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Efficacy of *Cichorium intybus* on Alanine Aminotransferase in Non-alcoholic Fatty Liver Disease: A Randomised Double-blind Clinical Trial with Placebo

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ABSTRACT: The present study assesses the efficacy of *Cichorium intybus* for the reduction of alanine aminotransferase (ALT) in Non-alcoholic fatty liver disease (NAFLD). This randomized double-blind clinical-trial with placebo study was carried out for 61 patients. One group received 500 mg/day of powder extract of root of C. intybus orally before breakfast for 8 weeks while the other group received starch. Their body mass index (BMI), triglyceride (TG), cholesterol (Chol), ALT, aspartate aminotransferase (AST), high density lipoprotein (HDL), low density lipoprotein (LDL), fasting blood sugar (FBS) and impaired glucose tolerance (IGT) were measured before and after the study. In *C. intybus* group, BMI, ALT, AST, IGT after treatments were reduced compare to baseline, P=0.03 for ALT and P=0.04 for AST, but there was no meaningful difference between the two groups (p≥0.5). C. intybus can reduce ALT in NAFLD but has no significant impact.

Keywords: Non-alcoholic fatty liver disease, Alanine aminotransferase, *Cichorium intybus*

INTRODUCTION

It is well known that liver as the largest internal organ in the body plays an important role in many essential physiologic processes. NAFLD is the most common chronic liver disease and is characterised by the accumulation of fat in the hepatocytes of patients having no history of over drinking alcohol (Masoodi, 2013). NAFLD is the major cause of up to 90% cases of elevated aminotransferase level when other causes of liver disease are excluded (Aguirre, 2014, Tabassum, Resuli, 2012). Up to now, 20-40% of individuals suffer from NAFLD and its prevalence is estimated to be around 5-30% in Asia (Iloon *et al.*, 2015). NAFLD comprises a wide spectrum of liver damages ranging from simple, uncomplicated steatosis to Steatohepatitis to advanced fibrosis and cirrhosis (Li, 2010, Zhang, 2008, Assy, 2009, Tsuruta, 2011, Zelber-Sagi, 2011). Nonalcoholic Steatohepatitis (NASH) forms part of a histological spectrum of NAFLD, which includes fatty liver in the absence of liver injury or inflammation. Recent studies have revealed that patients who develop NASH, most especially those with elevated ALT, are at increased risk of cirrhosis and hepatocellular carcinoma.

Available treatments for NAFLD and/or NASH include change of life style (weight loss and exercise), use of anti-oxidants (Vitamin E and C, Beanie), glutathione, acetyl cystein, L-methionine, S-adenosyl, insulin-sensitizing agents such as Metformin and Thiazolidinediones (Pioglitazone, Rosiglitazone), Gemfibrozil and Statins, Probiotics, orlistat and Sibutramine, Cytoprotectives such as Ursodeoxycolic acid, Pentoxifylline and Antifibrotic such as Losartane (Shavakhi *et al*., 2015, Thounaojam *et al.*, 2012). Although Thiazolidinediones and Metformin are the most widely studied pharmacological therapy but the results are inconsistent and in practice, weight gain and bone loss are the most challenging side effects of Thiazolidinediones therapy while anemia, nausea and vomiting area associated with Metformin (Cheung and Sanyal 2010). So, the management of NAFLD and/or NASH focuses on associated conditions in the absence of a treatment that would represent a standard of care.

Presently, both exercise and healthy weight loss, either separately or in combination therapy are recommended by American Gastroenterology Association as interventions for NAFLD and/or NASH. This current standard of care is difficult for many patients to achieve (Clark *et al*., 2002, Hashemi *et al*., 2009, Roberts and Yap 2006, Tsuruta, 2011). In recent years, several investigators have examined specific effects of plants traditionally used by native healers and herbalists for the treatment of liver diseases (Luper, 1998). There is an increase in the utilization of complementary and alternative medicine, especially herbal therapy, for patients with liver diseases. Complementary therapy, such as herbal drugs, has also been tested for the treatment of NAFLD and/or NASH.

Medicinal plants, once thought to belong to low-income families, have become popular today as dietary supplements among people of all social classes *C. intybus* belongs to the family Asteraceae (Composite). It is distributed in Europe, the Mediterranean region and Northern Asia.

