



The effect of silymarin on non-alcoholic fatty liver disease of children

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ABSTRACT

Introduction: Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver diseases of adults and its prevalence is increasing in children and adolescents. It is the most predisposing factor for liver cirrhosis. Due to complications of NAFLD, the treatment of this disease is essential. Since silymarin is recommended for adults recently, the effect of this herbal drug on children and adolescents was evaluated for treatment of NAFLD in children.

Methods: This is a clinical trial study, which was done in Shahrekord University of Medical Science in 2012-2013 on 5-16 years old children with NAFLD. The patients were randomly divided into two groups containing 20 patients in each group. Silymarin tablets were administered for 12 weeks in case group and changing life style including exercise was advised in both groups. Liver sonography and liver function tests (LFTs) were done after 12 weeks.

Results: Patients with higher grade of fatty liver were developed lower grade of fatty liver during intervention in case group based on sonography after 12 weeks ($P=0.001$). Meanwhile, no significant changes were observed in control group ($P=0.35$). Liver enzymes (alanine aminotransferase [ALT] and aspartate transaminase [AST]) ($P=0.001$, $P=0.025$, respectively) and triglyceride (TG) levels ($P=0.043$) were improved significantly but low-density lipoprotein (LDL) and high-density lipoprotein (HDL) did not change during intervention in case group.

Conclusion: Silymarin can improve fatty infiltration of liver and liver function in children and adolescents.

Implication for health policy/practice/research/medical education:

Silymarin can improve fatty infiltration of liver and liver function in children and adolescents, hence it might be beneficial for NAFLD patients.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver diseases of adults and is defined as evidence of hepatic steatosis, either by imaging or histology (1). The prevalence of this disease is 20%-30% in the general populations of Western countries. NAFLD is the most common predisposing factor for liver cirrhosis (2). Epidemiologic studies demonstrated prevalence of 21.5% for NAFLD in one group of Iranian adults and 7.1% in Iranian children (3). However, the prevalence of fatty liver is also enhancing in these age groups by increasing the incidence of obesity in children and adolescents. Due

to complications of NAFLD, the treatment of this disease is essential (4,5).

Several treatment options have been recommended for NAFLD, which have been reviewed elsewhere (6). The beneficial effect of silymarin was observed on alcoholic liver steatosis, which supports its efficacy in treatment of NAFLD. The positive effects of milk thistle (silymarin) were shown in terms of anti-inflammatory, antifibrotic and cytoprotective properties in liver (7). In a placebo-controlled trial in patients with NAFLD silymarin significantly improved biochemical markers of the patients (8). The beneficial effect of crude extract of

Silybum marianum with high polyphenolic content was elucidated on one group of nonalcoholic steatohepatitis patients (9). It was indicated that silymarin is superior to metformin and pioglitazone on improvement of fatty liver of adults (10).

Silymarin is extract of thistle herb seeds that is orally absorbed and excreted into the bile as sulfate-conjugate. It can also be used as a supplement for normal functioning of the liver because of its antioxidant property, lipid lowering and anti-inflammatory effects (11-13). It also can increase the detoxifying effect of liver by increment of glucuronidation activity and avoiding decrease glucuronic acid reserve of liver (14). The anti-inflammatory effect of silymarin is due to inhibiting leukotriene and prostaglandin synthesis, inhibit Kupffer cells, stabilizing mast cells and avoiding neutrophil migration in human body. This herbal medication can also increase hepatocyte protein synthesis, which needs for repairing liver tissue, slowing fibrosis process and even improvement of liver fibrosis (7).

There is no definite treatment for NAFLD till now so finding an effective treatment for this disease can prevent cirrhosis and end stage liver disease. Moreover, there is no study about silymarin effect on NAFLD of children. Due to the importance of NAFLD and its potential complications the purpose of this work is to investigate about this herbal drug on recovery of liver function tests and sonographic changes of these patients.

