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Original article

The effect of hydroalcoholic Berberis integerrima fruits extract on the lipid profile, antioxidant parameters and liver and kidney function tests in patients with nonalcoholic fatty liver disease

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is one of the hepatic disorders which is characterized by increasing fat deposits in the liver. This disorder may lead to elevation the activity of liver enzymes and is associated with obesity, dyslipidemia, hypertension, and type II diabetes. The aim of present study was to investigate the effect of *Berberis integerrima* extract on NAFLD patients compare to placebo.

Methods: The present clinical trial was performed on 42 NAFLD patients who were randomly divided into two groups. The case group received a capsule (750 mg) containing hydro-alcoholic extract of *Berberis integerrima* extract every 12 h for 2 months, while the control (placebo) group received a capsule containing cellulose. Baseline characteristics, biochemical factors, antioxidant parameter, functional liver and renal test were evaluated before and after the treatment.

Results: Comparison rate of different parameters in case group before and after treatment demonstrated that BMI, cholesterol, triglyceride, LDL-C, fasting blood glucose, liver enzymes and renal parameters such as aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were significantly decreased while HDL-C, glutathione peroxidase enzyme, and total antioxidant capacity were significantly elevated. The comparison of the mean parameters difference in two groups indicated that cholesterol, triglyceride, LDL-C, fasting blood glucose, liver enzymes and renal factors were significantly decreased, however HDL-C, glutathione peroxidase enzyme, and total antioxidant capacity were significantly increased in case group compared to control group.

Conclusion: The study findings revealed that the *Berberis integerrima* extract could reduce biochemical factors of blood, except HDL-C and increases total antioxidant capacity and glutathione peroxidase enzyme. Therefore, hydro-alcoholic extract of *Berberis integerrima* may be used as a great supplementary medicine in treating NAFLD.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a chronic disease whose prevalence is increasing throughout the whole world (Davoodi et al., 2012). This disease is characterized by excessive fat and triglyceride accumulation without any primary cause such as viral hepatitis, alcohol consumption, or drug-induced liver injury (Haga et al., 2015). Simple steatosis that is an early stage of fatty liver could progress towards non-alcoholic steatohepatitis

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(NASH), liver fibrosis, cirrhosis and liver cancer (Sookoian et al., 2016). NAFLD is a main part of metabolic syndrome (MetS) and is associated with obesity, diabetes, dyslipidemia, and insulin resistance (Kasper et al., 2015). It might be a risk factor for Heart ischemia, stroke and chronic kidney disease. So early detection and treatment of NAFLD could help to minimize these risks and improve the public health (Jamali et al., 2016).

In the last 50 years, various drugs such as Thiazolidinedione, vitamin D, Metformin have been suggested for treatment of NAFLD but these drugs have some side effects and also, they are considered a temporary treatment option (Singh et al., 2017). Scientists are trying to find an effective approach for this disease and nowadays their focus is on the traditional medicines as an alternative treatment.

Berberis integerrima belonging to *Berberidaceae* family is considered as an effective herbal medicine in liver dysfunction (Hosseinzadeh et al., 2013). Various ingredients including alkaloids, bioactive and phenolic compounds are reported for this plant. Bioactive compounds such as berbamine, berberuin, palmatine, oxyacanthine, malic acid, ascorbic acid, caffeic acid, ursolic acid, coumarin, beta carotene, and tannin are used in medical and food utilization (Ardestani et al., 2013). One of its main active alkaloids is berberin which has pharmacological properties including antimicrobial, anti-inflammatory, antihistaminic, anticholinergic, antipyretic, anticancer, and antioxidant (Rafiee et al., 2016). Some animal studies demonstrated that this alkaloid might be effective on regulating blood sugar and lipids. It could improve renal dysfunction and also reduce the oxidative stress (Ashraf et al., 2014; Bayani et al., 2016; Bhutada et al., 2010). Researchers reported that *Berberis integerrima* is used to alleviate insomnia, bronchial diseases, and liver disorder (Hosseinzadeh et al., 2013).

