

Effect of Silymarin Therapy on Liver Aminotransferase in Non-alcoholic Fatty Liver Disease

1. Iqbal Ahmed Memon 2. Muhammad Akbar 3. Ali Nawaz Bhurgri

1. Asstt. Prof. of Medicine, Liaquat University Hospital Jamshoro 2. Asstt. Prof. of Medicine, Isra University, Hyderabad 3. Asstt. Prof. of Pharmacology, Indus Medical College, Tando Muhammad Khan

ABSTRACT

Objective: The present study evaluated the effects of silymarin on blood glucose, blood lipids and liver amino-transferase (AST & ALT) in Non-alcoholic fatty liver disease (NAFLD).

Study Design: Randomized Placebo Controlled (double blind) Trial

Place and Duration of Study: This study was carried out at Consultant Clinic Cant area Hyderabad and Department of Medicine, Isra University Hospital Hyderabad from April 2012 to August 2013.

Materials and Methods: A sample of 64 subjects (33 cases and 31 controls) was selected for evaluating effects of silymarin. Subject selection observed the inclusion and exclusion criteria. Subjects with aspartate transaminase (AST) and alanine transaminase (ALT) >1.2 of normal were included. NAFLD diagnosis was confirmed by ultra-sonography. NAFLD cases were given silymarin (140 mg x2 tablets) daily for duration of 3 months. Controls received placebo. AST & ALT were checked after three months. Data variables were analyzed by SPSS version 21.0.

Results: Mean \pm SD of aspartate transaminase (AST) and alanine transaminase (ALT) before intervention were found as 73.2 ± 9.7 vs. 69.3 ± 17.6 IU/L⁻¹ ($p < 0.021$) and 92.1 ± 19.4 vs. 83 ± 15.6 IU/L⁻¹ in cases and controls ($p < 0.0001$) respectively. After three months the AST and ALT were found reduced compared to baseline. AST and ALT were found as 39.3 ± 7.5 IU/L⁻¹ and ALT to 39.3 ± 10.9 IU/L⁻¹ 35.9 ± 11.7 IU/L⁻¹ and 83 ± 15.6 IU/L⁻¹ in cases and controls respectively. Blood lipids and blood glucose also showed statistically significant differences ($p = 0.0001$).

Conclusion: Silymarin improves blood glucose, blood lipids and liver amino transferase in non-alcoholic fatty liver disease.

Key Words: Silymarin, NAFLD, Liver, Blood glucose, Blood lipids

Citation of article: Memon IA, Akbar M, Bhurgri AN. Effect of Silymarin Therapy on Liver Aminotransferase in Non-alcoholic Fatty Liver Disease. Med Forum 2015; 26(12):46-49.

INTRODUCTION

Non alcoholic fatty liver disease (NAFLD) is a metabolic disorder of characterized by abnormal fat deposition in liver parenchyma in the absence alcohol consumption. NAFLD clinically manifests as a metabolic disorder associated with obesity, systemic hypertension, hyperglycemia & diabetes mellitus and hyperlipidemia.^{1,2} Estimated prevalence of NAFLD in industrialized countries is estimated as 45% in the general population.³ NAFLD results in disordered liver metabolism and multiplies the risk of developing atherosclerosis manyfolds.⁴ Many factors have been implicated in the etiology and risk of NAFLD. The factors include the obesity, insulin resistance, adipokine inter-play, oxidative stress and bacterial overgrowth syndrome.⁵

A change in diet, dietary habits, physical exercise, brisk walking and a total change in life style improves the

NAFLD and guidelines recommend them for prevention.⁶ Different drugs have also been recommended which modify and change the natural course of liver injury in NAFLD through various mechanisms. Many drug agents are now available and prescribed in clinical practice. Anti-oxidants agents,⁷ metformin drug therapy,⁸ receptor sensitizers,⁷ Pioglitazone – a PPAR γ agonist,⁹ and the ezetimibe^{10,11} had been recommended and prescribed.