Patients and Methods

Subjects and protocol

This is a cross sectional, randomized and double blind clinical trial, which was done in out-patient departments of Shahrekord University of Medical Sciences from February 2012 till March 2013. This study was confirmed by Ethical committee of Shahrekord University of Medical Science and registered in IRCT website (code: IRCT2016080614882N4). The trial was conducted on 40 children and adolescents (5-16 years old) with NAFLD. These patients were referred from pediatricians and endocrinologists to pediatric gastroenterology and hepatology clinic because of their obesity or overweight that sonography and/or liver enzymes were suspicious to fatty liver. The diagnosis of NAFLD was based on history taking and precise physical examinations performed by the same pediatric, gastroenterologist, hepatologist, as well as sonography and laboratory evidences in favor of fatty liver.

NAFLD was considered as sonographic evidence of fatty infiltration of liver (G1-3) with or without abnormal liver function tests (LFTs) in non-alcoholic subjects (15). Other causes of fatty liver and abnormal LFT including Wilson disease, viral hepatitis, auto immune hepatitis, juvenile hemochromatosis, and other underlying diseases were excluded. Patients were also excluded in presence of evidence of cirrhosis, advanced liver disease, any underlying liver disease, diabetes mellitus, metabolic syndromes and previous treatment with other medications.

Forty children and adolescent were registered for the study with voluntary basis. They were divided to 2 groups, randomly.

Study design

Instruments with the following specifications were used to study different parameters: 1) Weight (Beurer digital GS34 Bargraph); 2) Height (wall meter, Seca 206 with 1mm accuracy); 3) Body mass index, BMI 4) Liver enzymes (Pars Azmoon kit); 5) Liver sonography (using an ultrasound multi-frequency curvilinear 3.5-5 MHz probe by Siemens Company, Sonoline G50 series, model number 7474922).

Period of treatment was 12 weeks. Patients who had inclusion criteria were assigned consecutive numbers. For odd numbers, silymarin and changing life style including exercise were considered. Meanwhile, only life style changes and exercise were considered for even numbers. The recommended 150-250 min/wk, walking according to American College of Sport Medicine and low fat as well as low carbohydrate diet, was used (16). Silymarin 70 and 140 mg tablets with brand name of Livergol (Goldaru company, Iran) (with dosage of 5 mg/kg/d, divided 3 dose with meal) were used.

Patients were not allowed to use other drugs for weight reduction or lipid lowering purposes. The patients of two groups had not significant difference in demographic variables including age, sex and BMI. Parents of children and adolescents with NAFLD provided written informed consent for voluntary arrival.

Follow up

Weight and BMI were measured again after the 12-week period. The sonography was done again by the same sonographer. LFTs and lipid profiles were checked in the same laboratory. Patients were evaluated each month for compliance to intervention, drug consumption and adverse effects of medication (allergic reaction, GI upset, loose stool).

Data analysis

Data analysis was done with SPSS software version 20 (IBM, Armonk, NY).

Results

Forty patients with NAFLD participated in the study and were divided into two groups; case and control. All patients completed the intervention. Anthropometric parameters of the two groups are shown in Table 1. Laboratory data of the two groups before and after the intervention are shown in Table 2. Variables of NAFLD were evaluated at the end of the intervention.

Grade of fatty liver

In the case and control groups, 10 patients had fatty liver grade 1 (50% vs. 50%), 8 and 10 patients, respectively, grade 2 (40% vs. 50%), and only in the case group, two patients (10%) grade 3. No statistically significant differences were

Table 1. Anthropometric measurements

| Variables | Silymarin group (mean ± SD) | Placebo group (mean ± SD) | Difference | P value ^a |
|---------------------------------------|-----------------------------|---------------------------|------------|----------------------|
| Age (y) ^a | 11.8 ± 3 | 10.5 ± 3.2 | 1.3 ± 0.2 | 0.2 |
| Weight (kg) ^a | 61.8 ± 16.7 | 60.3 ± 13.6 | 1.5 ± 3.1 | 0.76 |
| BMI (kg/m ²) ^a | 25.8 ± 4.1 | 24.3 ± 4.5 | 1.5 ± 0.4 | 0.29 |
| Height (cm) | 153.1 ± 13 | 157 ± 8.5 | 3.9 ± 4.5 | 0.27 |

^a P < 0.05 is statistically significant.

