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The Safety and Effectiveness of a Polysaccharide Extracted from *Rosa Canina* in Patients with NAFLD: A Randomized Trial

ABSTRACT

Objective: Non-alcoholic fatty liver (NAFL) is a common pathology of hepatocytes due to the accumulation of fat which is predominantly implicated in obesity. Due to the multifaceted characterization of fatty liver and no effective treatment, this study was aimed to assess the protective effect of a polysaccharide in NAFL patients.

Materials and methods: Polysaccharide fraction was isolated from *Rosa canina* and administered to 33 NAFL patients for 90 days. Demographic information, liver ultrasonography, and the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes were studied. Data were analyzed with the use of SPSS version 21 (SPSS, Inc.) All data were shown as means ± SDs and $p < 0.05$ was considered as significant.

Results: After 90-day prescription, the weight loss, reduced activity of ALT and AST as well as changing the echogenicity of the liver from grades of 3, 2, and 1 to 2, 1 and normal, respectively, were observed in patients compared to the baseline ($p \leq 0.05$). In addition, there were no visible side effects in patients during drug consumption.

Conclusions: The data indicated that polysaccharide fraction with no obvious side effects is significantly able to protect the liver from steatosis in patients with NAFL disease. However, further information is required to better conclude on the effectiveness of the isolated polysaccharide as a promising fatty liver drug. (Clin Diabetol 2022, 11; 4: 239–244)

Keywords: non-alcoholic fatty liver disease, polysaccharide, *Rosa canina*, alanine aminotransferase, aspartate aminotransferase

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Introduction

A spectrum of histopathological changes from fatty liver alone to severe non-alcoholic steatohepatitis (NASH) is represented in non-alcoholic fatty liver disease (NAFLD) which may potentially develop hepatic fibrosis and cirrhosis [1]. As the most common cause of chronic liver disease in developed and developing countries, it is reported that NAFLD affects 33.95% of the Iranian population [2, 3]. Considering the growing prevalence of NAFLD, this multifaceted metabolic disorder is closely

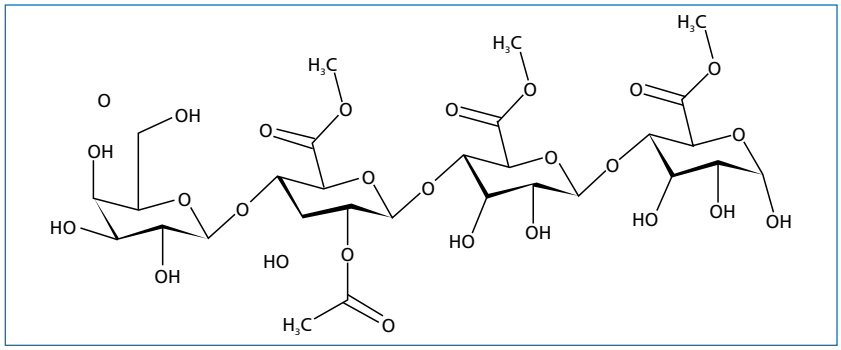

Figure 1. The Proposed Structure of Polysaccharide Obtained from Structural Analysis

associated with obesity affecting approximately 60% of the obese population [4]. Among 10 to 20 percent of NAFLD patients who progress to non-alcoholic steatohepatitis (NASH), there is a 10 to 20 percent chance of developing cirrhosis [5]. Higher rates of HCC and liver transplantation are representing the influence of the rise in NAFLD along with increased proportion with end-stage disease [6]. Evidence of hepatic steatosis, in the absence of secondary causes of fat accumulation encompassing steatogenic medications, extensive alcohol consumption, hepatitis C virus infection, Wilson disease, malnutrition, and hereditary disorders, is required for NAFLD diagnosis [7]. Although the exact pathophysiology of NAFLD is unknown, the "multiple hit" hypothesis illustrates the pathogenesis more accurately [8]. According to the multiple-hit hypothesis, dietary habits along with genetic and environmental factors leading to changes in gut microbiota, insulin resistance, and obesity may contribute to the development of NAFLD. Having that in mind, insulin resistance increases the fatty acid flux in the liver through alterations in adipose tissue lipolysis, adipokines, and inflammatory cytokines production, and an increase in hepatic de novo lipogenesis [9, 10]. In this line, the altered gut flora increases fatty acids production and small bowel permeability leading to raised circulatory levels of molecules and a proinflammatory state. In genetically predisposed subjects, these factors can lead to alterations in hepatocyte fat content and simple steatosis which is associated with abnormal triglyceride storage. Through manifold hepatocellular damage pathways, this condition can develop into a state of chronic hepatic inflammation and fibrosis [8, 11].