Silymarin is an herbal agent - a mixture of flavonolignans. Biochemical structure shows two diastereomers of “silybin”. Silymarin exerts anti oxidant through a novel pathway of stimulation of nuclear transcription. Silymarin stimulates the nuclear polymerase, and mRNA formation. Silymarin inhibits toxins entry into hepatocytes by blockade at cell membrane levels. Silymarin protects against free radical mediated hepatocytes injury. It also inhibits the lipid peroxidation cascade initiated by free radical.

Previous studies had reported over expression of superoxide dismutase gene on the hepatocytes, this way it exerts anti oxidant activity. Silymarin increases stores of glutathione and anti oxidant enzyme – the glutathione peroxidase (GPX).¹²

Correspondence: Dr. Iqbal Ahmed Memon,

Asstt. Prof. of Medicine, Liaquat University Hospital Jamshoro/Hyderabad

Contact No.: 0300-3019584

E-mail: dri.memon@yahoo.com

A search of Pakistani literature showed a limited experience of silymarin use in NAFLD, blood lipids and blood glucose in local population.¹³⁻¹⁵ The financial prosperity has begotten obesity epidemic in urban areas. The obesity is a risk for metabolic disorders including NAFLD. The NAFLD may be the earliest manifestation of metabolic syndrome. In near future, the NAFLD may be a public health problem.

The present study evaluated the effects of silymarin on blood glucose, blood lipids and liver amino-transferase (AST & ALT) in Non-alcoholic fatty liver disease (NAFLD).

MATERIALS AND METHODS

The present randomized clinical placebo controlled (double blind) trial was conducted at the Consultant Clinic Cant area Hyderabad and Department of Medicine, Isra University Hospital Hyderabad from April 2012 to August 2013. Subjects complaining of upper gut symptoms were screened for the liver fatty infiltrations.

A sample of 64 subjects (33 cases and 31 controls) was selected for evaluating effects of silymarin. Subject selection observed the inclusion and exclusion criteria. Merits and demerits of study were explained to study subjects. Volunteers who signed informed consent were selected. Subjects were informed that they can withdraw at any time. Volunteer subjects who satisfied the inclusion criteria for NAFLD were studied.

Subjects with aspartate transaminase (AST) and alanine transaminase (ALT) >1.2 of normal for last six months were included. NAFLD diagnosis was confirmed by ultra-sonography.^{13, 15} Subjects suffering viral hepatitis, history of alcohol use and hepatotoxic drug intake were excluded from study protocol. History of autoimmune disorders, diabetes mellitus, hypolipidemic drug intake, and diabetes mellitus was taken for exclusion.

Height, weight and body mass index (BMI) were noted. Subjects who satisfied the included criteria were block randomized into 2 groups – the cases and controls. NAFLD cases were given silymarin (140 mg x2 tablets) daily for duration of 3 months. Controls received placebo- identical appearing tablets. AST & ALT were checked after three months.

Participants were guided to take low fat low energy diet and regular physical exercise. Blood samples were collected from ante-cubital veins after three months therapy. Ethical issues were strictly observed. Data was noted in pre-structured questionnaire. Data variables were analyzed by SPSS version 21.0. (IBS-Corporation USA). Student's t-test and Chi square testing was used for numerical and categorical data respectively. Data was analyzed at CI of 95% interval ($p \leq 0.05$).

RESULTS

Age (mean \pm SD) in cases and controls was 49 ± 9.7 and 48 ± 8.9 years respectively ($p=0.07$). Male predominated over female and majority belonged to rural area. The baseline demographic data of study population is shown in table 1. BMI, blood glucose and blood lipids was observed between cases and controls ($p > 0.05$).

Mean \pm SD of aspartate transaminase (AST) and alanine transaminase (ALT) before intervention were found as 73.2 ± 9.7 vs. 69.3 ± 17.6 IUL⁻¹ ($p < 0.021$) and 92.1 ± 19.4 vs. 83 ± 15.6 IUL⁻¹ in cases and controls ($p < 0.0001$) respectively. After three months the AST and ALT were found reduced compared to baseline. AST and ALT were found as 39.3 ± 7.5 IUL⁻¹ and 39.3 ± 10.9 IUL⁻¹ 35.9 ± 11.7 IUL⁻¹ and 83 ± 15.6 IUL⁻¹ in cases and controls respectively. Blood lipids and blood glucose also showed statistically significant differences ($p=0.0001$).

