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Effect of Zizyphus Jujuba Fruits on Dyslipidemia in Obese Adolescents: a Triple-masked Randomized Controlled Clinical Trial

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Objective: This study aimed to assess the effects of Ziziphus jujuba (ZJ) fruit on controlling dyslipidemia in obese adolescents. **Methods:** This triple-blind randomized placebo-controlled clinical trial comprised 86 obese adolescents aged 12-18 years with dyslipidemia, i.e. serum low density lipoprotein- cholesterol (LDL-C) or total cholesterol (TC) or triglycerides (TG) equal or more than the age- and gender-specific 95th percentile or high density lipoprotein- cholesterol (HDL-C) less than 5th percentile. They were randomly assigned into two groups of equal number. Both groups received similar recommendations for dietary and physical activity habits. The case group received 5 grams of ZJ fruit powder three times a day for one month and controls took the same amount of a placebo powder. Fasting blood sugar, TC, LDL-C, HDL-C and TG were measured at the beginning and at the end of the trial. Data were analyzed using General linear method (multivariate) test. **Findings:** Overall, 70 participants (51% boys, mean age of 14±2) completed the trial. The two groups studied did not differ in terms of age, gender, weight and body mass index (BMI). After the trial, serum TC decreased significantly (195±37 mg/dl in controls vs. 170±29 mg/dl in cases, P=0.007) reduction. The corresponding figure was also significant for LDL-C (114±38 mg/dl vs. 104±22 mg/dl, respectively, P= 0.004). The changes in BMI and other lipids were not significant. **Conclusion:** This study suggest that ZJ`s fruits is generally well tolerated and may have potential favorable effects on serum lipid profile. While healthy lifestyle is the mainstay of controlling childhood obesity, this nutraceutical may be considered as a complementary treatment. Key words: Zizyphus, dyslipidemia, adolescence, obesity.

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1. INTRODUCTION

Chronic non-communicable diseases are the main cause of mortality worldwide (1, 2, 3). Both behavioral and biological factors related to chronic diseases persist from childhood to adulthood. Risk factors such as dyslipidemia, obesity and high blood pressure track from childhood to adulthood (4, 5). Atherosclerosis origins in childhood, and remains asymptomatic for many years. Therefore, measuring some related biomarkers which are expressed in the early stages of atherosclerosis can play an important role in primordial and primary prevention of chronic disease (2, 3).

Lifestyle modification is the mainstay of prevention and control of risk factors of chronic diseases, and using chemical medication is not without side effects, notably in the pediatric age group. Herbal plants contain considerable amount of complex materials that can have potential pharmacological therapeutic effects, and can be considered as a complimentary treatment of such risk factors as obesity and dyslipidemia. Traditional medicine is still widely practiced, and is well accepted by populations of various countries (6).

Common jujube, Zizyphus jujuba mill. (ZJ) is a plant native to Asia and southern Europe, which belongs to the Rhanmaceae family and has sweet date-like red eatable fruits. The fruits are cooling, digestible, tonic, aphrodisiac and laxative. They also remove biliousness, burning sensations, thirst and vomiting (4, 5). The ZJ fruits contain polysaccharides, amino acids, flavonoids, polyphenols, mucilage and pectin, a water-soluble fiber. Major characteristic constituents are Triterpene-like Alphitolic, Betulinic, Maslinic, oleanolic and saponins (4,6). There are several experimental and human studies indicating the efficacy and safety of ZJ fruits on various health disorders (5).

Controlling childhood obesity and its complications as dyslipidemia solely by lifestyle modification is usually difficult in the pediatric age group, thus using a safe complimentary medication

may be of help. In this study, the efficacy and safety of ZJ fruit was assessed for its possible effects on dyslipidemia in adolescents.

2. MATERIALS AND METHODS

This triple-masked placebo-controlled clinical trial which was conducted from July 2011 to June 2012 in Isfahan Cardiovascular Research Institute, affiliated to the Isfahan University of Medical Sciences, Isfahan, Iran.

After providing detailed oral information about the study, oral assent was obtained from participants and written consent from their parents. The study protocol was in accordance with the Declaration of Helsinki and was approved ethically by the board of human studies at the Isfahan University of Medical Sciences (Registration code: 388591). The study was also registered in the Iranian clinical trial registry, with the registration number of IRCT: 201109092306n1).

