation, and fibrogenesis caused by a number of pro-inflammatory adipocytokines, including tumor necrosis factor alpha (TNF-α)<sup>(15)</sup>.

In this study, there is a significant reduction in aminotransferase levels in vitamin E group compared to baseline, as Hoofnagle *et al*, in USA in which aminotransferase levels response were associated with decreased in NASH (P < 0.001), which used vitamin E (800 IU daily for 96 weeks)<sup>(12)</sup>. Another study was done by Kugelmas, in USA which vitamin E 800 IU qd for 96 weeks in patients with NASH showed improvement aminotransferase level<sup>(16)</sup>. Oxidative stress is considered a major contributor as the "second hit" in the pathogenesis of NAFLD and NASH, justifying the study of several antioxidants, like vitamin E in NASH treatment.

In our study, there is a significant reduction in Hs-CRP levels in both groups (vitamin E and pentoxifylline), that

reflect improvement in steatosis, lobular inflammation compared to baseline. This is comparable to study done by Claudia *et al.* <sup>(17)</sup>.

Pentoxifylline is an anti-TNF compound, it decreases the inflammation associated with NASH <sup>(18)</sup>. A study done by Elzafir *et al.* showed that a daily dose of 800 IU for 96 weeks of alpha-tocopherol improves inflammation <sup>(19)</sup>. Vitamin E is thought to decrease inflammation by acting as an antioxidant, thus decreasing oxidative cell damage <sup>(13)</sup>.

In our study, side effects were documented over the treatment period. In pentoxifylline group (nausea, heartburn, and dizziness) after 4 months of the drug uses, then to decrease the side effects, the dose of PTX titrated to (400 mg/day), as Wenjun *et al* study, in China <sup>(20)</sup>.

In vitamin E group (abdominal pain and blurred vision) after 3 months of the drug uses. Blurred vision was taken seriously referred to an ophthalmologist, and noted to be normal. In placebo group no adverse effect documented. During the study period, the patients were contacted regularly (every 2 weeks) to check up their adherence to LSM about exercise, diet, and the medication by asking them about the number of tablets left. Overall 26 cases were neither adherent to take medication regularly nor doing good LSMs.

In conclusion, Pentoxifylline and vitamin E were safe and well tolerated when given at the dose of 800mg and 800 IU daily respectively and significantly improve the biochemical parameters in non alcoholic fatty liver disease.

The authors disclose that they have no conflict of interest.

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