Table No. I: Baseline characteristics of cases and control subjects

	Cases (n=33)	Controls (n=31)	p-value
Age	49.0 \pm 9.70 years	48 \pm 8.9 years	0.071
Male	21 (63.6%)	21(67.7%)	0.091
Female	12 (36.3%)	10 (30.3%)	0.081
Rural population	22 (66.6%)	19 (61.2%)	0.072
Urban population	11 (33.3%)	12 (38.7%)	0.092
Weight (kg)	88.0 \pm 19.90	83.0 \pm 21.50	0.063
BMI (kgm ⁻²)	29.90 \pm 5.80	28.70 \pm 6.80	0.081
Postprandial blood glucose (mg/dl)	163.0 \pm 21.50	154.0 \pm 28.60	0.082
Triglycerides (mg/dl)	192.90 \pm 44.70	182.90 \pm 41.50	0.063
Total cholesterol (mg/dl)	199.10 \pm 23.80	198.30 \pm 21.40	0.08
HDLc (mg/dl)	37.10 \pm 8.10	36.90 \pm 9.50	0.092
LDLc (mg/dl)	95.30 \pm 17.50	97.20 \pm 15.30	0.063
VLDL(mg/dl)	41.0 \pm 9.40	43.0 \pm 14.60	0.074

Table No.2: Liver amino-transferase enzyme levels in cases and controls

	Cases		Controls		p-value
	Before intervention	After intervention	Before intervention	After intervention	
AST (IU/L)	73.20±9.70	39.30±7.50	69.30±17.60	35.90±11.70	0.02
ALT (IU/L)	92.10±19.40	39.30±10.90	83.0±15.60	51.20±19.10	0.001

DISCUSSION

Hepatoprotective mechanism of silymarin is now an established fact. Various underlying mechanisms have been proposed against oxidants in animal studies. Silymarin scavenges free radicals; therefore liver is an important site to be protected as free radicals are frequently formed there. Silymarin protects the phospholipids of cell membrane against free radical injury. Silymarin maintains cell membrane fluidity, and maintains cell membrane functions.¹⁶

Silymarin protects at sub cellular level through gene transcription. Silymarin facilitates gene transcription, mRNA formation and ribosomal translation. Thus silymarin produces new proteins which protect against toxic agents. Newly synthesized proteins may act as anti oxidant enzymes to neutralize free radicals and free radical mediated peroxidation of cell membrane phospholipids.¹⁷

Silymarin alleviates inflammation and exerts anti fibrogenic effects through inhibition of cytokine functioning.¹⁸⁻²²

Silymarin maintains cell membrane fluidity and permeability, thereby helps to maintain mitochondria functions and energy production. Enhanced mitochondria functioning increases cellular capability against xenobiotic induced liver injury.²³

Silymarin is reported to interfere directly with binding of toxins to cell membrane of hepatocytes. Thus the toxins mediated injury is minimized and cell functioning remains normal. Silymarin spares membrane transport proteins for normal cell functioning.²⁴

A previous clinical study has reported effects of silymarin against drug induced liver injury, alcohol toxicity, and viral hepatitis induced liver injuries.¹⁸

The present study reports that the silymarin improves liver functioning as evaluated by the improvement of hepatocytes enzyme levels. Silymarin also improves blood glucose and blood lipid levels. Our findings are in parallel to previous studies^{25,26}

Another previous study has also reported similar observations for silymarin.²⁷ A previous study reported that the silymarin reduces liver amino-transferase enzymes in NAFLD. Other liver functioning tests were also improved as reported by Hashemi.²⁸ The findings of above study are highly in keeping to our present study. Pro inflammatory cytokines are increased in NAFLD and they adversely affect the fat disposal in liver and promote fat deposition with hepatocytes. Lipid deposition sets up a vicious cycle which in turn increases the cytokine secretion.²⁸⁻²⁹ As regards blood lipids, present study reports an improvement in the