As regards the increasing prevalence of NAFLD recently, and also the lack of clinical study around the effect of *Berberis integerrima* fruits on NAFLD, the aim of this study is evaluation of the *Berberis integerrima* fruits effects on patients with NAFLD.

2. Materials and methods

2.1. NAFLD diagnosis and criteria for inclusion and exclusion

The present randomized double-blind clinical trial study was conducted on nonalcoholic fatty liver patients who referred to Ali-Ibn-Abitaleb Rafsanjan University Hospital and their disease was confirmed by increased liver enzymes and ultrasound of the liver tissue by an expert physician. Inclusion criteria included having the nonalcoholic fatty liver, elevated levels of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), the age range of 20–45 years and signing a declaration of consent. Patients using alcohol, daily *Berberis* and having allergy to *Berberis* and its compounds, patients with diabetes, high blood pressure, perceptual disorders, nephrotic syndrome, uremia ischemic heart diseases, chronic liver disease such as hepatitis, and pregnancy and lactation were excluded from the study (Hajiaghamohammadi et al., 2012).

2.2. Study design and blood sampling and laboratory methods

Forty-two patients were divided into two groups, randomly: control group received a gelatin capsule containing 750 mg cellulose every 12 h for two months and experimental group received a gelatin capsule containing 750 mg hydro-alcoholic extract of *Berberis integerrima* every 12 h for two months (Kashkooli et al., 2015). All patients in this study received Metformin and vitamin E as a standard treatment during the study.

The blood sampling was done before and after the study and blood samples were sent to a laboratory that had no information about the patients. In the laboratory biochemical factors, including fasting blood glucose (FBG), total cholesterol, triglyceride (TG), HDL-C, and LDL-C, functional liver test including ALT, AST, alkaline phosphatase (ALP), total and direct bilirubin, functional renal test such as urea and creatinine were measured by Pars Azmoon kits (Iran) and BT1500 system (Biotechnica, Italy). Antioxidant parameter such as glutathione peroxidase enzyme (GPX) (Zellbio, Germany, ZB-GPX-A48), malondialdehyde (MDA) (Zellbio, Germany, ZB-MDA-48A), and total antioxidant capacity (Zellbio, Germany, ZB-TAC-48A) were measured by antioxidant kits. Baseline characteristics such as height, weight, body mass index (BMI), blood pressure was measured before and after the study.

2.3. Hydro-alcoholic extract of Berberis preparation

Berberis fruits were taken from mountainous areas of Bardsir, Kerman, Southeast of Iran and confirmed by the Iranian Institute of Research & Development in Chemical Industries and they were extracted in that institute. The dried *Berberis integrrima* fruit powder (40 kg) was extracted with ethanol/ water (70/30) at 55 °C for 6 h. Then, the extract was filtered using a filter press, and after concentrating the extract in vacuo at 45–60 °C, the concentrated extract was powdered with a spray dryer.

750 mg of extract powder into gelatin capsules was poured.

2.4. Statistical analysis

All data were analyzed by SPSS statistical software V.22, independent two samples *t*-test and paired *t*-test. $P < 0.05$ was considered as statistically significant. To compare mean biochemical factors, total antioxidant capacity, enzymatic activity of glutathione peroxidase and malondialdehyde, independent two samples *t*-test was used before and after the intervention. Also, paired *t*-test was used to compare the mean of the factors studied in each group before and after the intervention. Kolmogorov-Smirnov nonparametric test was used to evaluate the normality of the distribution of quantitative variables in this study. Results will be reported for quantitative data (mean ± standard deviation) and for qualitative data (number [percentage]).

2.5. Ethics approval

This study was carried out with the permission of the Ethics Council of Rafsanjan University of Medical Sciences with the IR code: IR.RUMS.REC.1396.110.

3. Results

Forty-two patients entered the study, 21 enrolled in each group. Control group comprised 15 males and 6 females while the experimental group included 14 males and 7 females. Demographic characteristics of the experimental and control groups are shown in Table 1. The data showed that control and experimental group do not differ statistically and both groups match. Average age in experimental group was not different compared to control group (p: 0.574).

