The Impact of Combined Cranberry Supplementation and Weight Loss Diet on Inflammatory, Antioxidant and Apoptosis Biomarkers in Patients with Non-Alcoholic Fatty Liver Disease

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Research

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Abstract

Background

Non-alcoholic fatty liver disease (NAFLD) is a prevalent chronic liver diseases. The aim of this study was to evaluate the effect of combined weight loss diet and cranberry supplementation on inflammatory, antioxidant and apoptosis biomarkers in patients with NAFLD.

Methods

In this randomized, double-blinded, controlled clinical trial, 41 NAFLD patients were supplemented with either a 288-mg cranberry tablet or a placebo tablet for 12 weeks. Both groups followed a diet of 500–1000 calories less than the estimated energy requirements. Serum levels of Total antioxidant capacity (TAC), Malondialdehyde (MDA), Cytokeratin 18-M30 (CK-18 M30), Chemokine C-C motif ligand 2 (CCL2) and Tumour necrosis factor alpha (TNF- α) were measured at both baseline and the end of the study.

Results

Significant improvements in TAC were observed in the cranberry group and between the two groups ($p = 0.006$ and $p = 0.011$ respectively), but the changes in the placebo group were not significant ($p = 0.325$). There were no statistically significant differences in the serum levels of MDA, CK-18 M30, CCL2 and TNF- α between the cranberry and the placebo groups ($p > 0.05$).

Conclusions

It seems that daily consumption of cranberry supplement would be beneficial in increasing serum levels of TAC. Further studies are needed to investigate the effects of anti-inflammatory and antioxidant properties of cranberry on NAFLD.

Trial registration

: Iranian Registry of Clinical Trial. <http://www.irct.ir>: IRCT20150124020765N2, January 2019.

Background

Non-alcoholic fatty liver disease (NAFLD) is caused by an increase in the cellular content of free fatty acids; FFAs), resulting in excessive accumulation of triglycerides (TG) in the cytoplasm of hepatocytes (> 0.5%) without the over-consumption of alcohol [1]. NAFLD that embrace a broad spectrum of physio-pathological conditions from simple steatosis to non-alcoholic steatohepatitis (NASH), is highly related to

obesity, diabetes, insulin resistance, hypertension, hyperlipidemia, and metabolic syndrome [2]. The prevalence of NAFLD is high worldwide and estimated to be about 24% [3]. The pathophysiology of NAFLD is complex and it has multiple manifestations/complications [4]. In terms of pathogenesis of NAFLD, it has been shown in both “two-hit” model and “multi-parallel hit” hypothesis that hepatic inflammation, oxidative stress, necrosis, apoptosis, and finally, fibrosis are caused by excessive fat accumulation in the hepatocytes [5]. A fundamental intervention for the management of NAFLD is Lifestyle modification such as having healthy eating patterns and regular exercise [6]. Many studies have been shown that in these patients the serum and liver contents of free radical oxidation products increase and the total antioxidant capacity decreases [5]. Therefore, following healthy eating patterns [7] along with antioxidant and anti-inflammatory supplements [8] could be helpful in better improvement to NAFLD, given that oxidative stress and inflammation play a role in its pathogenesis [9]. Available evidence show that food ingredients such as phytochemicals, vitamins and minerals exhibit anti-inflammatory, antioxidant and immune-regulating activity in the body [10–15]. One of the classes of phytochemicals are phenolics, which include flavonoids and polyphenol [16]. Researchers have been shown that these phytochemicals have ability to scavenge free radicals, reducing inflammation, modifying the lipid profile, lowering blood pressure, and reducing platelet aggregation [17–20]. Cranberries (*Vaccinium Macrocarpon*) are rich in polyphenols such as flavonols, catechins, anthocyanins, resveratrol, organic acids, B-type proanthocyanidins (PACs) and a high amount of rare A-type PACs [21] and according to the United States Department of Agriculture (USDA), they have the highest free radical scavenging ability [5]. Previous studies showed some effects of cranberry bio-actives include reducing inflammation in humans and in vitro, reducing blood markers of oxidative stress in humans [22] and abating of hepatic inflammation and steatosis in mice fed a high-fat diet [23]. Impact of consumption low-calorie cranberry juice on decreasing cardiometabolic risk in overweight middle-aged population was investigated by a clinical trial. There was a significant improvement in C-reactive protein (CRP) for intervention group after 8 weeks of evaluation [24].