The eligibility criteria consisted of being aged 12-18 years, having a body mass index (BMI) equal or more than the age - and gender-specific 95th percentile, which is confirmed to be appropriate for Iranian children and adolescents, and having dyslipidemia. The latter was defined as serum total cholesterol (TC) or low density lipoprotein - cholesterol (LDL-C) or triglycerides (TG) equal or more than the age - and gender-specific 95th percentile or high density lipoprotein- cholesterol (HDL-C) lower than 5th percentile (7). Those individuals with syndromal appearance, any chronic disease or receiving any medication were not included to the study (Figure 1) (8, 9).

Participants were selected by convenient sampling from among individuals referred to the above mentioned department. All of them received recommendations for healthy eating habits and regular physical activity. Then they were randomly assigned to two groups by using simple randomization sampling method. We recommended the case group to use one sealed sachet of ZJ powder (5 grams) three times a day for one month and the placebo group were asked to take equal amounts of a placebo powder in identical package for the same time period.

The sample size was calculated as 35 in each groups (cases and controls) to have a 90% chance of detecting a difference in mean cholesterol change of 10 mg/dl at the 5% level of significance, assuming the standard deviation of total cholesterol as 15.47mg/dl from a previous study (10).

Preparing dosage form

This process was done in the research laboratories of the School of Pharmacy and Pharmaceutical sciences at the Isfahan University of Medical Sciences. Plant material was collected from Kouhpayeh district located in Isfahan province (Iran). It was taxonomically identified by an academic herbal botanist.

Dry fruits of ZJ plant were frozen at -18C and then powdered with an ordinary mechanical grinder. Then, the seeds of ZJ fruits were withdrawn, and finally the powder was packed in 5-gram sachets.

Measurement of pectin and total phenol in the ZJ fruits

Ethanol 96° and distilled water were used for the extraction (11). We used the Water house and coworkers method (12) for determining the total phenol content of the pulp of ZJ. Figure-2 shows the steps for determining the amount of pectin in 10g ZJ powder.

The placebo was matched to the study drug for size, color, and taste and contained microcrystalline cellulose. Parents were asked to contact our clinic two weeks after the beginning of the trial and to report the compliance of their children for using the medication, as well as any side effect of the medication used. We followed the children's compliance by regular phone calls. The findings of those participants who did not take their medication regularly were excluded from the statistical analysis.

Physical examination and laboratory tests

Weight and height were measured by calibrated instruments and under standard protocols. The body mass index (BMI) was calculated by dividing weight by the height squared (kg/m²). Participants were instructed to fast for 12 hours before blood sampling and their compliance for fasting was con-firmed by interviewing them before sampling. Five milliliter of blood samples were collected from ante-cubital vein. Fasting blood sugar (FBS), TC, TG, LDL-C, and HDL-C were measured by standard enzymatic methods by standard kits (Pars Azmoun, Iran) using a fully automatic auto-analyzer (Hitachi 902, Japan All measurements of the baseline survey were repeated with the same method at the end of the trial.

Statistical analysis

Statistical calculations were performed by the Predictive Analytic Software (PASW) Statistical Package ver.18.0.1 (Chicago, USA), by using (K-S) test, Chi-Square test, independent sample T-test, and General linear model (GLM). All data passed the Kolmogrov-Smirnov test to assess their normal distribution. Differences of the two groups for the quantitative data of lipid profile were analyzed by Independent Sample T-Test and nominal demographic data were analyzed using Chi-Square test. As shown in Table 1, at the baseline survey, mean FBS have significant differences between two groups, therefore it was considered as a covariant factors in the final analysis performed by GLM test. P-value of less than 0.05 was considered as statistically significant.

3. RESULTS

Overall, 70 of 86 individuals (81 (93%) completed the trial according to the study protocol. The main reasons for the attrition were taking sachets irregularly, low desire to consume sachets, using chemical drugs during the study period, and irregular follow up.

Demographic parameters

The baseline characteristics of the subjects study participants in ZJ and placebo group are shown in Table1. The mean age of students in ZJ group was13.7 ± 2 years and 14.3 ± 2 years in placebo group. Before starting the trial, there was no significant difference between the two groups in terms of age, gender ratio, weight and BMI; however, as illustrated in Table 1, the FBS factor has noticeably different values among them (p<0.05). Therefore FBS was considered as a covariant factor in the final analysis.