It is one of the effective drugs for the treatment of liver diseases (Ziamajidi *et al.*, 2013). *C. intybus* has been tested for its ability to reduce plasma and liver cholesterol, and is also utilised for liver complaints. Its medicinal parts are the dried leaves and roots and no side-effect reported has been reported except skin sensitivity. It has the ability to target hyperglycemia, hyperlipidemic and insulin resistance in NAFLD and/or NASH in experimental set-ups (Asl, 2014, Muthusamy, 2010).

C. intybus was reported as the best treatment choice in NAFLD and/or NASH since it has been recommended by TPM physicians (Avicenna, 2005). Moreover, to the best of our knowledge, it has not yet been utilised in patients with NAFLD and/or NASH. So, the aim of this study was to investigate the effects of *C. intybus* on ALT in NAFLD and/or NASH.

MATERIALS AND METHODS

A. Participant and study setting

This study was a double blind, randomised, clinical trial carried out on 61 patients with NAFLD and/or NASH. The patients seeking treatment came voluntary to the TPM Clinic and the Daro-Shafaye Imam Mojtaba affiliated to Shahid Beheshti University of Medical Sciences. Appropriate work-ups were performed by the gastroenterologist based on the current standards. Thirty one patients in *C. intybus* group and thirty patients in placebo group were recruited from March 2014 to October 2015. The study protocol was approved by the ethics committee of Shahid Beheshti University of Medical Sciences. The study protocol was registered at the Iranian Registry of Clinical Trials, registration code: IRCT2013121615825N1.

Being 18 to 70 years of age, having been diagnosed with fatty liver in sonography with a minimum of grade 1 and abnormal ALT and not taking medications affecting fatty liver were the inclusion criteria for the study.

Exclusion criteria include Patients with severe acute or chronic diseases (liver, heart or renal failure), infectious liver diseases (positive hepatitis B surface antigens, anti-hepatitis C virus), autoimmune liver diseases, pregnant/lactating women, senility to *C. intybus*, alcohol consumption, diabetic patient treated with insulin, fatty oxidation disorder, and willingness to discontinue the study for any reason.

B. Herbal drug preparation

The root of the plant was purchased from a traditional herbal medicinal store and processed under the supervision of a pharmacognosy professor at Shahid Beheshti University of Medicinal Sciences. To prepare the herbal drug, *C. intybus* root was cleaned, extracted with water, concentrated, and standardized according to phenol content, the dried powder was filled in 250 mg capsules. The placebo containing corn starch was also prepared in separate capsules. Both the drug and placebo capsules were coded.

C. Intervention

The experiment was explained to potential subjects, and they were asked to provide written informed consent before participating in the study. The selected patients were advised to take a low-fat, low carbohydrate diet, to do regular sport activities; and to lose weight more than 4 kg. The patients were advised to take 2 capsules every day, 30 min before breakfast for the duration of 8 weeks and those in the placebo group received placebo.

D. Follow up

During the study, patients were asked about the side effects of the drug. All patients had the following tests before and after the treatment sonography and blood test (ALT, AST, FBS, TG, Chol, HDL, LDL, and IGT).

E. Statistical analyses

Data were analysed using the SPSS software version 16.0 (SPSS Inc., Chicago IL., and USA). Comparisons between the two groups were performed with the Independent Sample t-test and Chi-Square (or Fisher's Exact) test. Comparison within groups was done with paired t-test. For assessment of ALT1,2, FBS1,2 and AST2, which do not have a normal distribution, Mann-Whitney U test were used (1: before intervention 2: after intervention).

RESULTS

In this study, a total of 342 patients presenting with NAFLD and/or NASH were evaluated; from which, 72 patients were eligible and commenced the intervention. A total of 31 patients (27 male and 4 female) in the *C. intybus* and 30 (24 male and 6 female) in the placebo group completed the observation and were analysed. Demographic characteristics are presented in Table 1.

Table 1: Demographic characteristics of the *C. intybus* and placebo groups.

Group	N	Age (yr) Mean (±SD)	Sex Number (percent)		Weight (kg) Mean (±SD)	BMI Mean (±SD)
			Male	Female		
C. intybus	31	41.2(±12.5)	27(87.1%)	4(%12.9)	93.7(±13.7)	31 (±4.1)
Placebo	30	41.8(±11.4)	24(80%)	6(20%)	89.7(±17.1)	30.5(±3.9)

Both groups were similar in terms of age, sex, and weight. The frequency of variables was the same for both groups in the beginning of the study. During the study, nine patients dropped-out: four patients from the *C. intybus* group and three patients from the placebo group because they couldn't take the second medical test; one patient from the *C. intybus* group due to travel and one from the placebo group because of pregnancy (Fig. 1).