Average of anthropometric, biochemical, and antioxidant parameters in patients with NAFLD have no difference in two groups before the treatment and the experimental and control group also matched (Table 2).

Table 1
Demographic characteristics of case and control groups.

Parameters		Groups		P value
		Case group	Control group	
Age (year)		33.52 ± 6.67	34.90 ± 8.69	0.574
Sex (female/male)	Male	14 (66.7)	15 (70)	0.819
	Female	7 (33.3)	6 (30)	
Family history of liver disease	Yes	3 (14.3)	6 (30)	0.277
	No	18 (85.7)	14 (70)	

Table 2
Comparison of different parameters in case and control groups, before treatment and comparison of average difference before and after treatment in case and control groups.

Parameters	Groups					
	Case vs. Control.			Case vs. Control. Average difference before and after treatment		
	Before treatment					
	Case	Control	P value	Case	Control	P value
Height (cm)	168.86 ± 7.64	173.75 ± 7.41	0.054	0.00	0.00	1.00
Weight (kg)	73.83 ± 10.08	80.48 ± 10.24	0.053	−1.05 ± 1.37	−0.33 ± 1.04	0.066
BMI (kg/m^2)	25.85 ± 2.63	26.66 ± 2.91	0.352	−0.37 ± 0.50	−0.12 ± 0.35	0.073
Waist (cm)	93.41 ± 5.3	96.85 ± 8.36	0.124	−1.83 ± 1.89	−0.75 ± 1.45	0.047
Systolic blood pressure (mmHg)	123.62 ± 6.98	122.30 ± 6.97	0.548	−3.29 ± 6.02	−1.25 ± 4.28	0.221
Diastolic blood pressure (mmHg)	80.95 ± 5.61	80.10 ± 4.23	0.587	−1.57 ± 4.37	−0.4 ± 3.58	0.354
MDA (mM/L)	4.09 ± 0.89	4.25 ± 0.74	0.542	−1.54 ± 0.76	−0.48 ± 0.40	<0.001
Total antioxidant capacity (mM/L)	1.38 ± 0.28	1.32 ± 0.23	0.446	0.59 ± 0.32	0.29 ± 0.23	0.001
GPX (IU/mg)	256.10 ± 39.16	253.50 ± 45.96	0.388	38.71 ± 25.61	14.50 ± 27.02	0.005
FBG (mg/dl)	103.76 ± 6.86	99.30 ± 8.20	0.066	−20.7 ± 4.38	−10.65 ± 4.21	<0.001
Total cholesterol (mg/dl)	250.19 ± 51.68	277.15 ± 48.54	0.150	−44.57 ± 14.90	−10.90 ± 12.20	<0.001
HDL-C (mg/dl)	46.38 ± 5.06	44.90 ± 4.62	0.332	8.43 ± 3.06	1.80 ± 8.09	0.003
LDL-C (mg/dl)	133.05 ± 20.11	122.35 ± 21.73	0.110	−20.71 ± 11.29	−7.00 ± 8.09	<0.001
TG (mg/dl)	224.43 ± 83.90	233.05 ± 61.74	0.711	−29.81 ± 28.92	−10.00 ± 9.77	0.015
Urea (mg/dl)	29.10 ± 6.98	34.40 ± 9.13	0.053	−2.86 ± 3.47	−2.30 ± 4.05	0.638
Creatinine (mg/dl)	1.29 ± 0.21	1.27 ± 0.28	0.839	−0.09 ± 0.18	−0.01 ± 0.15	0.133
AST (U/L)	52.95 ± 10.04	52.35 ± 9.89	0.848	−15.14 ± 9.92	−6.50 ± 8.84	0.005
ALT (U/L)	54.52 ± 10.31	54.55 ± 9.81	0.993	−15.14 ± 7.30	−8.40 ± 6.17	0.003
ALP (U/L)	239.19 ± 53.02	232.60 ± 44.21	0.669	−18.57 ± 32.83	−0.10 ± 25.22	0.041
Total bilirubin (mg/dl)	1.04 ± 0.33	1.10 ± 0.30	0.564	−0.08 ± 0.14	−0.04 ± 0.09	0.214
Direct bilirubin (mg/dl)	0.29 ± 0.09	0.29 ± 0.10	0.888	−0.04 ± 0.08	0.01 ± 0.60	0.066

3.1. Comparison of different parameters in case group, before and after treatment

The average of anthropometric, biochemical, and antioxidant parameters, before treatment in the case group were compared with those after treatment through the paired T test (Table 3).