Table 2 illustrates the laboratory findings at the end of the trial in both

groups. P-values were calculated by General Linear Model (Multivariate) test. GLM multivariate test showed that A after 1 month administration of powdered ZJ fruits sachets, LDL-C, TC and FBS levels had significant differences but other measured parameters variables showed no change compared to the placebo group.

4. DISCUSSION

This trial, which to the best of our knowledge is the first of its kind in the pediatric age group, revealed that daily consumption of 15g ZJ fruits for one month can decrease the serum levels of TC and LDL-C in dyslipidemic adolescents.

Regarding that the pulp of ZJ has a considerable amount of sugar, i.e. fructose, galactose and glucose, the impact of this fruit on FBS was also evaluated and revealed a small but significant change of it in the limited time period of the study.

The formation of foam-cells in human macrophages has an important role in the initial origination of atherosclerosis so that prevention of these cells formation is considered to have a key role in its prevention or treatment (13, 14). Fujiwara et al. found that the pulps and seeds of ZJ have a noticeable inhibitory effect on the formation of foam-cells in comparison with fifty other plants. This effect was attributed to tri-terpenoids such as Oleanonic acid, Pomolic acid, Pomonic acid and tri-terpenoids containing a carboxylic acid at C-28 (15). Pectin, a kind of soluble fiber (SF), is a crucial structural compound found in the cell wall of all fruits and vegetables, which has a considerable positive impact on the blood cholesterol level (16, 17, 18). In the current trial, individuals in the drug group consumed 15g of ZJ powder containing 300 mg pectin every day. There are three theories justifying the influence of pectin on cholesterol level: 1. increasing fecal bile acid excretion which is the consequence of preventing bile acid re-absorption from small intestine; 2. Slowing down of the digestion of glucose and other macro-nutrients absorption by pectin, which results in a decreased insulin level since there is a correlation between the falling of choles-

terol synthesis and blood insulin level; and 3. the production of short chain fatty acid (SCFA) from SF in the caecum and colon which is mediated by the anaerobic bacteria fermentation process and can deplete plasma cholesterol by inhibiting hepatic cholesterol metabolism (19). The intake of polyphenol-rich foods has cardio-protective effects because of their antioxidant, vasodilator, anti-inflammatory, anti-fibrotic and antiapoptotic characteristics (20, 21). According to the Folin-ciucalteo method (12), subjects took 165mg of polyphenols in each day of the study. Antioxidants modify the function of veins by decreasing the thickness of intima layer and media carotid layer, which may decline the risk of cardiovascular diseases (22, 23). Procyanidin B2, Epicatechin and Quercetin are some of the flavonoids existing in the fruits of ZJ (6).

Study limitations and strengths:

The main limitation of this trial is that we did not measure any biochemical factor for assessment of the compliance of participants. Moreover, the duration of the trial was 4 weeks, and by longer follow up, we might reach to more favorable changes in other serum lipids. The

strengths of this trial are its novelty in the pediatric age group and its triple-masked design.