In *C. intybus* group, BMI, ALT, AST, IGT after treatments were reduced compare to baseline, P=0.03 for ALT and P=0.04 for AST. TG, Chol, and FBS reduced but there was no meaningful difference.

The grade of steatosis before and after treatment in sonography was not statistically significant (P≤0.5). In placebo group, BMI, ALT, AST after treatment were reduced statistically significant in compare with the baseline. TG, Chol and IGT also were reduced but there was no statistically significant difference (Table 2 and Fig. 2). The grade of steatosis before and after treatment in sonography was not statistically significant (P≥0.5). Normalization of ALT in *C. intybus* group was 48.4% and 36.7% in placebo group, but there was no statistically significant difference between two groups. Side-effects were not seen in two groups.

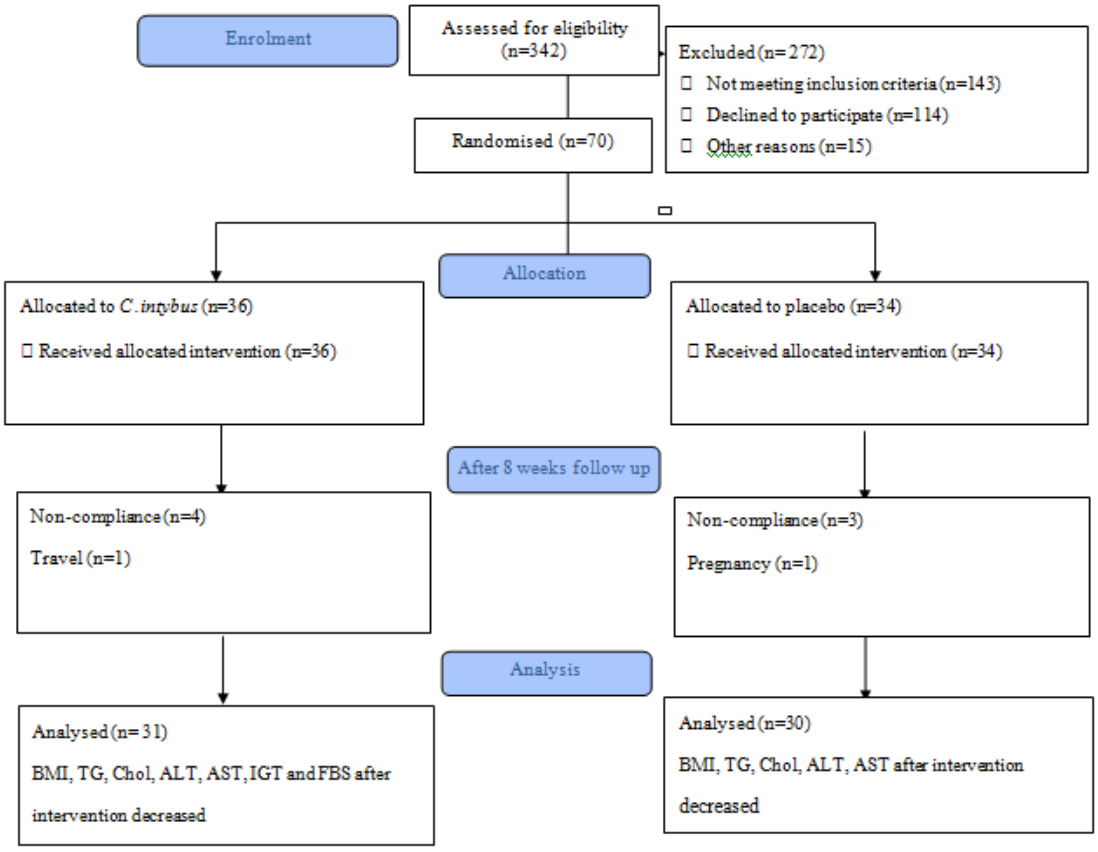

Fig 1. Study flow diagram.

Table 1: Lab data before and after intervention.

