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ORIGINAL ARTICLE

Effects of Almond Hydro-Alcoholic Extract on Body Fat and Serum Lipids in Male Sprague Dawley Rats Receiving High Fat Diet: an Experimental Study

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ABSTRACT
Obesity and hyperlipidemia are important risk factors for a variety of diseases, including diabetes, cardiovascular disease and some cancers. Many strategies are used for treatment of obesity including diet therapy, exercise, pharmacotherapy, and herbal treatments. Nuts especially sweet almonds are controversial herb products for their effects on weight and body fat, therefore we studied the effect of sweet almond extract on body weight, serum cholesterol, lipoproteins, triglycerides, and body fat levels in male Sprague Dawley rats. 40 mature male Sprague Dawley rats were divided into 5 groups of 8 rats including: Positive control group(PC) that were fed with normal diet without any intervention, negative control group(NC) receiving just a high-fat diet and three other groups receiving high fat diet and hydro-alcoholic almond extract. These three groups received 10, 25 and 50 mg/Kg body weight hydro-alcoholic extract of sweet almond(LE, ME, HE groups respectively). After 8 weeks of intervention, serum cholesterol, triglycerides and high density lipoprotein cholesterol (HDL-C) levels of rats were measured. Rats were killed after anesthesia and corpus fat percentages were determined chemically. Data were compared using one way ANOVA and Scheffe post-hoc test. The study findings showed a greater weight gain in groups receiving high fat diet compared with group received a normal diet. The levels of body fat percent showed a non-significant reduction in groups receiving almond hydro-alcoholic extract compared with NC group. The concentration of serum triglycerides in HE group reduced significantly compared with NC group (99±69 mg vs. 222±56 mg; $p<0.05$), whereas no significant differences were observed in the serum high-density lipoprotein and total cholesterol of groups. The study findings show that sweet almond extract can reduce body fat, serum triglycerides and may also be effective in preventing obesity.
Keywords: *Obesity, Sweet almond, Cholesterol, Triglycerides, High density lipoprotein (HDL), Rat*

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INTRODUCTION

Obesity has become one of the most important health problems throughout the world [1] that affects all population groups regardless of sex, age, race, income or education level [2]. It is associated with increased adult morbidity through predisposing to conditions such as insulin resistance, lipoprotein abnormalities, and diabetes mellitus type II, cardiovascular disease, deep vein thrombosis, elevated blood pressure [3] and colon cancer [4].

Obesity treatment is one of the most challenging health problems worldwide and imposes great costs to countries economy [1]. Therefore many studies are focused to find new treatments specially, diet related treatments of obesity [2]. lifestyle interventions(Calorie restriction, increasing physical activity) [2], pharmacotherapy, and surgery [5], herbs and their natural compounds are used for obesity management but because of the low efficiency and undesired side effects [18] treatment is not optimal with ischemic heart disease (IHD) [6] and obesity prevalence's [7, 8]. Epidemiologic studies have shown a negative correlation between nuts consumption, particularly almonds

Almond is rich in monounsaturated fats (mainly ω-9, about 10 g/ounce) [9], arginine [10], and is a relatively good source of polyunsaturated free fatty acids (PUFA) (3.5 g/ounce) [1].
almond, in particular, is rich in many tocopherols, including α- tocopherol, the most active form of vitamin E, which has shown potent antiatherogenic and antilipidemic effects [11, 12]. Although some Clinical studies showed negative association between almond consumption and body fat [6, 7, 13], other studies reported no such effects [14].
In human studies confounding, inter-correlated exposures, unclear dietary changes, and the absence of precise assessment methods makes the conclusions limited and biased the studies [15].Controversies on the effects of almond on body weight, body fat percent and blood lipids is increasing [16] and it appears that to clarify the ambiguity about the issue, needs more controlled experimental studies. However we had not found any experimental study on the effects of almond extracts on the body fat and blood lipids. Therefore we have studied the effects of hydro-alcoholic effects of almonds on body fat percent, and blood lipids of male Sprague Dawley rats receiving high fat diet.

MATERIALS AND METHODS

Animals: 40 mature male Sprague Dawley rats were randomly allocated in five different groups and kept at the animal house under the 50-60 percent humidity, 18-22 degree centigrade room temperature and 12/12 hours of dark/light cycle.
Diets: The basic diet was received from Pars Company, Tehran, Iran. A group of rats was fed basic diet as control group (CG). Four other groups were fed a high fat diet composed of 30 percent fat (20 percent vegetable oil and 10 percent animal fat added to the basic diet). All rats fed the same amounts of diets.
Almond extract: Five kilograms sweet almond were powdered using electric nut grinder. The powder was soaked in 50/50 percent water and ethanol mixture at room temperature for 24 hours. The mixture was filtered using No. 1 Whatman paper filter and then concentrated using rotary. The filtrate was dried in 45 degree centigrade incubator and was used as a 100 percent concentration extract. Concentrations of 10, 25 and 50 mg of almond extract in distilled water was prepared and were fed to the three groups of rats through gavage for 8 weeks. The fifth group just received high fat diet without almond extract as negative control (NC).
Measurements: serum lipids were measured using Pars-Azmoon Company kits (Tehran, Iran), body fat percent was measured using kjeldahl method as described by Malekzadeh *et. al.*[17].
Statistical Analysis: Data higher or lower than two standard deviation from group mean removed from analysis. After controlling for normality distribution and variance equality, data were compared between groups using one way ANOVA. If any significant differences were observed between groups the Scheffe post hoc test has been used to find the different groups. Significant probability value cut point was considered as 0.05.