This placebo-controlled, double-blind, randomized clinical trial study was designed to explore the possible role of cranberry supplementation on inflammatory and oxidative markers in patients with NAFLD because of the anti-inflammatory and antioxidant properties of cranberry and role of inflammation and oxidative stress in pathogenesis of NAFLD.

Materials And Method

Samples and study design

In this randomized double-blind and placebo-controlled clinical trial, the patients with NAFLD were recruited from Ahvaz Golestan hospital. Totally, 50 eligible patients were recruited. A signed consent form was collected from all subjects. The recruited subjects were randomly allocated into control (n = 25) and intervention (n = 25) groups based on the block design. Age 18 years or older, BMI of 25–5 kg/m², patients with confirmed NAFLD (the grade of steatosis higher or equal to 2 at ultrasonography) were the inclusion criteria and history of significant alcohol intake (more than 10 mL/day for women and 20 mL/d

for men); smoking habits; subjects affected by other liver diseases, cardiovascular, respiratory, kidney disorders, malignancies, and diabetes mellitus,, pregnancy or breastfeeding, medication in the previous 6 months, supplementation with antioxidants or vitamins, weight loss over the past 3 months, metabolism and endocrine disorders pregnancy or breastfeeding, medication in the previous 6 months, supplementation with antioxidants or vitamins, weight loss over the past 3 months, metabolism and endocrine disorders were the exclusion criteria. Subjects in the intervention and control groups received either cranberry or placebo tablets for 12 weeks. The color, size and weight of cranberry and placebo tablets were similar. All subject received two tablets; one tablet after lunch and another one after dinner. Cranberry tablets were purchased from Shari Nutraceutical Co., Tehran, Iran. Each tablet contained 144 mg of Vaccinium macrocarpon extract with at least 36 mg proanthocyanidine (equal to 13 g dried cranberry fruit), while composition of the remaining 144 mg was unknown. Placebo tablets contained 288 mg of starch. Both groups followed a diet of 500–1000 calories less than the estimated energy requirement. Dietary intakes were obtained from three 24 h dietary recalls (1 weekend day and 2 weekdays). Physical activity levels were assessed by the metabolic equivalent of task (MET) questionnaire.

This clinical trial study was registered in the “Iranian Registry of Clinical Trials” with the IRCT number of IRCT20150124020765N2.

Biochemical And Anthropometric Measurements

At baseline, 6 weeks and after 12 weeks, all subjects underwent anthropometric measurements: Height was measured to the nearest 0.1 cm using a non-stretched tape measure. Weight, body fat (BF), and body mass index (BMI) were measured using a bioelectrical impedance analysis (OMRON device BF-511). Waist circumference (WC) (the widest area between the lower rib and the superior iliac crest) was also measured to the nearest 0.1 cm. A fasting blood sample (12 ml) was collected from subjects at baseline and the end of intervention. Blood samples were centrifuged at 3500 rpm for 10 min and then the supernates were stored at -70°C until analysis. The serum samples were used to analysis TAC, MDA, TNF- α , CK-18 M30 and CCL2. Serum levels of CCL2, CK-18 M30, TNF- α and TAC were measured using ELISA method by laboratory kits (Biotech Day Crystal for CCL2, CK-18 M30, TNF- α ; and LDN, PLabor Diagnostika Nord GmbH, Germany for TAC). Serum MDA levels were assessed utilizing the thiobarbituric acid reactive substances (TBARS).

Statistical analysis

Considering 95% confidence with an estimated standard deviation and difference of the fasting blood glucose (16) the sample size was calculated and 25 subjects in each group were determined. The statistical analyses were performed using SPSS (version 19). The results presented as mean $\pm$ SD. P-value lower than 0.05 was considered as significant. The Kolmogorov-Smirnov test was used to assess the normal distribution of variables. Independent samples comparisons in terms of quantitative variables

were performed using two independent samples t tests and repeated measures ANOVA, as appropriate. In order to analyze nutrient intakes, "Nutritionist IV" software was applied.