Data	group	Pre intervention		Post intervention				
				Between groups analysis		Before-after analysis		
		Mean (SD)	P value	Mean (SD)	P value		Decrease Mean (SD)	P value
ALT	C.intybus	77.6(32.3)	0.06	62.3(59.7)	0.9		15.3(54.1)	0.03
	placebo	61.4(15.7)		48.6(20.4)			12.8(22.8)	0.004
AST	C.intybus	45.4(14.8)	0.02	39.2(29.1)	0.05		6.2(30)	0.04
	placebo	37.8(10)		28.3(8.9)			9.5(10.1)	<0.001
Weight	C.intybus	93.7(13.7)	0.3	91.1(12.9)	0.3		2.6(4)	0.001
	placebo	89.7(17.1)		87.4(16.9)			2.3(3.5)	0.001
FBS	C.intybus	98.4(16.5)	0.01	95.5(10.6)	0.1		2.9(11.2)	0.1
	placebo	113.1(27.5)		115.9(48.2)			-2.8(38.2)	0.6
TG	C.intybus	176.6(100.4)	0.2	163.9(105.9)	0.2		12.7(90.7)	0.4
	placebo	216.1(148)		194.1(112.2)			22(127.9)	0.3
Chol	C.intybus	195.1(38.6)	0.6	194.2(38.6)	0.4		.8(25.9)	0.8
	placebo	191.5(28.5)		187.4(29.2)			4 (33.5)	0.5
HDL	C.intybus	42.4(10.8)	0.8	42(11.7)	0.1		.4(8.5)	0.7
	placebo	42(10)		38.3(6.9)			3.6(9.9)	0.06
LDL	C.intybus	117.7(36.2)	0.2	122.9(30.3)	0.1		-.5.2(34.8)	0.4
	placebo	108.7(26.1)		113.7(23.6)			-4.9(31.5)	0.3
IGT	C.intybus	125.4(32.6)	0.01	118.8(22.1)	0.01		6.5(22.2)	0.03
	placebo	159.2(67.7)		160.4(84)			-1.2(44.7)	0.5

DISCUSSION

NAFLD and/or NASH are considered as a part of metabolic syndrome including obesity, hyperinsulinemia, insulin resistance, hypertriglyceridemia and hypertension. These are the most common causes of unexplained continuous increase in serum ALT level. Although studies have indicated various treatments consisting of life style modification and medications, but there has been no standard therapy for NAFLD and/or NASH (Popkin, 2011). In the present study, *C. intybus*, as a medicinal plant in TPM, was utilised in NAFLD and/or NASH patients and its efficacy was compared with placebo. After 8 weeks, ALT became normal in 48.4% of patients in *C. intybus* group and 36.7% in placebo group; although there was no statistically significant difference between two groups. The improvement of ALT in our study was in line with the study of Hoofnagle, (2013) which demonstrated that ALT became normal in 48% of patients who used vitamin E. Therefore, it seems the antioxidant effect of *C. intybus* is effective in reducing ALT.

In addition, results of the current study was in line with the study of Ahmed *et al*, which showed that the anti-hepatotoxic activity of *C. intybus* roots reduces the levels of hepatic enzymes (Zafar *et al.*, 1998). The results from the study of Resuli *et al* have demonstrated that ALT became normal in 86% of patients by using Metformin. Although Metformin was more effective in reducing ALT than *C. intybus*, but Metformin has some side effects such as vomiting and diarrhea, while in our study, any side effect was not reported.

