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ORIGINAL ARTICLE

Effect of Ethanolic Extract of Walnut Leaves on Lipid Profile and Atherogenic index in Hypercholesterolemic Rats

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ABSTRACT

Aims: To determine the effect of Ethanolic extract of walnut leaves on lipid profile i.e seum total cholesterol, triglycerides, Low density and high-density lipoprotein. Atherogenic index in Hypercholesterolemic rats was calculated using serum TC/HDL and LDL/HDL ratios.

Methodology: Total 30 male Sprague Dawley rats were further divided into three groups (C, HC and EE) comprising ten rats per group. Group 1 (Control group, C). Group 2 (Hypercholesterolemic control, HC) and Group 3 (Ethanolic Extract Group EE) were fed high fat diet for 8 weeks before administration of walnut leaf extract. Ethanolic extract (EE) of walnut leaves was given in a dose of (200mg/kg) through gavage needle once daily for four weeks. Blood Sampling was done at the beginning (Baseline, after week 8 and week 12 to perform lipid profile.

Results: Serum mid- cholesterol levels (08 weeks) in HC were significantly raised to 164±7.90 mg/dl ($p<0.001$) as compared to 57.75±6.64mg/dl), which confirmed the development of hypercholesterolemia**.** Post-cholesterol levels of EE (after 12 weeks) was decreased to 52.8±4.42 mg/dl compared with 153.2±5.92 mg/dl in HC group in ($p<0.001$). Group 3 had significantly lower levels of of serum triglycerides, LDL and high levels of HDL cholesterol ($p<0.001$) as compared to HC group 2 and 12 weeks versus 8 weeks of EE of walnut supplementation. The atherogenic index calculated by serum TC/HDL and LDL/HDL ratios were significantly reduced ($p<0.001$).

Conclusion: Ethanolic extracts of walnut leaves have hypolipidemic effects decreasing serum cholesterol, triglycerides, LDL, serum TC/HDL and LDL/HDL ratios while elevating the good cholesterol HDL.

Keywords: Atherogenic ratio, Cholesterol, Dietary Lipids, Lipoprotein metabolism, Walnut supplementation

INTRODUCTION

One of the leading causes of death affecting millions of people worldwide in both developing and developed countries is cardiovascular disease and a major risk factor is Hyperlipidemia. According to CDC, nearly 12% of adults aged 20 and older had total cholesterol higher than 240 mg/dL, and about 17% had high-density lipoprotein (HDL, or "good") cholesterol levels less than 40 mg/dL in 2015–2018.[1] In U.S. 54.5%, or 47 million (slightly more than half of adults) are taking cholesterol medicine.[2] This is a modifiable cause ofs cardiovascular disease such as coronary heart disease, diabetes, and cancer.[3] It is managed by diet modification and use of lipid lowering agents. Several lipid lowering drugs are used such as bile acid sequestrants, HMG-CoA reductase inhibitors, fibrates. Life style medicine is a new evolving branch of medicine which stresses on use of nutritional interventions for disease prevention and treatment.[4] Walnut (Juglans regia L,) is a medicinal plant that belongs to the family juglandaceae and is cultivated in China, Japan, South Asia, in South Eastern Europe and United States. Different parts of juglans regia such as kernel, shell, leaves, septum, bark, epicarp have been used in pharmaceutical and cosmetic products.[5] Previous studies have investigated walnuts for its antimicrobial, antiaging, anti-tussive and anti-proliferative activity[6,7,8,9]. A major walnut component Alpha-linolenic acid, is metabolized into bioactive oxylipins, which decreases cholesterol and protects from myocardial infarction and arrhythmia[10].The purpose of this study was to evaluate the effect of ethanolic extract of walnut leaves on lipid profile along with LDL/HDL and atherogenic ratios(TC/HDL) in hypercholesterolemic rats.

METHODOLOGY

This randomized control study was conducted at Islamic International Medical College, Riphah International University, Islamabad in collaboration with Animal Housing Facility at National Institute of Health (NIH) from Januaury to June 2015 after approval by Ethics Review Committee of Islamic International Medical College, Riphah International University. A total of 30 Sprague Dawley rats weighing 250-300gms were divided equally in three groups and kept for 3 months in housing facility of NIH. The rats were supplied with water and food at a room temperature 24+2 C under 12 hours light and dark cycle after acclimatization for one week on standard rat diet and water ad libitum.[11,12] Group 1 was normal Control (C). Group 2 Hypercholesterolemic control (HC) was fed for initial eight weeks with high fat diet (17% of calories from carbohydrates, 25% from proteins and 58% of calories from fats) to induce hypercholesterolemia and then fed on standard rat diet till the study was completed. Group 3 was Ethanolic extract (EE) group was fed for initial eight weeks with high fat diet and was given ethanolic extract of walnut leave in a dose of 200 mg/ kg daily with the help of gavage needle for 4 weeks. Walnut leaves were collected from Muzaffarabad, Azad Kashmir, identified, authenticated, coded and kept under voucher # 157 at the Herbarium, Quaid-i-Azam