Various anti-oxidant and anti-inflammatory properties of herbal medicine make it a suitable choice in the management and prevention of liver disorders [12]. *Rosa canina* (*R. canina*), a member of the *Rosaceae* family, that grows in different regions of the world is well-known for its usage in different metabolic and inflammatory conditions such as arthritis, gout, sciatica, fever, influenza, common cold, kidney and the lower urinary tract diseases, gallstones and diabetes [13, 14].

Clinical rationale for the study

Interestingly, a few clinical trials have introduced *R. canina* powder or extracts as an anti-obesity agent reducing abdominal obesity along with fasting blood glucose (FBG) and cholesterol levels [15, 16]. Manifold components of *R. canina* fruits have been detected including various phytochemical compounds [17]. The current experimental trial aimed to investigate the effect of *R. canina* purified oligosaccharide on patients with NAFLD through evaluating changes in the liver enzymes and grading of the disease.

Materials and methods

Drug preparation

The purified fraction was prepared using flash column chromatography and HPLC from ripe fruits of *Rosa canina* according to Rahimi et al. [18]. In addition, high performance liquid chromatography diode array detector tandem mass spectroscopy (HPLC-DAD MS/MS), infrared radiation (IR), and nuclear magnetic resonance (NMR) systems were applied to characterize the structure of isolated fraction (Fig. 1). For administration, each capsule with 400 mg polysaccharide fraction was prepared.

Patients and administration

In an experimental preclinical trial, patients diagnosed with nonalcoholic fatty liver disease (NAFLD) were allocated by the research team to find out the safety and efficacy of an isolated polysaccharide from *Rosa canina* in the treatment of NAFLD.

A total of 33 NAFLD patients who were diagnosed by ultrasonography and biochemical experiments were recruited from Taleghani hospital, Kermanshah, Iran. Inform consent was obtained before initiation of therapy. The criteria for inclusion in the study were grade 1–3

fatty liver in sonography, alanine aminotransferase (ALT) more than 30 for men and more than 20 for women, aged 30–65 years, BMI > 25, no use of supplementary antioxidants during one month before the start of the study, no fasting, weight loss and gain during 3 months from the start of the study as well as no evidence of any other acute and chronic heart, kidney, liver (hepatitis B and C) and infectious disorders, diabetes, or other ischemic pathologies. Patients who were pregnant or lactating, taking methotrexate, amiodarone, tamoxifen, sodium valproate, corticosteroids and drugs that affect weight and liver within the past 3 months were excluded from the study. Outcome variables including liver ultrasonography, ALT, and aspartate aminotransferase (AST) were measured at baseline and the end of 3 months of the administration. The administrative dose of the drug was three capsules after meals daily for three months. There were no reports of serious adverse effects in patients prescribed isolated polysaccharide throughout the study.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) measurement

The measurement of AST and ALT enzymatic activities were performed with commercial kits (Pars Azmoon), by using an autoanalyzer (AVIDA 1800 chemistry system; Siemens), in the blood plasma of patients.

Ultrasonography

Fatty liver was evaluated using abdominal ultrasonography by an expert radiologist. To determine the grading of fatty liver, a modified method of Kurtz was considered [19]. In this line, participants were divided into normal and fatty liver including grade 1 (mild), grade 2 (moderate), and grade 3 (severe) impaired parenchyma of the liver diagnosed as brighter texture than the renal parenchyma [20].

Statistical analysis

Data were analyzed with the use of SPSS version 21 (SPSS, Inc.). All data were shown as means ± SDs and $p < 0.05$ was considered as significant. Descriptive statistics were used for the description of data. Qualitative variables were described with frequency and percentage and quantitative variables were assessed by using mean and standard deviation. Wilcoxon test was used to compare quantitative variables. In order to check the compliance of the variables with the normal distribution, the Kolmogorov-Simonov test was used.

Ethical permission

This study was reviewed and accepted by ethics committee from Kermanshah University of Medical Sciences with certification number 3004267.