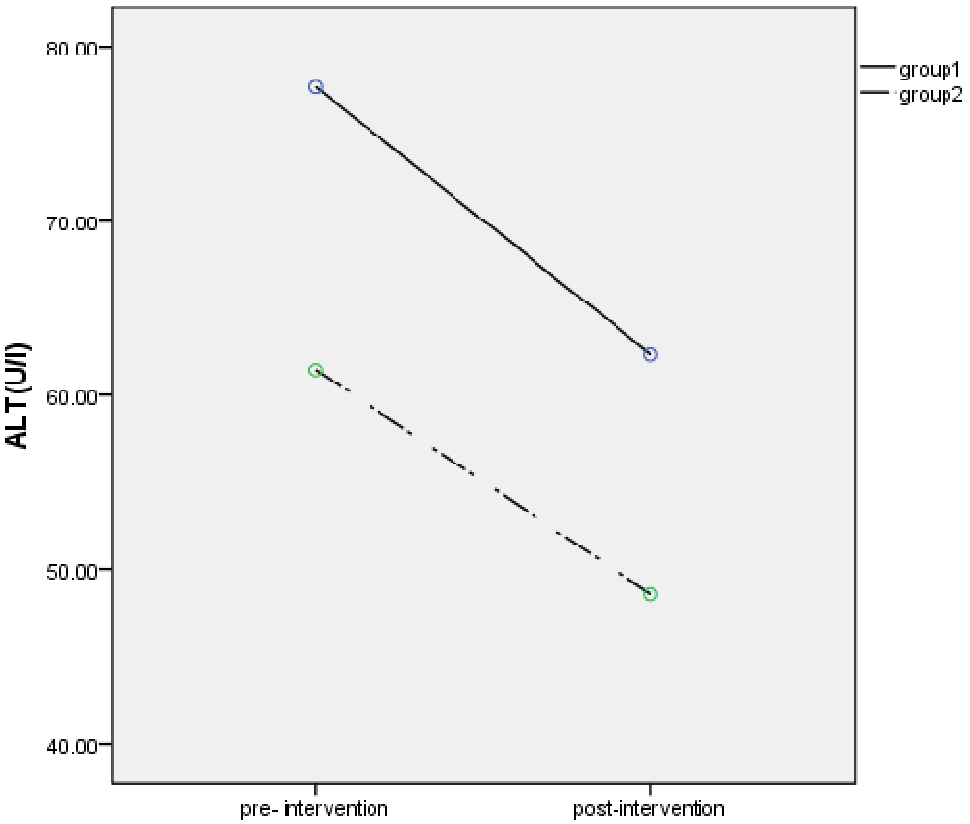

Fig. 2. Comparison of ALT between *C. intybus* (Group 1) and placebo (Group 2) before and after intervention.

In addition, Resuli *et al* demonstrated that ALT became normal in 82% of patients on low fat diet.

In our study, low fat diet was recommended as one of life style modifications in both groups, moreover on low fat diet, participants were advised to take a low carbohydrate diet, do regular sport activities; so as to lose weight. Therefore, these interventions could be the reasons for the absence of any significant difference in ALT reduction between the two groups. Furthermore, results of the present study indicated that in *C. intybus* groups, levels of TG, Chol, AST, IGT and FBS were reduced after treatment; nevertheless, there was no statistically significant difference between two groups ($p \geq 0.5$). Although the life style modifications can reduce TG, Chol, ALT, AST and FBS, but since the reduction of ALT in *C. intybus* group was higher than in placebo group, it might be attributed to the effects of *C. intybus* on reducing lipid profile and hepatic enzymes. Studies have suggested that anti hepatotoxic activity and useful effects of *C. intybus* on hepatic enzymes might be attributed to esculetin (Asl, 2014). This study demonstrated that *C. intybus* in patients with NAFLD and/or NASH was well tolerated and no side effects were reported in all the study. In present study, the useful effects of *C. intybus* in reducing lipid profile and hepatic enzymes were less than expected based on the TPM manuscripts; which might be due to inadequate dose of *C. intybus* capsules or unsuitable form of the drug, or short duration of the study (Hajaghamohammadi *et al.*, 2008, Hajiaghamohammadi *et al.*, 2012).

In this study, the different doses of the C. intybus were not evaluated, perhaps increasing the dose of this compound may have better effects on the liver biochemistry profile and histological features, moreover, for ease in taking the medication and to avoid the bitter taste of *C. intybus*, capsule form was chosen, while in TPM manuscripts *C. intybus* decoction was prescribed. Since studies have demonstrated the correlation between reduced transaminase levels and improved histology of liver after the fatty liver treatment, it might be better if liver histology can be evaluated before and after study, therefore we suggest liver biopsy in subsequent studies (Hoofnagle *et al.*, 2013). It is suggested that more clinical trials with more study population and longer evaluation time should be carried out to assess the effect of C. intybus on NAFLD and/or NASH; also, it might be better in further studies if the efficacy of life style modifications is evaluated separately.

CONCLUSION

The results of this study shows that *C. intybus* formula can reduce ALT as well as TG, Chol, AST, IGT and FBS in patients with NAFLD and/or NASH; nevertheless, further studies with more study population and longer evaluation time are necessary to better assess the efficacy and safety of *C. intybus* on NAFLD and/or NASH.

ETHICAL APPROVAL

The study protocol was approved by Ethics Committee of Shahid Beheshti University of Medical Sciences; number: 143.

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