University, Islamabad, Pakistan. They were dried,grounded into powder and soaked in 95% ethanol. The solution was filtered using Whatmann filter paper # 1 and dried in rotary evaporator at 55°C at research laboratory of RIPS, Islamabad. The extract obtained was in the form of a dark brown semi-solid sticky paste was stored in air tight glass bottles, protected from light and kept in refrigerator at 2-8 o C to be used throughout the experiment.[13,14] Blood samples (1.5ml) were taken by intracardiac sampling at 8 and 12 weeks and All the samples were placed in the centrifuge machine (Hettich, EBA-20) and were centrifuged at 3000 rev/min for 15min and serum was separated[15]. Total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides and Atherogenic index (TC/HDL and LDL/HDL) were measured using Merck kits (Germany-lot no 17895) on Selectra E Automated chemistry analyzer by colorimetric method[16]. Ratios were calculated as TC/HDL and LDL/HDL. The data was analyzed using SPSS 19(statistical package for social sciences). All data was shown as mean ±S.E.M and student t-test was applied between groups 2 (HC) and group 3 (EE). p-value of < 0.05 was considered as statistically significant.

RESULTS

During the experiment blood lipid profile was analysed in C, HC, and EE groups at the start of experiment (baseline), after 08 weeks & 12 weeks respectively. These variables are presented in table I shown below

Table 1: Lipid Profile variables in mg/dl in C, HC and EE groups at the start of experiment (Pre-Cholesterol), after 08 weeks (Mid-Cholesterol) & after 12 weeks (Post-Cholesterol) in Sprague Dawley rats.

Variables	Groups	Baseline	08 weeks	12 weeks
Serum cholesterol levels (mg/dl)	C	60.75	57.75	58.5
	HC	62.65	164.8**	153.2
	EE	72.66	170.5**	52.8**
Serum triglycerides levels (mg/dl)	C	65.8	69.88	60.25
	HC	60.54	150.63**	130.5
	EE	72.45	158.65**	50.63**
Serum LDL levels (mg/dl)	C	26.13	25.12	24.62
	HC	27.33	52.25**	45.25
	EE	25.83	52.34**	21.5**
Serum HDL levels (mg/dl)	C	26.62	26	23.75
	HC	28.54	23.12	22.75*
	EE	23.44	25.66*	26.8*

C=control, HC= hypercholesterolemic, EE= ethanolic extract. All values are expressed as mean+-SD.

*p<0.05 is considered significant

**p<0.001 is considered significant on comparison with hypercholesterolemic group.

The mid cholesterol levels in HC were 164.8mg/dl which were significantly raised (p<0.001) as compared to the mid-cholesterol levels of Control group i.e 57.75mg/dl which confirmed the development of hypercholesterolemia. Ethanolic extract (EE) group had significantly reduced (p<0.001) post cholesterol levels to 52.8mg/dl as compared to post cholesterol levels of hypercholesterolemic control of 58.5mg/dl. The mid-TG levels in HC and EE were significantly raised (p<0.001) as compared to mid-TG levels of control group. Ethanolic extract group had significantly low (p<0.001), post-TG levels (50.63 mg/dl) as compared with post-TG levels of hypercholesterolemic control (60.25mg/dl). Serum LDL levels (mg/dl) were analyzed in C, HC and EE groups at the start of experiment (Pre-LDL), after 08 weeks (Mid-LDL) & after 12 weeks (Post-LDL) as shown in table I. Ethanolic group had significantly lowered the post LDL levels to 21.5mg/dl (p<0.001) as compared to post LDL levels of 24.62 mg/dl in hypercholesterolemic control. EE had significantly raised post HDL levels to 26.8mg/dl as compared to post HDL levels of 23.75mg/dl hypercholesterolemic control. Serum TC/HDL ratios were calculated in C, HC & EE groups at the start of experiment (Pre-TC/HDL), after 08 weeks (Mid-TC/HDL) & after 12 weeks (Post-TC/HDL) and are presented in Fig 1.

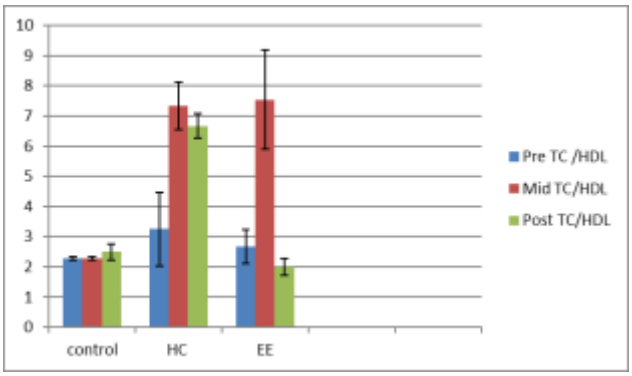

Fig 1: Serum Mid-TC/HDL (after 08 weeks) was significantly raised (p<0.001) in both HC & EE groups. Post-TC/HDL (after 12 weeks) was significantly raised in EE group only. C=control, HC= hypercholesterolemic, EE= ethanolic extract. All values are expressed as mean+-SD.

