Original Article

Comparison of Whole Walnut, Ethanolic and Aqueous Walnut Leaves Extract on Lipid Profile and Atherogenic Index in Hypercholesterolemic Rats

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ABSTRACT

Objective: To compare the effect of whole walnut and walnut leaf extract (Aqueous and Ethanolic) on lipid profile and atherogenic index in Hypercholesterolemic rats.

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the Islamic International Medical College, Riphah International University, in collaboration with National Institute of Health and Citilab, Islamabad, Pakistan from January to June 2015.

Materials and Methods: A total of 50 Sprague Dawley rats were in divided in five groups, HC (hypercholesterolemic control), WW (Whole walnut), AE (Aqueous extract), and EE (Ethanolic extract). Serum cholesterol, triglycerides, LDL, HDL and atherogenic ratios LDL/HDL and TC/HDL were measured at baseline, 8 and 12 weeks respectively after inducing hypercholesterolemia. Data was analyzed by ANOVA and student t test using SPSS version 19. P value of <0.05 was considered statistically significant.

Results: WW in comparison to AE group decreased serum triglycerides (p=0.01) and serum total cholesterol in (p=0.02). WW in comparison to EE group decreased only serum triglycerides significantly among all lipid profile parameters (p=0.00). A significant decrease in serum total cholesterol (p=0.00) and an increase in serum HDL (p=0.00) by EE was observed compared to AE group.

Conclusion: WW, AE and EE have specific effects on individual lipid profile parameters which is hypolipidemic as all three decreased bad cholesterol and raised the good HDL lipoproteins. Further research can delineate this therapeutic potential in cardiovascular diseases.

Key Words: Atherogenic index, Hypercholesterolemia, Juglans regia, Triglycerides, Walnuts

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INTRODUCTION

Cardiovascular diseases cause worldwide morbidity and is a leading cause of death in United States.[1] Abnormal lipid profile due to unhealthy lifestyle, diet and stress contributes to this situation. Since high cholesterol levels does not necessarily cause any symptom so many people live with it undiagnosed and untreated.

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Total cholesterol level of more than 200mg/dl is defined as hypercholesterolemia and 38% of adults in America and increases risk of stroke and heart disease.[2] Out of all the lipoproteins that circulate in blood, Low Density Lipoprotein is considered as the most dangerous. A recent large population-based study in Denmark found a higher incidence of myocardial infarction and atherosclerotic cardiovascular disease with raised small dense LDL.[3] As the field of lipidology is expanding based on increasing clinical trials and genetic epidemiology studies, serum triglycerides remains a valuable predictor of the atherosclerotic disease.[4] VLDL also transports cholesterol and has shown it to contribute fifty percent of risk among all apo-B containing lipoproteins. The good cholesterol containing lipoproteins are called High-density lipoproteins. There is recent data on HDL proteome which is unveiling them as useful markers on cardiovascular protection.[5] Serum HDL is a promising marker as more mechanistic insights into HDL metabolism are unveiling.[6] The ratios of total cholesterol to HDL and LDL/HDL are said to be best

predictors of cardiovascular disease and is more sensitive and specific than individually used LDL, total cholesterol, and HDL levels.[7]

Lipid lowering agents are used to treat hypercholesterolemia. However, use of medicinal plants for their therapeutic potential are gaining importance for being safe and cost effective.[8] Walnut (Juglans regia L,) belongs to family juglandaceae and is produced in South Asia, Japan, China, and United States. Walnut fruit as well as kernel, shell, leaves, septum, bark, epicarp have all been shown to possess anti oxidative and anti-inflammatory properties.[9]

The objective of this study was to compare the effect of whole walnut as well as walnut leaves extract (both aqueous and ethanolic) on lipid profile with ratios of LDL/HDL and TC/HDL in rats with raised cholesterol levels.