Table 2. Different clinical parameters in case and control groups^a

| | ALT (U/L) | | | AST (U/L) | | | TG (mg/dL) | | | LDL (mg/dL) | | | HDL (mg/dL) | | |
|-----------|-----------|----------|-------|-----------|----------|-------|------------|-------------|-------|-------------|-------------|-----|-------------|-----------|------|
| | Before | After | P | Before | After | P | Before | After | P | Before | After | P | Before | After | P |
| Silymarin | 47.3±5.4 | 36.4±6.6 | 0.015 | 49.1±2.2 | 31.5±1.3 | 0.025 | 203.7± 5.7 | 198.8± 13.8 | 0.043 | 121.8±4.4 | 118.4± 11.4 | 0.1 | 45.5±9 | 50.7±13.4 | 0.11 |
| P | 0.09 | 0.043 | | 0.43 | 0.003 | | 0.04 | 0.034 | | 0.14 | 0.34 | | 0.99 | 0.98 | |

Abbreviations: AST, aspartate aminotransferase; ALT, alanin aminotransferase; LDL, Low-density lipoprotein; HDL, High-density lipoprotein; TG, triglyceride.

^a Normal ranges: AST & ALT < 40 U/L, TG < 150 mg/dL, LDL < 100 mg/dL, HDL > 60 mg/dL.

Table 3. Grades of fatty liver

| Time | Grade group | Case No. (%) | Control No. (%) | P value |
|---------|-------------|--------------|-----------------|---------|
| Before | 1 | 10 (50) | 10 (50) | 0.54 |
| | 2 | 8 (40) | 10 (50) | |
| | 3 | 2 (10) | 0 (0) | |
| After | 1 | 15 (75) | 11 (55) | 0.44 |
| | 2 | 2 (10) | 5 (25) | |
| | 3 | 3 (15) | 4 (20) | |
| P value | | 0.001 | 0.35 | |

observed between the two groups ($P=0.44$). Table 3 shows the results after the treatment; 15 patients in the case group (75%) and 11 patients (55%) in the control group had grade 1 fatty liver. Thus, the number of the patients with fatty liver grade 1 increased from 10 to 15 patients in the case group. Therefore, some patients with higher grade of fatty liver developed lower grade of fatty liver after the intervention based on sonography ($P=0.001$). Meanwhile, as seen in Table 3, no significant changes were observed in the control group ($P=0.35$).

Aspartate transaminase level

According to Table 2, no statistically significant difference in aspartate transaminase (AST) level was observed before and after the treatment in the control group. Meanwhile, significant differences were observed in the intervention group ($P=0.025$).

Alanine aminotransferase level

At the beginning of the study, there was not statistically significant difference between the two groups ($P=0.09$). As shown in Table 2, level of alanine aminotransferase (ALT) decreased significantly in silymarin group ($P=0.015$) at completion of the treatment. In contrast to silymarin group, no significant changes were observed in the control group ($P=0.02$).

Triglyceride level

Mean triglyceride (TG) levels were statistically different between the two groups before the intervention ($P=0.034$). TG levels also decreased statistically in both silymarin ($P=0.043$) and control ($P=0.040$) groups at completion of the intervention. As seen in Table 2, low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol levels were not significantly different before and after the intervention in both groups.

Body mass index

BMI did not change significantly before and after the treatment (25.8 ± 4.1 and 25.2 ± 3.8 , respectively).