3.2. Anthropometric characteristics

Data showed no change in the height of patients. Systolic and diastolic blood pressure of the case group were reduced after treatment and this reduction for systolic blood pressure was significant, while for diastolic blood pressure it was not significant. Mean weight, waist and BMI significantly reduced after treatment with hydro- alcoholic Barberry fruits.

3.3. Biochemical factors

Mean serum FBG level in case group was 103.76 ± 6.86 mg/dl before treatment and diminished to 83.05 ± 7.02 mg/dl after treatment and this reduction was statistically significant (p: 0.001). Total cholesterol, TG, LDL-C of the case group were 250.19 ± 51.68 mg/dl, 224.43 ± 83.90 mg/dl, 133.05 ± 20.11 mg/dl respectively and after treatment these parameters reached 205.62 ± 45.01 mg/dl, 194.62 ± 60.51 mg/dl, 112.33 ± 15.13 mg/dl respectively ($p < 0.05$). Before treatment, HDL-C was 46.38 ± 5.06 mg/dl and significantly increased to 54.81 ± 4.80 mg/dl (p: 0.001).

3.4. Antioxidant parameters

Mean serum MDA level was 4.09 ± 0.89 μm/l in case group before treatment and it reached 2.54 ± 0.72 μm/l after treatment and this reduction was significant ($p < 0.05$). GPX and total antioxidant capacity averages were 265.10 ± 39.16 U/ml and 1.38 ± 0.28 μm/l respectively and these parameters elevated to 303.81 ± 26.64 U/ml and 1.98 ± 0.29 μm/l after treatment. These data showed that GPX and total antioxidant capacity averages significantly increased after treatment ($p < 0.05$).

3.5. Functional liver and renal test

The average of serum ALT, AST and ALP level were 54.52 ± 10.31 U/L, 52.95 ± 10.04 U/L, 239.19 ± 53.02 U/L, respectively, in case group before treatment and the rate of these parameters decreased to 39.38 ± 5.32 U/L, 37.81 ± 5.34 U/L, 220.62 ± 30.94 U/L, respectively after treatment and these declines were statistically significant ($p < 0.05$). Before treatment of case group, total and direct bilirubin were 1.04 ± 0.33 mg/dl and 0.29 ± 0.09 mg/dl, respectively which reduced to 0.96 ± 0.23 mg/dl and 0.25 ± 0.07 mg/dl and these rates were significantly different. Average of serum urea and creatinine level, respectively were 29.10 ± 6.98 mg/dl and 1.29 ± 0.21 mg/dl in case group before treatment and declined to 26.24 ± 4.55 mg/dl and 1.20 ± 0.19 mg/dl, respectively and these decreases were statistically significant $p < 0.05$.

Table 3
Comparison of different parameters in case and control groups, before and after treatment.