blood lipids which is an important finding and is in confirmation to above studies. Previous studies reported the liver amino-transferase was correlated positively with the pro inflammatory markers such as the C-reactive proteins. Hence, in present study if liver amino-transferase were reduced it means it might be due to a reduction in pro inflammatory cytokines. Our finding is of clinical importance and is consistent to previous studies.³⁰⁻³³ Seemingly, it may be concluded that the Silymarin inhibits inflammatory cytokine release and thereby exerts hepatoprotective effects. Inhibition of cytokines improves liver fat deposition and facilitates fat disposal into circulation. Other underlying mechanisms may be working and need further elucidation. The limitations of present study are that we were not able to perform liver biopsy due to human ethical issues. The present study reports that the administration of silymarin improves liver parenchyma functioning in patients with NAFLD..

CONCLUSION

The present study reports that the silymarin improves blood glucose, blood lipids and liver amino transferase in non-alcoholic fatty liver disease. Liver amino-transferase are enzymes of hepatocytes cytoplasm compartment, hence present study concludes silymarin improves liver functioning at cellular levels which needs further elaboration.

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Petta S, Muratore C, Craxi A. Non-alcoholic fatty liver disease pathogenesis: The present and the future. *Dig Liver Dis* 2009; 41: 615-25.
2. Solhi H, Ghahremani R, Kazemifar AM, Yazdi ZH. Silymarin in treatment of non-alcoholic steatohepatitis: A randomized clinical trial. *Caspian J Intern Med* 2014;5 (1):9-12.
3. Younossi ZM, Otgonsuren M, Venkatesan C, Mishra A. In patients with Non-Alcoholic Fatty Liver Disease (NAFLD), metabolically abnormal individuals are at a higher risk for mortality while metabolically normal individuals are not. *Metabolism* 2013; 62: 352-60.
4. Liu CJ. Prevalence and risk factors for non-alcoholic fatty liver disease in Asian people who are not obese. *J Gastroenterol Hepatol* 2012; 27: 1555-60.