Table 1. Demographic and Baseline Disease Characteristics of Patients with NAFLD[1]

Age [y]	41.98 ± 7.68
Sex (%)	
male	36.4
female	63.6
Marital status (%)	
single	15.15
married	84.85
Job (%)	
governmental	45.5
freelance	9.1
housewife	36.4
student	9.1
Serum liver enzymes [IU/L]	
AST	39.75 ± 16.8
ALT	48.96 ± 29.02
Smoking status (%)	
current smoker	12.1
non-smoker	87.9
BMI [kg/m^2]	25.20 ± 3.82
Physical activity (%)	
daily walking	33.3
athletic	57.6
none	9.1

[1] Values are means ± SDs otherwise indicated; ALT — alanine aminotransferase; AST — aspartate aminotransferase; BMI — body mass index

Results

A total of 33 NAFLD patients were enrolled into the study. Thirty subjects (90.9%) completed the trial and were included in the final analysis and there were three missing data. The subjects reported no history of adverse effects, hypersensitivity reactions, and/or any abnormal events during the trial. Baseline demographic characteristics of the subjects are summarized in Table 1. More subjects were female (63.6%) and the age range of 21 to 54 years. Minimum and maximum BMI values were 20 and 40 kg/m^2. Reductions in serum concentrations of ALT ($p = 0.008$) and AST ($p < 0.002$) after 90 days treatment were significant (Fig. 2). At the end of the study, 86.6 % of patients showed a reduction in the grade of fatty liver. So, there was a significant improvement in NAFLD grade ($p < 0.001$) (Tab. 2).

Discussion

In this trial, the protective effect of an isolated fraction with a polysaccharide structure was evaluated for the first time in patients with fatty liver disease. Administration of the isolated polysaccharide for 90

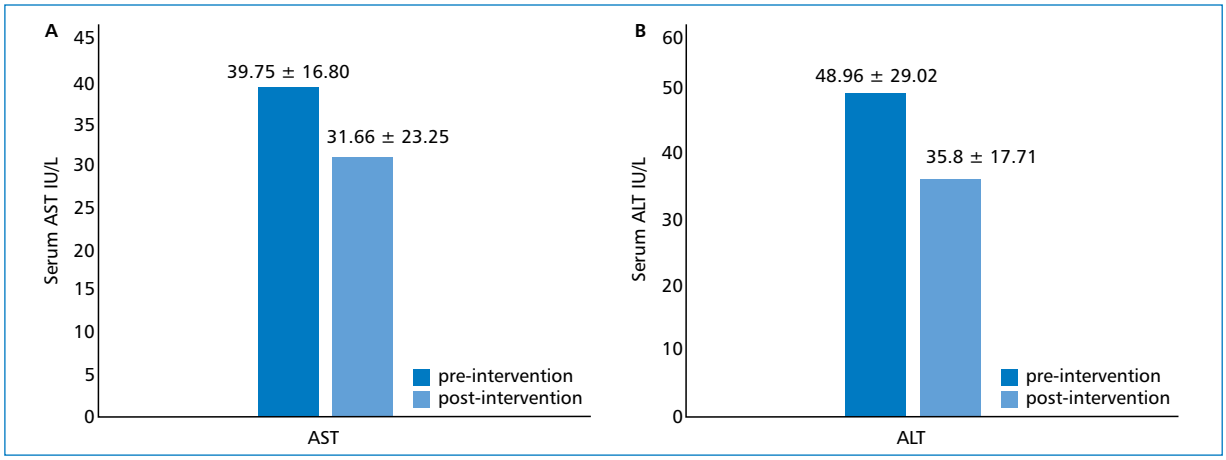

Figure 2. Changes in the Serum Liver Enzymes AST (A), and ALT (B). On the basis of Wilcoxon paired rank test there were significant differences between pre- and post-intervention liver enzymes. ALT ($p = 0.008$), AST ($p < 0.002$)

Table 2. Comparison of Pre- and Post-Invention Grades of Liver Steatosis Assessed by Ultrasonography