Parameters	Groups					
	Case group			Control group		
	Before treatment	After treatment	P value	Before treatment	After treatment	P value
Height (cm)	168.86 ± 7.64	168.86 ± 7.64	1.000	173.75 ± 7.41	173.75 ± 7.41	1.000
Weight (kg)	73.83 ± 10.08	72.79 ± 9.94	0.002	80.48 ± 10.24	80.15 ± 10.20	0.179
BMI (kg/m^2)	25.85 ± 2.63	25.48 ± 2.56	0.003	26.66 ± 2.91	26.54 ± 2.82	0.151
Waist (cm)	93.41 ± 5.3	91.57 ± 5.2	<0.001	96.85 ± 8.36	96.10 ± 8.25	0.032
Systolic blood pressure (mmHg)	123.62 ± 6.98	120.33 ± 3.14	0.021	122.30 ± 6.97	121.05 ± 5.00	0.207
Diastolic blood pressure (mmHg)	80.95 ± 5.61	79.38 ± 3.92	0.115	80.10 ± 4.23	79.70 ± 3.66	0.623
MDA (mM/L)	4.09 ± 0.89	2.54 ± 0.72	<0.001	4.25 ± 0.74	3.77 ± 0.65	<0.001
Total antioxidant capacity (mM/L)	1.38 ± 0.28	1.98 ± 0.29	<0.001	1.32 ± 0.23	1.62 ± 0.31	<0.001
GPX (IU/mg)	256.10 ± 39.16	303.81 ± 26.64	<0.001	253.50 ± 45.96	268.0 ± 33.65	0.027
FBG (mg/dl)	103.76 ± 6.86	83.05 ± 7.02	<0.001	99.30 ± 8.20	88.65 ± 7.14	<0.001
Total cholesterol (mg/dl)	250.19 ± 51.68	205.62 ± 45.01	<0.001	277.15 ± 48.54	216.25 ± 41.7	0.001
HDL-C (mg/dl)	46.38 ± 5.06	54.81 ± 4.80	<0.001	44.90 ± 4.62	46.70 ± 10.86	0.387
LDL-C (mg/dl)	133.05 ± 20.11	12.33 ± 15.13	<0.001	122.35 ± 21.73	115.35 ± 20.47	<0.001
TG (mg/dl)	224.43 ± 83.90	194.62 ± 6051	<0.001	233.05 ± 61.74	223.05 ± 48.01	0.036
Urea (mg/dl)	29.10 ± 6.98	26.24 ± 4.55	0.001	34.40 ± 9.13	32.10 ± 5.97	0.020
Creatinine (mg/dl)	1.29 ± 0.21	1.20 ± 0.19	0.033	1.27 ± 0.28	1.26 ± 0.18	0.772
AST (U/L)	52.95 ± 10.04	37.81 ± 5.34	<0.001	52.35 ± 9.89	45.85 ± 7.74	0.004
ALT (U/L)	54.52 ± 10.31	39.38 ± 5.32	<0.001	54.55 ± 9.81	46.15 ± 7.31	<0.001
ALP (U/L)	239.19 ± 53.02	220.62 ± 30.94	0.017	232.60 ± 44.21	232.50 ± 33.07	0.986
Total bilirubin (mg/dl)	1.04 ± 0.33	0.96 ± 0.23	0.013	1.10 ± 0.30	1.07 ± 0.27	0.085
Direct bilirubin (mg/dl)	0.29 ± 0.09	0.25 ± 0.07	0.044	0.29 ± 0.10	0.30 ± 0.11	0.716

3.6. Comparison of different parameters in control group, before and after treatment

The average of different parameters, before and after treatment in patients who received cellulose is shown in Table 3. The data were compared to each other through paired T test.

3.7. Anthropometric characteristics

Mean height, weight, waist and BMI, after treatment in control group did not show any differences compared to these parameters before treatment ($p > 0.05$). Systolic and diastolic blood pressure in control group were slightly reduced, but this reduction was not significant ($p > 0.05$).

3.8. Biochemical factors

In control group, mean FBG was 99.30 ± 8.20 mg/dl before treatment and significantly declined to 88.65 ± 7.14 mg/dl after treatment ($p < 0.001$). Average of serum total cholesterol, TG, and LDL-C level were respectively, 277.15 ± 48.54 mg/dl, 233.05 ± 61. 74 mg/dl, and 122.35 ± 21.73 mg/dl before treatment and these factors reached to 216.25 ± 41.71 mg/dl, 223.05 ± 48.01 mg/dl, 115.35 ± 20.47 respectively, after treatment in control group. These data showed significant reduction in total cholesterol, TG and LDL-C after treatment ($p < 0.05$). Although average of serum HDL-C level was increased after treatment, it was not statistically significant.