5. Santos RD, Agewall S. Non-alcoholic fatty liver disease and cardiovascular disease. *Atherosclerosis* 2012; 224: 324-5.
6. Tan HH, Chang JP. Non-alcoholic fatty liver disease. *Proceedings of Singapore Healthcare* 2010; 19: 36-50.
7. Socha P, Horvath A, Vajro P. Pharmacological interventions for nonalcoholic fatty liver disease in adults and in children: a systematic review. *J Pediatr Gastroenterol Nutr* 2009; 48: 587-96.
8. Nair S, Diehl AM, Wiseman M, Farr GH, Perrillo RP. Metformin in the treatment of non-alcoholic steatohepatitis: a pilot open label trial. *Aliment Pharmacol Ther* 2004; 20: 23-8.
9. Tiikkainen M, Häkkinen AM, Korsheninnikova E, Nyman T, Mäkimattila S, Yki-Järvinen H. Effects of rosiglitazone and metformin on liver fat content, hepatic insulin resistance, insulin clearance, and gene expression in adipose tissue in patients with type 2 diabetes. *Diabetes* 2004; 53: 2169-76.
10. Park H, Shima T, Yamaguchi K, Mitsuyoshi H, Minami M, Yasui K, et al. Efficacy of long term ezetimibe therapy in patients with nonalcoholic fatty liver disease. *J Gastroenterol* 2011; 46: 101-7.
11. Cacciapuoti F, Scognamiglio A, Palumbo R, Forte R, Cacciapuoti F. Silymarin in non alcoholic fatty liver disease. *World J Hepatol* 2013 March 27; 5(3): 109-13.
12. Wellington K, Jarvis B. Silymarin: a review of its clinical properties in the management of hepatic disorders. *Bio Drugs* 2001; 15: 465-89.
13. Kazemifar AM, Hajaghamohammadi AA, Samimi R, et al. Hepatoprotective property of oral silymarin is comparable to n-acetyl cysteine in acetaminophen poisoning. *Gastroenterol Res* 2012; 5: 190-4.
14. Hashemi SJ, Hajiani E, Sardabi EH. A placebo-controlled trial of silymarin in patients with nonalcoholic fatty liver disease. *Hepat Mon* 2009; 9: 265-70.
15. Hajaghamohammadi AA, Ziaee A, Oveisi S, Masroor H. Effects of metformin, pioglitazone, and silymarin treatment on non-alcoholic fatty liver disease: a randomized controlled pilot study. *Hepat Mon* 2012; 12: 1-6.
16. Sonnenbichler J, Zetl I. Biochemical effects of the flavonolignan silibinin on RNA, protein and DNA synthesis in rat liver. *Progr Clin Biol Res* 1986; 213:319-31.
17. Muriel P, Mourelle M. Prevention by Silymarin of membrane alterations in acute CCl₄ liver damage. *J Appl Toxicol* 1990; 10:275-9.
18. Saller R, Meier R, Brignoli R. The use of Silymarin in the treatment of liver diseases. *Drugs* 2001; 61:2035-63.
19. Dehmlow C, Eahard J, Goot HD. Inhibition of Kupffer cells as an explanation for the hepatoprotective properties of silibinin. *Hepatol* 1996; 23:749-54.
20. Saliou C, Rihn B, Cillard J, Okamoto T, Packer L. Selective inhibition of NF-B activation by the flavonoid hepatoprotector Silymarin in Hep G2. *FEBS Lett* 1998; 440: 8-12.
21. Johnson VJ, Osuchowski MF, He Q, Sharma RP. Physiological responses to a natural antioxidant flavonoids mixture, Silymarin, in BALB/c mice: II. Alterations on thymic differentiation correlate with changes in cmyc gene expression. *Planta Med* 2002; 68:961-5.
22. Gebhardt R. Oxidative stress, plant derived antioxidants and liver fibrosis. *Planta Med* 2002; 68:289-96.
23. Munter K, Mayer D, Faulstich H. Characterization of a transporting system in rat hepatocytes: studies with competitive and non-competitive inhibitors of phalloidin transport. *Biochem Biophys Acta* 1986;860:91-8.
24. Faulstich H, Jahn W, Wieland T. Silybin inhibition of amatoxin uptake in the perfused rat liver. *Arzneimittelforschung* 1980; 30:452-4.
25. Solhi H, Ghahremani R, Kazemifar AM, Hoseini Yazdani Z. Silymarin in treatment of non-alcoholic steatohepatitis: a randomized clinical trial. *Caspian J Intern Med* 2014; 5(1): 9-12
26. Masoodi M, Rezadoost A, Panahian M, Vojdanian M. Effects of Silymarin on Reducing Liver Aminotransferases in patients with non-alcoholic fatty liver diseases. *Govareh* 2013;18:181-5.
27. Hajaghamohammadi AA, Ziaee A, Rafiei R. Hepatoprotective herbal drug, Silymarin from experimental pharmacology to clinical medicine: A Randomized Controlled Clinical Trial. *Hepat Mon* 2008; 8:191-5.
28. Hashemi SJ, Hajiani A, Sardabi EH. A placebo-controlled trial of Silymarin in patients with nonalcoholic fatty liver disease. *Hepat Mon* 2009; 9: 265-70.
29. Harmon RC, Tiniakos DG, Argo CK. Inflammation in nonalcoholic steatohepatitis. *Expert Rev Gastroenterology Hepatol* 2011; 5:189-200.
30. Kulkarni YA, Yele VU, Addepalli V, Kulkarni KS. Non alcoholic fatty liver disease: Introspection. *Archives* 2012; 3:104-12.
31. Choi S, Diehl AM. Role of inflammation in nonalcoholic steatohepatitis. *Curr Opin Gastroenterol* 2005; 21:702-7.
32. Neuman G, Sagi R, Shalitin S, Reif S. serum inflammatory markers in overweight children and adolescents with nonalcoholic fatty liver disease. *Isr Med Assoc J* 2010; 12:410- 5.
33. Kerner A, Avizohar O, Sella R, Bartha P, Zinder O, Markiewicz W, et al. Association between elevated liver enzymes and C-reactive protein: possible hepatic contribution to systemic inflammation in the metabolic syndrome. *Arterioscler Thromb Vasc Biol* 2005; 25:193-7.