Results

In this study, 9 out of 50 patients were excluded due to non-adherence to diet and medication. Therefore, 21 subjects in the group receiving cranberry supplements and 20 in the placebo group remained in the study. Patient compliance in this randomized clinical trial study was 82%. The characteristics of subjects are shown in Table 1. No statistically significant difference were seen between two groups in terms of their demographic characteristics or their baseline biomedical, and anthropometric measurements ($p > 0.05$). Table 2 shows the dietary intake and anthropometric indices of the two groups. Statistical analysis showed that after 12 weeks, the weight, BF, BMI, and WC significantly decreased in both groups ($p < 0.05$). There was no significant difference in weight, BMI, BF, and WC between the two groups ($p > 0.05$). No significant differences were also observed between two groups for dietary data including the intakes of energy, and macronutrients at the end of the study.

Table 1
Baseline characteristics and dietary intakes of study participants.

Characteristics	Placebo group (n = 21)	Cranberry group (n = 20)	<i>p</i> ^a
Age (years)	40.00 ± 9.91	43.55 ± 11.51	0.296
Gender	7	11	0.215
Female (N)	14	9	
Male (N)			
Weight (kg)	89.66 ± 13.39	89.94 ± 11.58	0.944
Height (m)	1.68 ± 0.08	1.67 ± 0.12	0.758
BMI	31.67 ± 4.72	32.46 ± 6.09	0.645
WC (cm)	105.64 ± 9.19	106.52 ± 11.15	0.783
BF (%)	40.12 ± 11.01	39.46 ± 10.72	0.847
MET (h/d)	35.77 ± 4.81	33.68 ± 2.44	0.090
TAC(mmol/L)	1.42 ± 0.50	1.21 ± 0.47	0.197
MDA (nmol/mL)	13.14 ± 7.11	12.28 ± 6.33	0.686
CK-18 M30 (ngl/mL)	16.01 ± 7.88	18.76 ± 7.06	0.248
(MCP1) CCL2 (ng/L)	851.05 ± 875.96	916.94 ± 374.78	0.669
TNF-α (ng/L)	433.98 ± 300.22	547.13 ± 242.38	0.193
Energy (kcal/day)	2714.98 ± 799.72	2743.85 ± 706.31	0.903
Carbohydrate (g/day)	340.15 ± 90.84	364.51 ± 121.15	0.469
Protein (g/day)	96.94 ± 33.76	83.66 ± 35.69	0.228
Fat (g/day)	104.10 ± 55.04	95.9 ± 19.40	0.535
BMI: body mass index; BF: body fat, WC: waist circumference MET: metabolic equivalent of task, TAC: total antioxidant capacity, MDA: malondialdehyde, CK-18 M30: cytokeratin 18-M30, CCL2: chemokine (C-C motif) ligand 2 (CCL2), TNF-α: tumour necrosis factor alpha			
Values are means ± SD, unless otherwise indicated.			
p < .05 was considered significant.			
^a P values indicate comparison between the variables between 2 groups			

Table 2
Anthropometric measurements and dietary intakes at the baseline, 6 weeks and after 12 weeks

Characteristics	Placebo group (n = 21)	Cranberry group (n = 20)	^b pvalue group	^b pvalue Time & group
Weight (kg)	39.13 ± 66.89	58.11 ± 94.89	71.0	0.05
Baseline	98.11 ± 20.86	60.11 ± 19.88		
6 weeks	65.11 ± 50.84	.6410 ± 85.85		
12 weeks	0 > .001	0 > .001		
p ^a				
BMI (kg/m²)	72.4 ± 67.31	09.6 ± 46.32	75.0	16.0
Baseline	12.4 ± 44.30	20.6 ± 85.31		
6 weeks	32.4 ± 87.29	02.6 ± 04.31		
12 weeks	0 > .001	0 > .001		
p ^a				
WC (cm)	19.9 ± 64.105	15.11 ± 52.106	0.59	32.0
Baseline	39.8 ± 19.102	95.12 ± 55.105		
6 weeks	24.8 ± 04.101	33.11 ± 60.103		
12 weeks	0 > .001	0 > .001		
p ^a				

BMI: body mass index; **BF:** body fat; **WC:** waist circumference

Values are expressed as means ± SD.

p < 0.05 was considered significant.