3.9. Antioxidant parameters

In control group, mean serum MDA level was 4.25 ± 0.74 μm/l before treatment and significantly reduced to 3.77 ± 0.65 μm/l after treatment ($p < 0.05$). Average of GPX and total antioxidant capacity were 253.50 ± 45.69 U/ml, and 1.32 ± 0.23 μm/l respectively, before treatment and increased to 268.00 ± 33.65 U/ml and 1.62 ± 0.31 μ m/l after treatment. These enhancements were statistically significant in control group ($p < 0.05$)

3.10. Functional liver and renal test

In control group mean serum ALT, AST and ALP level were diminished after treatment and the rate of reduction for ALT and AST was significant ($p < 0.05$) while the rate of reduction for ALP was not significant (p: 0.98). Mean serum urea and creatinine level reduced in control group after receiving cellulose. Although serum urea level had a significant reduction ($p < 0.05$), serum creatinine level did not show any significant difference (p: 0.77). Average of serum total and direct bilirubin level in control group did not have any significant difference before and after treatment ($p > 0.05$).

3.11. Comparison of the average difference (before and after treatment) of different parameters

As shown in Table 2, the average differences of the various parameters in case and control groups were compared to each other. The average of weight difference and BMI difference in case group had no significant difference compared to control group, however, the average of waist difference in case group was significantly more than control group $p < 0.05$). In systolic and diastolic blood pressure no differences were observed between two groups ($p > 0.05$).

3.12. Biochemical factors

The average of FBG difference was −20.71 ± 4.38 mg/dl in case group and was −10.65 ± 4.21 mg/dl in control group. The comparison of these two averages showed a significant different ($p < 0.05$). In case group the mean of cholesterol difference was −44.57 ± 14. 90 mg/dl while in control group it was −10.90 ± 12.20 mg/dl and this difference was significant ($p < 0.05$). The mean of LDL, HDL and TG differences were more in case group compared to control group ($p < 0.05$).

3.13. Antioxidant parameters

The average difference of MDA, GPX and total antioxidant capacity was −1.54 ± 0.76 μm/l, 0.59 ± 0.32 μm/l, and 38.71 ± 25.

61 U/ml, respectively in case group which had a significant difference with control group.

3.14. Functional liver and renal test

The mean difference of ALT, AST and ALP was −15.14 ± 7.30 U/L, −15.14 ± 9.92 U/L, and −18.57 ± 32.83 U/L, respectively in case group and these mean differences were −8.40 ± 6.17 U/L, −6.50 ± 8.84 U/L, and −0.10 ± 25.22 U/L, in control group which indicates a significant difference ($p < 0.05$). The average urea and creatinine differences in control group had no significant difference with control group ($p > 0.05$). In the average total and direct bilirubin no differences were observed between two groups.

4. Discussion

Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease with the worldwide prevalence that can lead to liver cirrhosis, liver cancer, and eventually death (Tanaka et al., 2019). NAFLD is defined as fatty accumulation in the liver, which can progress to cirrhosis, and hepatic cancer cells (HCC). Therefore, in the early stages of the disease before it progresses to cirrhosis and liver inflammation, fatty accumulation is the main pathological process (Mehta et al., 2016). Currently, the exact pathogenesis of NAFLD has not been identified. However, it is often associated with obesity and metabolic disorders such as insulin resistance, diabetes, hypertension, dyslipidemia, atherosclerosis and inflammation (Younossi et al., 2016). The oxidative stress caused by the imbalance between pro-oxidants and anti-oxidants leads to many pathological events in the liver. Oxidative stress is an important mechanism in the pathogenesis of NAFLD (Su et al., 2016). The mitochondria are required for energy production through the oxidative phosphorylation of glucose and fat in the liver. Particularly, because of that mitochondria are vulnerable to ROS relative to oxidative stress, maintaining mitochondrial function is vital for the survival of cells due to oxidative stress (Paradies et al., 2014). In fact, significant evidence suggests that mitochondrial dysfunction is directly related to the NAFLD pathogenesis. Previous studies have shown that ROS modifies the redox conditions of the liver cells and activates the kinases that are sensitive to redox reactions and ultimately lead to liver steatosis. Therefore, regulation of mitochondrial oxidative stress by antioxidants may be a new strategy for the treatment of NAFLD (Rolo et al., 2012).