RESULTS

Findings showed a higher weight gain in groups receiving high fat diet. No significant difference was showed between weight gain in groups of almond extracts (Table 1) and negative control group.
Percent of corpus fat was not also significantly different however almond extract receiving groups showed lower percent of body fat compared with positive control and negative control (table 2).

Table 1: Mean and standard deviation of weight gain in rats receiving different levels of almond extract and high fat diet

Group	Weight gain(gr) Mean±SD	CI for 95%	
		LB	UB
PC	625±3*	3.3	8.8
LE	58±15.6**	45.2	71.4
ME	45±23.4**	23.7	67
HE	58±34**	38	78.4
NC	54±19**	34.9	74.3

ANOVA F=11.25 p<0.0001 Groups differently signed are significantly different

Table 2: Mean and standard deviation of corpus fat percent in rats receiving different levels of almond extract

group	Number	Corpus fat percent Mean±SD	CI for 95 percent	
			LB	UB
PC	6	18.2±4.9	13	23
LE	7	17.6±3.2	14.6	20.7
ME	8	15.5±3.3	12.7	18.3
HE	5	15.6±3.5	11.25	19.9
NC	7	19.9±2.2	17.8	21.9

F=2.4 P>0.05 No Significant different was shown

Serum triglyceride levels in groups receiving high fat diet were significantly higher than positive control group($p<0.05$), there is a linear negative relationship between almond extract concentration and serum triglyceride levels in groups receiving almond extract(table 3). In addition, group receiving 50 mg /kg almond extract showed a significantly lower serum triglyceride than negative control ($p<0.05$).

Table 3: Mean and standard deviation of Serum triglycerides in rats receiving different levels of almond extract and high fat diet.

group	Number	Serum Triglyceride(mg/dl) Mean±SD	CI for 95 percent	
			LB	UB
PC	8	48±28**	60.9	107.3
LE	8	153±73	91.9	214.3
ME	7	135±68	72.8	197.9
HE	8	99±69**	42	157
NC	6	222±56*	163	281.9

F=5.02 p<0.002 *, ** Groups differently signed are significantly different

Serum cholesterol data are presented in table 4. Serum cholesterol levels in groups receiving high fat diet are higher than positive control except for 50 mg extract group that showed a great reduction in serum cholesterol($p<0.07$). Post hoc analysis showed a significantly higher serum cholesterol in group receiving 25 mg/kg extract compared with positive and 50 mg receiving group($p<0.05$)

Table 4: Mean and standard deviation of serum cholesterol in rats receiving different levels of almond extract

Group	Number	Serum Cholesterol(mg/dl) Mean±SD	CI for 95 percent	
			LB	UB
PC	8	46±22.5	27.7	65.2
LE	8	59±27	37.2	82
ME	7	80±15	66.5	94
HE	8	45±34	16.5	72.9
NC	6	79±45	32.5	126.8

ANOVA F=2.46 p<0.07

The mean and standard deviation of serum HDL-C is shown in table 5. The group that received higher extract concentration of almond showed lower serum HDL-C, although the observed different is not statistically significant.

Table 5: Mean and standard deviation of serum HDL cholesterol in rats receiving different levels of almond extract

group	Number	HDL-C(mg/dl) Mean±SD	CI for 95 percent	
			LB	UB
PC	**8**	39±15	52	26/9
LE	**8**	57±31	82/4	31
ME	**7**	55±10/5	64/7	45/5
HE	**8**	33±26	54/7	11/7
NC	**6**	46±12/5	59	32/6

ANOVA F=1.7 p=0.17

DISCUSSION

This experiment carried out to test the effects of almond hydro-alcoholic extract on body weight, body fat, and serum lipids in rats receiving high fat diet. The study findings showed that almond extract has no effect on weight gain but it may reduce body fat, serum TG and HDL-C. Serum cholesterol is also reduced with higher intake of almond extract (50 mg/kg) as compared with other groups that received high fat diet.

Jia et.al in their study on the effects of almond oil showed lipid reducing effects on rats. They reported reductions in total cholesterol, triglyceride and low density lipoprotein, and increased serum high density lipoprotein content(18) that is accordance with the present study findings except for HDL-C.

Fraser et al in their study on human subjects reported that receiving 320 extra calories from almond in diet was not associated with significant weight gain [14]. Wien et al also reported cases receiving sweet almond plus low calorie diet had greater weight loss compared with cases receiving only low calorie diet [19]. Further they reported a reduction in serum HDL-C in almond receiving group [19] that is accordance with this study finding.

Dietary almond consumption may reduce fat and energy absorption, through its fiber content [10]. In addition high concentrations of arginine in sweet almond may have beneficial effects on blood lipids [20]. Almond has a high content of polyunsaturated fatty acids including oleic and linoleic acid [21]. Previous studies have shown that these fatty acids have reducing effects on serum cholesterol [22, 23]. PUFA intakes suppress adipocyte differentiation and down regulate adipocyte P2 and adipsin genes [24].The fatty fraction of Almond contains also plant sterols with anti-oxidant and cholesterol lowering effects [7]. Plant sterols, polyphenolics and high unsaturated fats have hypocholesterolemic effects [25].

Almond consumption as a dietary constituent has already shown a reducing effect on serum cholesterol in human subjects [11, 12, 19, 26] but the effects of its extracts on serum lipids and body fat was not reported yet.

CONCLUSION

our findings is convincing for some healthy effects of almond such as serum triglyceride and body fat reduction, but we suggest that the study should be done with higher sample size, longer duration and different types of extracts to determine its hypocholesterolemic effects. Also in-vitro studies are need to determine the mechanisms underlying confounding effects of almond extract on serum lipids especially on HDL-C.

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