^a P values indicate comparison within groups.

^b P values indicate comparison between the changes of each variable between 2 groups.

Characteristics	Placebo group (n = 21)	Cranberry group (n = 20)	^b pvalue group	^b pvalue Time & group
BF (%)	01.11 ± 12.40	72.10 ± 46.39	99.0	0.22
Baseline	96.9 ± 13.37	38.10 ± 76.37		
6 weeks	39.9 ± 19.34	27.10 ± 54.34		
12 weeks	0 > .001	0 > .001		
p ^a				
Energy (kcal/day)	799 ± 9.2714	706 ± 8.2743	0.69	0.80
Baseline	344 ± 5.1956	569 ± 3.2063		
6 weeks	395 ± 5.1749	329 ± 1794		
12 weeks	0 > .001	0 > .001		
p ^a				
Carbohydrate (%)	60.7 ± 18.51	64.6 ± 54.52	0.40	0.69
Baseline	40.6 ± 84.52	37.6 ± 25.52		
6 weeks	48.6 ± 94.52	25.7 ± 23.56		
12 weeks	0 > .001	0 > .001		
p ^a				
Protein (%)	69.4 ± 54.15	02.4 ± 86.14	0.38	0.58
Baseline	86.4 ± 68.16	77.4 ± 97.14		
6 weeks	28.5 ± 08.16	70.3 ± 31.14		
12 weeks	005.0	007.0		
p ^a				

BMI: body mass index; **BF:** body fat; **WC:** waist circumference

Values are expressed as means ± SD.

p < 0.05 was considered significant.

^a P values indicate comparison within groups.

^b P values indicate comparison between the changes of each variable between 2 groups.

Characteristics	Placebo group (n = 21)	Cranberry group (n = 20)	^b pvalue group	^b pvalue Time & group
Fat (%)	49.10 ± 28.33	28.7 ± 60.32	0.32	0.93
Baseline	82.11 ± 48.30	6.44 ± 78.32		
6 weeks	8.53 ± 98.30	52.6 ± 29.46		
12 weeks	0 > .001	0 > .001		
p ^a				
BMI: body mass index; BF: body fat; WC: waist circumference				
Values are expressed as means ± SD.				
p < 0.05 was considered significant.				
^a P values indicate comparison within groups.				
^b P values indicate comparison between the changes of each variable between 2 groups.				

Table 3 shows the inflammatory, antioxidant and apoptosis biomarkers at baseline and after 12 weeks for the two groups. Significant improvements in TAC were observed in the cranberry group and between the two groups ($p = 0.006$ and $p = 0.011$ respectively), but the changes in the placebo group were not significant ($p = 0.325$). The mean MDA was reduced (but not significantly) in the cranberry group after intervention, moreover no significant differences were seen in the serum levels of MDA between the cranberry and the placebo groups ($p > 0.05$). Also both within cranberry and placebo groups, there were no significant changes in the mean levels of CK-18 M30 post intervention compared with baseline ($p > 0.05$). There were no statistically significant differences in the serum levels of CCL2 and TNF- α between the cranberry and the placebo groups ($p > 0.05$).

Table 3
Biochemical parameters at the baseline and after 12 weeks.

Parameter	Baseline	12 weeks	p ^a	Change	p ^b
TAC(mmol/L)	1.42 ± 0.50	1.20 ± 0.67	0.325	-0.21 ± 0.99	0.011
Placebo	1.21 ± 0.47	1.73 ± 0.72	0.006	0.52 ± 0.75	
Cranberry					
MDA(nmol/mL)	13.14 ± 7.11	15.52 ± 14.59	0.512	2.38 ± 16.34	0.252
Placebo	12.28 ± 6.33	9.95 ± 3.67	0.208	-2.33 ± 8.00	
Cranberry					
CK-18 M30 (ngl/mL)	16.01 ± 7.88	16.27 ± 6.84	0.914	0.26 ± 10.94	0.316
Placebo	18.76 ± 7.06	18.12 ± 7.18	0.736	-0.63 ± 8.29	
Cranberry					
(MCP1) CCL2 (ng/L)	851.05 ± 875.96	960.16 ± 591.13	0.585	109.10 ± 900.31	0.535
Placebo	916.94 ± 374.78	890.02 ± 410.38	0.752	-26.91 ± 375.66	
Cranberry					
TNF-α (ng/L)	433.98 ± 300.22	512.57 ± 305.90	0.443	78.59 ± 460.21	0.316
Placebo	547.13 ± 242.38	502.12 ± 252.72	0.505	-45.01 ± 296.46	
Cranberry					
TAC: Total antioxidant capacity, MDA: Malondialdehyde, CK-18 M30: Cytokeratin 18-M30, CCL2: Chemokine (C-C motif) ligand 2 (CCL2), TNF-α: Tumour necrosis factor alpha					
Values are expressed as means ± SD.					
p < 0.05 was considered significant.					
^a P values indicate comparison within groups.					
^b P values indicate comparison between the changes of each variable between two groups.					