Discussion

NAFLD refers to a wide spectrum of diseases, including augmented fat content in the liver (steatosis), inflammatory change, non-alcoholic steatohepatitis (NASH), and potentially fibrosis and cirrhosis (17). It is one of the most prevalent liver diseases among adults and the most common predisposing factor for liver cirrhosis (2). However, the prevalence of fatty liver in these age groups is increasing by rising obesity in children and adolescents. NAFLD is due to chronic liver disease, cirrhosis, and ESRD. NAFLD is now recognized as a component of metabolic syndrome, including hyperlipidemia, glucose intolerance, hyperinsulinism, and obesity. Risk and severity of NAFLD are increased through a number of metabolic syndrome components (17). Treatment of NAFLD includes appropriate weight loss, elimination of objective drugs and toxins, control of metabolic disorders, such as diabetes and hyperlipidemia. Several studies have been conducted on diet and exercise as well as some medications with inconsistent results (18).

The present study confirmed the effect of silymarin on improvement of NAFLD in the children and adolescents. Unlike similar studies conducted in Iran and other countries on adults, this research was conducted on children. Our findings confirm that silymarin can exert

beneficial effect on improvement of NAFLD in children aged 5-16 years. Hashemi et al in a placebo-control study suggested that silymarin could be significantly effective on biochemical improvement and in decreasing the ALT and AST levels in patients with NASH because of its antioxidant property (8).

Hajiani et al in a comparative study between silymarin and vitamin E concluded that both medications could improve sonographic findings and liver enzymes because of antioxidant effect of vitamin E and antioxidant, anti-inflammatory, and hepatocyte membrane-stabilizing actions of silymarin (19). Mitochondria are the main sites of lipids metabolism and oxidative phosphorylation in the liver. Oxidative stress can cause mitochondrial dysfunction in NASH. The excess of free fatty acids increases mitochondrial H₂O₂ production. Then, this compound oxidizes mitochondrial membranes that regulate the activity of uncoupling protein 2 and carnitine palmitoyl transferase-1 (20). Silymarin with antioxidant properties can be effective in prevention and treatment of this disease.

Cacciapuoti et al showed that silymarin administration for 6 months caused significant reduction in liver steatosis, according to sonography, and liver enzymes (AST and ALT) (21). In our study, use of silymarin for three months led to optimal results in the light of sonographic findings and liver enzymes. It seems that use of silymarin for longer periods can lead to more optimal findings.

It has been shown that silymarin exerts therapeutic effects on markers of NAFLD in adults (10). Silymarin can reduce serum cholesterol and TG as well (8). In our study, TG levels of the patients significantly decreased but no change was observed in LDL and HDL cholesterol. The patients in the present study had slightly abnormal cholesterol levels but strikingly high TG levels before the treatment. This may be due to differences in dietary regimen or eating habits between adults and children.

Based on the findings, 50% of the patients had grade 1 fatty liver before silymarin treatment in the intervention group. This figure increased to 75% after the treatment. In the control group, 50% and 55% of the patients had grade 1 fatty liver before and after liver treatment, respectively. More clearly, silymarin could significantly decrease the grade of fatty liver in terms of physical activity alone.

There was no significant change in BMI after 12 weeks, which is consistent with another study (10). Therefore, longer treatments are needed to decrease BMI. Studies on children with longer treatments, which have already done on adults, can complement the available evidence and help draw more documented conclusions about silymarin effect on fatty liver in children. The results of this study show that silymarin is an effective herbal drug with few adverse effects and relatively good tolerance. Since a relatively small number of cases were investigated in this study because of lower prevalence of this disease in children compared to adults and the duration of the study (3 months) was not long enough, the results of the current work can be complemented by the study of similar cases

in different countries.

Conclusion

This study confirmed that silymarin could improve biochemical and sonographic indices of NAFLD in children and adolescents.

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Authors' contributions

SR and MB prepared the first draft and RR edited it. All authors read and confirmed the manuscript.

Conflict of interests

The authors declare no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) were completely observed by the authors.

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