There is no specific drug available for this disease, however, losing weight and treatment of hyperlipidemia can help to reduce the risk of NAFLD (Kashkooli et al., 2015). In spite of modern medical advances, there is always a partial defect in the treatment of commonly used drugs concerning side effects and drug resistance. So nowadays, the use of herbal medicines has increased significantly. Regarding the many studies which evaluate the extract of *Berberis integerrima's* effects on NAFLD, in this study we try to investigate the effect of hydroalcoholic extract of *Berberis integerrima* on lipid profile, antioxidant parameters and functional liver and kidney tests in patients with non-alcoholic fatty liver. The results showed that the rate of different parameters before and after treatment such as BMI, cholesterol, triglyceride, LDL-C, fasting blood glucose and some functional liver and renal factors such as ALT, AST and ALP were significantly decreased while HDL-C, glutathione peroxidase enzyme, and total antioxidant capacity were significantly elevated in case group. The comparison of the mean parameters difference in two groups indicated that cholesterol, triglyceride, LDL-C, fasting blood glucose, functional liver and renal factors were significantly decreased, however HDL-C, glutathione peroxidase enzyme, and total antioxidant capacity were significantly increased in case group in comparison to control group.

A study in 2016 evaluated the effects of copper oxide nanoparticles and hydroalcoholic extracts of *Berberis vulgaris*, Descurainia sophia and Silybum marianum on catalase, glutathione peroxidase, and malondialdehyde concentration in male diabetic rats. They reported that *Berberis integerrima* extract reduces the negative effects of oxidative stress, induced by copper oxide nanoparticles in diabetic rats, by increasing the concentration of glutathione peroxidase enzyme (Mohamadifard et al., 2016). These results were similar to our results that revealed hydroalcoholic extract of *Berberis integerrima* enhanced significantly the glutathione peroxidase enzyme, and total antioxidant capacity in patients with NAFLD.

Another study examined *Berberis vulgaris* effects on oxidative stress and liver injury in lead-intoxicated mice and it was observed in treated rats with aqueous extract of barberry, the levels of liver factors (AST and ALT) and total cholesterol (TC), total bilirubin (TB) and glutathione peroxidase enzymes were normalized. Therefore, they concluded that the aqueous extract of *Berberis integerrima* has significant effects on lead-induced oxidative stress and liver damage (Laamech et al., 2017;14(1).). These results were consistent with our results and the probable mechanism of this plant's action may be that, by enhancing the antioxidant status, it confers the ability to chelate the lead and neutralize free radicals.

Berberis integerrima contains different types of phytochemicals compounds such as alkaloids, flavonoids, tannins and phenols, which are responsible for various antioxidant activities. The fruits, roots and stems of *Berberis integerrima* contain these compounds. Therefore, the possible mechanism for the regeneration of enzymes such as GPX, free radical purging and high antioxidant activity of *Berberis integerrima* is due to the presence of vitamin C, malic acid and tannins (Laamech et al., 2017).

Previous research demonstrated the effect of *Berberis vulgaris* extract on transaminase activities in non-alcoholic Fatty liver disease. They measured the weight, liver transaminases levels and lipid profiles of the control and treatment groups before, during, and after the treatment. It was shown that *Berberis vulgaris* extract significantly reduced the liver enzymes (ALT and AST), weight, triglycerides and cholesterol. They stated that more experiments about this extract can be helpful in treatment of the NAFLD patients. This study was similar to our study in some measured tests but they evaluated the other extract of *Berberis vulgaris* whose results were consistent with our results. However, in their study no changes in fasting blood glucose, HDL-C and LDL-C were observed, so this part of their results were inconsistent our results (Kashkooli et al., 2015;15(2).). Hypolipidemic effects are induced by inhibiting the synthesis of cholesterol and triglyceride through activating the pathway of AMPK, followed by the inhibition of acetyl-CoA carboxylase (ACC) in liver cells (Brusq et al., 2006). Studies have shown that the effect of *Berberis integerrima* on these enzymes is due to its antioxidant properties and its compounds, thereby leading to liver protection (Kashkooli et al., 2015). *Berberis integerrima* contains organic acids and phenolic compounds such as anthocyanin and carotenoid pigments as well as polyphenolase, phenolase and glycosidase enzymes, and various alkaloids such as berbamine, berberine and palmatine in different parts of the plant. These compounds have antioxidant effects due to interactions with different molecules (Lewis and Mohanty, 2010).