Discussion

The present study was conducted to evaluate effects of cranberry supplementation and weight loss diet on markers of inflammation (TNF-α and CCL-2), oxidative stress (MDA and TAC) and hepatic cell apoptosis (CK-18 M30) in NAFLD patients. Supplementing a weight loss diet with cranberry for a period of 12 weeks could significantly increase TAC. However, changes in other variables remained insignificant. Different studies have been conducted on the effects of cranberry with or without dietary interventions on

these markers with different intervention types (extract, juice, tablet, etc), variable doses and different findings.

Cytokine imbalances occur in the “second hit” of NAFLD. Therefore the issue has gained a considerable attention as target for therapeutic interventions [25]. Glisan et al evaluated effects of polyphenol enriched cranberry extract (CBE) on markers of hepatic inflammation in HFD-fed obese rats and found that CBE can decrease hepatic protein levels of TNF- α and CCL-2, as well as hepatic mRNA levels of toll like receptor-4 (TLR-4) and nuclear factor κ B (NF κ B) [22]. Another study was performed to evaluate possible anti-fibrotic effects of cranberry nutraceuticals in high fat cholesterol diet induced (HFCD)-NAFLD rats. The results showed that cranberry could alleviate markers of oxidative stress (MDA, glutathione, catalase and superoxide dismutase), inflammation (TNF- α , IL-6 and NF κ B) and improved markers of insulin resistance [26]. Apoptosis is a key mechanism in the progression of steatosis to NASH and apoptosis markers are related with histologic severity of NAFLD [27]. CK-18 M30 is a well-known substrate of caspase activity during apoptotic hepatocyte death [28] and has been shown to have a high accuracy in differentiating NAFLD from control subjects [29]. Accordingly, in the current study, CK-18 M30 was assessed as a marker of apoptosis which did not significantly change during intervention. Studies regarding anti-apoptotic effects of cranberries are few. However, some studies have been conducted on other polyphenol rich compounds in this area. In a randomized clinical trial, 44 participants were given either 250 ml bayberry juice or placebo, twice a day for 4 wks. Bayberry consumption could significantly improve markers of inflammation and apoptosis including polypeptide specific antigen and CK-18 M30 [30]. In another study, 14 days of dark chocolate consumption, as a source of polyphenols, led to a significant reduction in CK-18 M30 in NAFLD patients [31]. According to findings, multistage processing of fruit extraction leads to a considerable loss in phytochemical content through thermal degradation and polyphenol oxidation which could have been considered as a reason for the null findings. Therefore, future research should focus on comparisons between different forms of cranberry supplements.

On the other hand, a significant improvement in TAC was observed in intervention group which indicates possible anti-oxidative effects of cranberry supplements in NAFLD patients. Oxidative stress is a result of an imbalance between pro-oxidants and anti-oxidants and plays a crucial role in the pathogenesis of NAFLD [32]. A considerable amount of research has been conducted regarding anti-oxidative effects of berries. In one study mulberry treatment in HFD rats significantly suppressed hepatic reactive oxygen species (ROS) overproduction and mitochondrial oxidative stress [33]. Another research team investigated effects of raspberry on obese diabetic (db/db) mice for 8 weeks. The findings showed that raspberry intake could improve antioxidant status and lessen IL-6 in treatment group [34]. Results of a double blind randomized trial showed that 4 weeks supplementation with maqui berry (delphinol) significantly reduced markers of oxidative stress (ox-LDL and urinary F2-isoprostane) in intervention group [35]. Wild blueberry consumption significantly improved postprandial oxidative stress in male subjects. Oxygen radical absorbance capacity (ORAC) assay and the total antioxidant status (TAS) were evaluated as markers of oxidative stress in the study [36]. On the other hand, in a randomized controlled trial, 40 post-menopausal women consumed either 22 grams of blueberry or placebo for 8 weeks. Blood

markers of oxidative stress, inflammation and antioxidant defense did not change in blueberry group after 8 weeks [37].