Pre-intervention grades n (%)		Post-intervention grades n (%)[2]		Changes of pre- to post-intervention grades of liver steatosis; n (%)[1]			
				Grade 1 to not fatty liver	3 (10.0%)	**Grade 2 to not fatty liver**	1 (3.3%)
		Not fatty liver	7 (23.3)	**Grade 1 to 1**	2 (6.6%)	**Grade 2 to 3**	1 (3.3%)
Grade 1[1]	6 (18.2)	**Grade 1**	10 (33.3)	**Grade 1–2 to 1**	1 (3.3%)	**Grade 2–3 to 1**	3 (3.3%)
Grade 1–2	4 (12.1)	**Grade 1–2**	1 (3.3)	**Grade 1–2 to not fatty liver**	2 (6.6%)	**Grade 2–3 to 2**	6 (20.0%)
Grade 2	7 (21.2)	**Grade 2**	9 (30.0)	**Grade 1–2 to 2–3**	1 (3.3%)	**Grade 2–3 to not fatty liver**	1 (3.3%)
Grade 2–3	11 (33.3)	**Grade 2–3**	2(6.7)	**Grade 2 to 1–2**	1 (3.3%)	**Grade 3 to 2–3**	1 (3.3%)
Grade 3	5 (15.2)	**Grade 3**	1 (3.3)	**Grade 2 to 1**	4 (13.3%)	**Grade 3 to 2**	3 (10.0%)

[1]grade 1 — mild fatty liver changes; grade 1–2 — mild to moderate fatty liver changes; grade 2 — moderated fatty liver changes; grade 2–3 — moderate to severe fatty liver changes; grade 3 — severe fatty liver changes
[2]data are expressed as frequency percent. On the basis of Wilcoxon paired rank test there were significant differences between pre- and post-intervention grades of liver steatosis ($p = 0.001$)

days could normalize the activity of hepatic enzymes and improve destructed texture of fatty liver from grade 1, 2 and 3 to normal, 1 and 2, respectively, in non-alcoholic fatty liver (NAFL) patients. In addition, 90-day consumption of the fraction was not associated with any adverse effects on the whole body of patients. In this line, Sadeghi *et al*. showed that the hydro-alcoholic fruit extract of *R. canina* has hepatoprotective effects against CCl4-induced hepatic damage which was probably mediated by modulation of oxidative stress [21]. Another study showed the protective effects of *R. canina* on tamoxifen-induced fatty liver in rats [22].

According to our previous studies, *Rosa canina* extract, powder and isolated fraction promoted the regenerative potential and the promising anti-diabetic effects in the pancreas from animal models and *in vitro* pancreatic cell lines [18, 23–25]. The protective effects of *Rosa canina* were also examined in animal models of atherosclerosis and fatty liver in our lab. (Data were not published.) *R. canina* powder or extracts were reported in clinical trials as anti-obesity agents that could reduce abdominal obesity, FBG and cholesterol levels [15, 16]. On the other hand, several studies have suggested that isolated polysaccharide derivatives from algae, mushrooms and plants imply the convenient protective effects against non-alcoholic fatty liver disease [26–29]. This scenario opens exciting new possibilities to hypothesize whether isolated herbal fractions such as isolated polysaccharide from *R. canina* are effective in clinical trials.

Given that fatty liver disease remains incompletely characterized, research is in progress to develop an acceptable therapeutic strategy. Considering the low adverse effects of natural products, the effects of antioxidants, phytochemicals, vitamins, and fatty acids were examined in several clinical trials [30–33]. To our knowledge, this is the first evidence in which the protective effect of an isolated polysaccharide was studied in

the clinical trial. In fact, lowering body weight is hardly achieved in obese people [34]. Surprisingly, our data showed that studied patients with no fasting regimens obtained lower body weight than the baseline of the study. Furthermore, normalization of serum ALT and AST levels following the trial period has been associated with improved liver echogenicity.

The study was associated with some cons and pros aspects. The main advantage of the study was to evaluate the palliative effect of the isolated polysaccharide in patients for the first time. What is more, no side effects were reported in all studied patients. On the negative side, some disadvantages limited our study. At first, the placebo group was not considered in the trial. Secondly, pathological and biochemical analyses are needed to check for correct explication of the results. Therefore, further clinical trials are necessary to better clarify the protective and therapeutic effects of the isolated fraction in the alleviation of fatty liver.

Conclusions

The culmination of these observations illustrates the promising protective and therapeutic effects of the isolated polysaccharide with no striking side effects in NAFL patients.

Conflict of interest

None declared.

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