The probable mechanism of this plant in association with the reduction of cholesterol level inhibits intestinal absorption and interfers with intracellular cholesterol micellarization and reduces the absorption and secretion of cholesterol in enterocytes. Additionally, *Berberis integerrima* significantly increases the mRNA levels of carnitine palmitoyltransferase (CPT1a), microsomal triglyceride transfer protein (MTTP), and LDL-R in the liver.

A current research evaluated Zereshk-e-Saghir, a combination of several herbs such as *Berberis integerrima*, on tetrachloride-induced liver damage in rats. The results showed that the level of liver enzymes AST, ALT and ALP decreased. Also, in treated group the lipid peroxidation reduced and the levels of glutathione and total antioxidant capacity were maintained (Sarhadynejad et al., 2016). These results confirmed our results.

In the present study, the results showed that fruit extract of *Berberis integerrima* decreases fasting blood glucose. Hemmati et al. (2016) investigated the effects of an ethanolic extract of *Berberis vulgaris* fruits on hyperglycemia and related gene expression in streptozotocin-induced diabetes in rats. They expressed that *Berberis integerrima* extract increased total antioxidant levels and expression of glucokinase (GK) and decreased glucose 6-phosphatase (G6P) expression. These results indicated that barberry can improve hyperglycemia in diabetic rats by inhibition of gluconeogenesis. (Hemmati et al., 2017). These results were in agreement with our results

A study was performed in diabetic rats to evaluate the effect of ethanolic extract of *Berberis integerrima* fruit on tissue changes and biochemical markers of liver injury. The results showed that glucose level and the AST, ALT and ALP enzymes decreased in metformin and extract groups (Rahimi-Madiseh et al., 2017). These results were similar to those of the present study. The suggested mechanism for reducing glucose by barberry may imitate insulin activity or prevent the death of beta cells, and may also destroy damaged beta cells or other mechanisms such as stimulating glucose uptake by peripheral tissue, inhibiting the production of endogenous glucose or inhibiting the activation of gluconeogenesis in the liver and muscles. The main mechanism of this plant in treating diabetes may be its activity as an alpha glycosidase inhibitor.

It has also been reported that decrease of liver triglycerides leads to progression of insulin sensitivity and protection against type 2 diabetes. Insulin resistance theory, which is the main mechanism for liver steatosis, states that insulin resistance leads to fatty accumulation in the liver cells through two mechanisms: 1- Increase in lipolysis, which increases the amount of free fatty acids. 2- Hyperinsulinemia that increases beta-oxidation in the mitochondria through increasing the absorption of fatty acids in the liver cells, resulting in fatty accumulation in the liver cells (Lewis and Mohanty, 2010). Therefore, presumably, *Berberis integerrima* reduces cholesterol and triglycerides, leading to insulin sensitivity and improved NAFLD-induced diabetes.

5. Conclusion

The result of the present study revealed that the hydroalcoholic extract of *Berberis integerrima* could have an antioxidant property and a protective effect on liver, hypolipidemic, and hypoglycemic through the reduction of liver damage marker and renal function factor and also increase of antioxidant parameters. Therefore, this plant may improve dyslipidemia, diabetes and oxidative stress which are related to non-alcoholic disease. Based on the present study more studies are needed to evaluate the related molecular pathways to antioxidant properties, hypolipidemic, and hypoglycemic of *Berberis integerrima* fruit extract. In addition, in vitro studies on liver cell lines may clarify the effects of barberry on NAFLD.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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