Health benefits of fruits and vegetables have been demonstrated in nutrition not only for their vitamins and minerals, but also their phytochemical components [38]. The American cranberry (species *Vaccinium macrocarpon*) has been particularly considered a health fruit for centuries [39]. Cranberries as a uniquely rich source of phytochemicals, contain over 150 phytochemicals with flavonoids as the most predominant component. Some cranberry flavonoids include anthocyanins, proanthocyanidins, catechins, organic acids, resveratrol and flavonols which are responsible for the fruit's color and sour astringent flavor [40]. Several in vivo animal models have confirmed anticarcinogenic, antitumorogenic, antiangiogenic, anti-inflammatory and antioxidant properties of cranberry polyphenols [41]. NAFLD has been linked to gut dysbiosis and metabolic endotoxemia which are the initial triggers of inflammatory cascade [42]. NF- κ B is a key regulator in this cascade and has the potential to control the production of pro-inflammatory cytokines including TNF- α and IL-6 [43, 44]. Cranberries as a great source of polyphenols might exert as prebiotics which can have immunomodulatory and anti-inflammatory effects by interacting with gut microbiota [45]. In one study, dietary cranberry supplementation in a mouse model of IBD, not only suppressed colonic levels of pro-inflammatory cytokines (IL-1 β , IL-6 and TNF- α) but also increased the abundance of beneficial gut bacteria including *Lactobacillus* and *Bifidobacterium* [46]. It has also been shown that phenolic compounds can suppress IL-1 β secretion and exert anti-inflammatory effects through inhibition of cyclo-oxygenase and lipoxygenase activity [47]. Antioxidant properties of cranberry are attributed to free radical scavenging properties of polyphenols against ROSs as well as inhibition of lipid and protein oxidation [48]. According to studies, cranberry supplementation has also the potential to decrease NO synthase activity, improve homocysteine levels, and endothelial function, thus suppressing oxidative stress [49].

Conclusion

In conclusion, this randomized, double-blind, placebo controlled trial indicate that 288 mg/d of cranberry supplementation in addition to weight loss diet may not change MDA, CK-18 M30, CCL2 and TNF- α , but it would be beneficial in improvement serum levels of TAC in NAFLD patients. Further studies are needed to investigate the effects of anti-inflammatory and antioxidant properties of cranberry on NAFLD.

Abbreviations

NAFLD

non-alcoholic fatty liver disease, NASH:non-alcoholic steatohepatitis, BMI:body mass index, BF:body fat, WC:waist circumference, MET:metabolic equivalent of task, TAC:total antioxidant capacity, MDA:malondialdehyde, CK-18 M30:cytokeratin 18-M30, CCL2:chemokine (C-C motif) ligand 2 (CCL2), TNF- α :tumour necrosis factor alpha, CRP:C-reactive protein, PACs:proanthocyanidins, USDA:United States Department of Agriculture, TG:triglycerides, TBARS:thiobarbituric acid reactive substances, CBE:enriched cranberry extract, TLR-4:toll like receptor-4, NF κ B:nuclear factor κ B, HFCD:high fat cholesterol diet

induced, ROS:reactive oxygen species, ORAC:Oxygen radical absorbance capacity, TAS:total antioxidant status (TAS)

Declarations

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Authors' contributions

MMS, FR and RH were involved in the conception and design of the study. ASH, BH and PA contributed in methodology. ASH performed the data analysis and interpretation. RH participated in the conduction of the study and manuscript drafting. MMS supervised the development of the work. All authors read and approved the final manuscript.

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Availability of data and materials

The data gathered and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the ethics committee of the Research Deputy of Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1397.678).

Consent for publication

No personal data is noted herein.

Competing interests

The authors have declared that there is no conflict of interest.

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