MATERIALS AND METHODS

This study was conducted at Islamic International Medical College, Riphah International University and National Institute of Health (NIH) after ethical approval. A total of 50 male Sprague Dawley rats were divided into control (C), hypercholesterolemic control (HC,) whole walnut (WW), aqueous (AE) and ethanolic extract (EE) groups. C was fed on standard rat diet throughout the study. HC was fed for initial eight weeks with high fat diet and then fed on standard rat diet till the study was completed. High fat diet (HFD) was 17 % of calories as carbohydrates, 25% as proteins and 58% calories as fat and 2% cholesterol powder[10]. WW was fed for initial eight weeks with high fat diet and then given 10% whole walnut feed (powder) mixed in standard feed daily.EE and AE were given extracts of 100gm powder of walnut leave in 1000ml of 95% ethanol and distilled water respectively.[11]

Sampling was done at baseline, 08 week & 12 weeks. Blood was centrifuged at 3000 rev/min for I5 min to obtain serum. Serum total cholesterol (TC), Serum triglycerides (TG), serum LDL and serum HDL were analyzed using Merck Germany kits (lot no 17895) on Selectra E Automated chemistry analyzer.[12] All data was shown as mean ±S.E.M. ANOVA and student t test were applied using SPSS 19. A p-value of < 0.05 was considered as statistically significant.

RESULTS

Comparison of lipid profile parameters in all groups at the end of experiment as shown in table 1. Comparison of WW and AE showed that aqueous extract significantly reduced TC and TG levels in both the groups as shown in table 2. Comparison of WW and EE revealed that EE significantly reduced only TG levels (p=0.00) as shown in table 3. Comparison of AE and EE revealed significant reduction of serum TC levels (p=0.00). Serum HDL levels were significantly increased in EE group as shown in table 4.

Table No.1: Comparison of lipid profile in all groups at the end of 12 weeks.

		Sum of Squares	df	Mean Square	F	p value
TC (mg/dl)	Between Groups	66265.150	4	16566.288	613.404	.000
	Within Groups	945.250	35	27.007		
	Total	67210.400	39			
TG (mg/dl)	Between Groups	34859.650	4	8714.913	86.848	.000
	Within Groups	3512.125	35	100.346		
	Total	38371.775	39			
LDL (mg/dl)	Between Groups	3360.400	4	840.100	118.383	.000
	Within Groups	248.375	35	7.096		
	Total	3608.775	39			
HDL (mg/dl)	Between Groups	68.900	4	17.225	7.188	.000
	Within Groups	83.875	35	2.396		
	Total	152.775	39			
TC/HDL ratio	Between Groups	136.504	4	34.126	438.670	.000
	Within Groups	2.723	35	.078		
	Total	139.227	39			
LDL/HDL ratio	Between Groups	8.131	4	2.033	70.321	.000
	Within Groups	1.012	35	.029		
	Total	9.143	39			

*= $p < 0.05$ is considered significant

Table No.2: Comparison of lipid profile in WW and AE groups

Groups		Mean ±SD	p-value
TG (mg/dl)	WW	76.13±4.74	.001*
	AE	3.30±14.63	
TC (mg/dl)	WW	51.88±6.10	.02*
	AE	45.00±4.56	
LDL (mg/dl)	WW	22.75±2.43	0.15
	AE	1.25±1.48	
HDL (mg/dl)	WW	25.50±1.64	0.36
	AE	24.87±0.83	
TC/HDL ratio	WW	2.03±0.28	0.11
	AE	1.84±0.15	
LDL/HDL ratio	WW	0.89±0.13	0.41
	AE	0 .85±0.06	

*= p <0.05 is considered significant

Table No.3: Comparison of lipid profile in WW and EE groups

Groups		Mean± SD	p-value
TG (mg/dl)	WW	76.1±4.703	0.00***
	EE	50. 6±11.73	
TC (mg/dl)	WW	51.8±6.108	0.71
	EE	52.8± 4.428	
LDL (mg/dl)	WW	22.7± 2.430	0.20
	EE	21.5±1.060	
HDL (mg/dl)	WW	25.5±1.690	0 .23
	EE	26.5± 1.510	
TC/HDL ratio	WW	2.03±0.288	0.71
	EE	1.991±0.212	
LDL/HDL ratio	WW	0.89± 0.135	0.11
	EE	0.80± 0.648	