5. CONCLUSION

It is concluded that not only the daily use of 15g of ZJ fruits for 1 month is almost well tolerated in teenagers, but also has positive effects on reducing serum TC and LDL-C, which are important factors for the prevention or treatment of developing atherosclerosis from childhood. Further studies with other daily doses of ZJ and longer time period of usage with larger sample size is recommended.
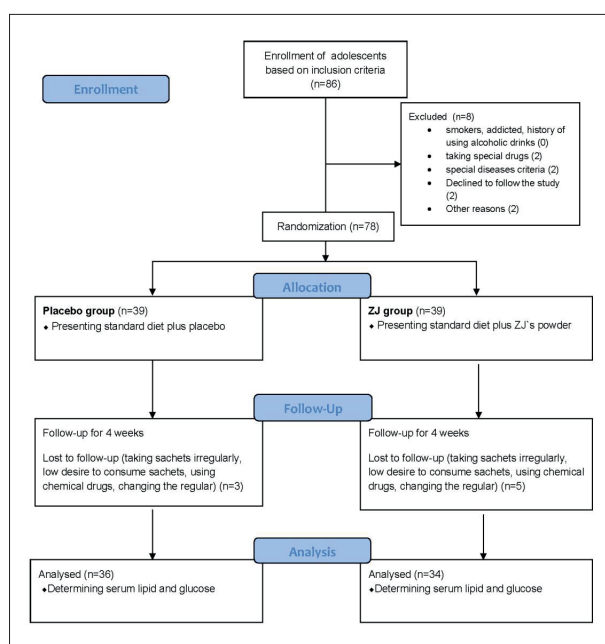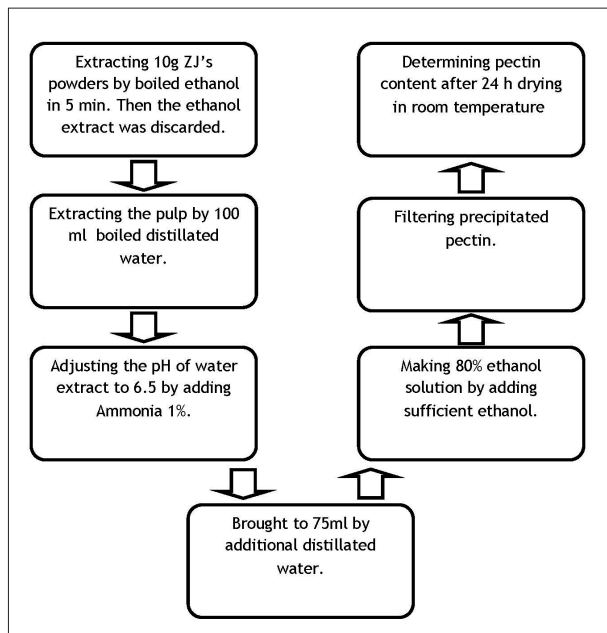
	No(%) of participant-stients		Mean (SD)		Cholesterol (mg/dl)			Triglycerides (mg/dl)	Fasting Blood Sugar (mg/dl)
	Male Boys	Female Girls	Age(yr)	BMI (kg/m2)	Total	HDL	LDL		
Placebo Group	17 (47)	19 (51)	14.3 (2)	23.6 (4.5)	198.1 (37.5)	41 (9.8)	136 (52)	162.6 (54.6)	91.8 (11.2)
ZJ Group	19 (55)	15 (45)	13.7 (2)	24.4 (3.7)	190.7 (36)	44 (9.7)	114 (38.5)	168 (70)	83.1 (7.7)
P-value	0.759		0.276	0.452	0.413	0.211	0.052	0.723	<0.001

Table1. Demographic andlaboratory parameters Baseline characteristics of participants the subjectsat the beginning of studyin ZJand placebo group.

	Mean (SD)					
	BMI (kg/m²)	Cholesterol (mg/dl)			Triglycerides (mg/dl)	FBS (mg/dl)
		Total	HDL	LDL		
Placebo	24.1 (4.5)	195.4 (37)	42.7 (9.7)	114.9 (38.6)	157.5 (47.9)	89.9 (10)
ZJ`s fruits	23.7 (3.5)	170 (29.4)	39.6 (11.8)	104.7 (22.4)	132.2 (49.7)	84.3 (7.6)
P-value	0.386	0.007	0.3	0.004	0.071	0.008
*: Data are presented as mean (SD)						

Table 2. Body mass index MI and Llaboratory findings *at the end of the trial in both groups studied.

Firgure 1. Consort diagram of the study

Figure 2. Practical steps for determing the amount of pectin in ZJ powder

Figure 3. Laboratory parameters findings of ZJ participants usingwho consumed 5 grams Zizyphusof Ziziphus jujuba fruit per day before and after the study

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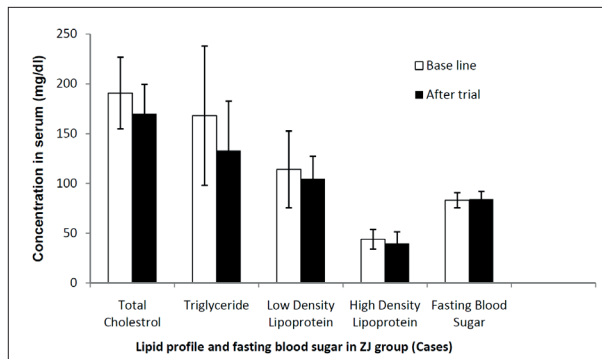7. Kliegman RM, Stanton B, Geme JS, Schor N, Behrman RE. Nelson Textbook of Pediatrics: Expert Consult Premium Edition - Enhanced Online Features and Print: Elsevier - Health Sciences Division, 2004.
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