*= p <0.05 is considered significant

Table No.4: Comparison of lipid profile in AE and EE groups

Groups		Mean± SD	p-value
TG (mg/dl)	AE	53.33± 14.60 8	0.68
	EE	50.62± 11.73	
TC (mg/dl)	AE	45.00± 4.56	0 .00*
	EE	52.83± 4.40	
LDL (mg/dl)	AE	21.25± 1.480	0 .70
	EE	21.50± 1.060	
HDL (mg/dl)	AE	24.87± 0.830	0.01*
	EE	26.50± 1.510	
TC/HDL ratio	AE	1.84± 0.153	0.14
	EE	1.99±0.21 2	
LDL/HDL ratio	AE	0.85± 0.065	0.19
	EE	0.80± 0.068	

* = p <0.05 is considered significant

DISCUSSION

Comparison of lipid profile amongst all groups revealed significant (p=0.00) difference as shown in table1. Further comparison between WW, AE and EE groups indicate that TG, TC, LDL, TC/HDL and LDL/HDL ratio are all not uniformly reduced in all three groups but have specific effects on each lipid profile parameter as shown in tables 2,3 and 4. HDL levels were significantly increased in only EE group (26.5 mg/dl; p=0.01) in comparison with AE group, along with decrease in TC (p=0.00) as shown in table 4. EE in comparison with WW whole walnuts significantly decreased only serum TG (p= 0.00) as shown in table 3. WW in comparison to AE reduced both serum TC (p=0.02) and TG (p=0.01) as shown in table 2. Similar results are reported by Uti DE et al, whereby ethanol extract of walnuts decreased total lipid, phospholipids, triacylglycerol, and cholesterol concentration in adipose, kidney, brain and heart tissues of obese rats fed.[13-15]

Sun Y et al found a decrease in serum LDL as well as cardiac markers in MI with use of walnut kernel extracts.[16] Walnut leaves consist of components like fiber, folic acid, calcium, potassium, vitamin E and C, magnesium, plant protein (e.g., arginine) and polyphenols along with fatty acids. Unsaturated fatty acids and polyphenols have antioxidant properties in rats with diabetic nephropathy and lung injury. [14,15] The possible mechanism for lowering lipid levels by walnut leaves is that phenolic acids and flavonoids. Pedunculagin is a polyphenol which belongs to the class ellagitannin. Its hydrolysis yields ellagic acid and urolithins Ellagitannins are antioxidant and anti-inflammatory in cancer, cardiovascular, and neurodegenerative diseases.

A recent study conducted by Liu X et al conducted on three large cohorts of men and women using food frequency questionnaires for four years found walnuts consumption associated with decreased risk of coronary artery disease and stroke.[17] They report lipid lowering effect due to another antioxidant substance called quercitin and cholorogenic acid. They reduce synthesis of cholesterol in the liver through suppression of HMG COA reductase enzyme and thus causes increased release of cholesterol in bile. The phenolic compounds isolated from nuts by HPLC fingerprinting have recently been shown to have antioxidant properties responsible for reducing serum cholesterol as reported by Olabiyi AA et al.[18]

Recent large-scale studies in metabolomics and lipidomic fields are investigating the complex metabolism of lipids in chronic inflammatory diseases and diabetes. Side by side nutraceuticals such as walnuts have gained special attention due to unique composition of omega 3 and omega 6 fatty acids as well as antioxidant polyphenols.

The strength of this study is the comparative analysis of three groups WW, AE and EE which has not been done before and shows differential effects on lipid

profile which can be further explored. Further studies can be carried out using different doses and timings of walnut intake as well as designing a different combination of macronutrients to understand the impact on lipid profile. Beneficial effect of walnuts using newer markers of cholesterol metabolism at molecular level offers more for future research.

CONCLUSION

The present study suggests that whole walnuts in comparison with aqueous and ethanolic extract of its leaves decreased both serum total cholesterol and triglycerides. Moreover, the ethanolic extract increased serum HDL compared to aqueous extract.

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Conflict of Interest: The study has no conflict of interest to declare by any author.

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