The mid-TC/HDL ratio in Group HC (7.33) and EE (7.54) groups were significantly raised (p<0.001) as compared to mid- TC/HDL ratio of control group (2.27). Ethanolic extract post TC/HDL ratio was significantly reduced to 1.99 as compared to post TC/HDL ratio of hypercholesterolemic control group. Serum LDL/HDL ratio in C, HC & EE group at the start of experiment (Pre-LDL/HDL), after 08 weeks (Mid-LDL/HDL) & after 12 weeks (Post-LDL/HDL) and are shown in figure 2.

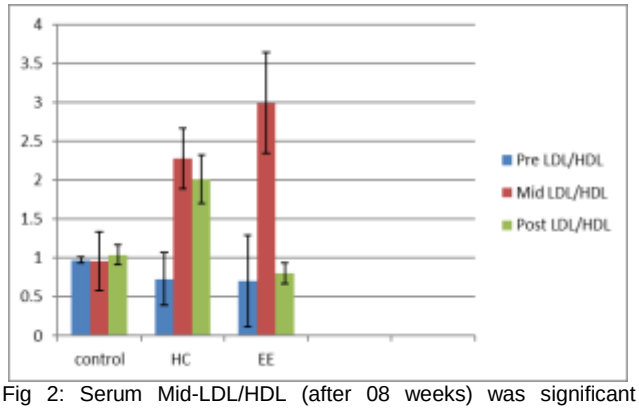

Fig 2: Serum Mid-LDL/HDL (after 08 weeks) was significant (p<0.001) in both HC & EE groups. Post- LDL/HDL (after 12 weeks) was significant (p<0.001) in EE group only when compared to HC. C=control, HC= hypercholesterolemic, EE= ethanolic extract. All values are expressed as mean+-SD.

The mid-LDL/HDL ratio in Group HC (2.28) and EE (2.99) were significantly increased ($p<0.001$) as compared to mid- LDL/HDL ratio of control group (0.96). Ethanolic extract group had significantly lowered the post LDL/HDL ratios to 0.8 ($p<0.001$) on comparison with post-LDL/HDL ratio of hypercholesterolemic control group (1.04).

DISCUSSION

The present study showed the anti hypercholesterolemic effect of ethanolic extract of walnut leaves in rats causes significant reduction in serum cholesterol, TGs, LDL, TC/HDL and LDL/HDL ratios and significant increase in HDL levels. High levels of lipid in blood are said to be one of the predominant risk factors for atherosclerosis, stroke and hypertension which are serious health issues worldwide. Our results are consistent with Jelodar et al who fed cyclohexane extract of walnut leaves to diabetic rats. They found beneficial effect on body weight, fasting blood glucose, lipids profile, antioxidant enzyme activities. After 28 days of feeding extract by oral gavage, the activity of antioxidant enzymes significantly increased in treated groups compared with diabetic control. Fasting Blood glucose, Total cholesterol, Triglycerides, LDL-c, VLDL-c, homocysteine, and MDA level were decreased while increased levels of HDL-c were reported in diabetic rats[17]. Bamberger C et al found positive association between walnut intake and improvements in plasma lipids in a randomized, controlled, prospective, cross-over study in 194 healthy non-smoking participants of age more than 50 years. They found fasting cholesterol (walnut vs. control: −8.5 ± 37.2 vs. −1.1 ± 35.4 mg/d, $p = 0.002$), non-HDL cholesterol (−10.3 ± 35.5 vs. −1.4 ± 33.1 mg/dL; $p \leq 0.001$), LDL-cholesterol (−7.4 ± 32.4 vs. −1.7 ± 29.7 mg/dL; $p = 0.029$), triglycerides (−5.0 ± 47.5 vs. 3.7 ± 48.5 mg/dL; $p = 0.015$) and HDL-cholesterol and lipoprotein (a) did not change significantly[18].Many studies have therefore been carried out on the use of natural food materials in the prevention and treatment of hyperlipidemia and other chronic non-communicable diseases in an attempt to use nutrition as a lifestyle modification[19].Of 1113 different food items that were tested for their antioxidant contents, walnuts were ranked second place [20]. Walnuts have a unique fatty acid composition with abundant polyunsaturated fatty acids (PUFA) which includes linoleic acid and α-linolenic acid and phenolics. When PUFA are metabolized, they form oxylipins as a result of cyclooxygenases (COX) – producing prostaglandins and thromboxane. lipoxygenases (LOX) – producing hydroperoxides and their associated downstream products. Oxylipins are the primary mediators of PUFA action, regulating inflammation, glucose metabolism and cardiovascular functions[21]. A Recent study highlighted the molecular mechanism of lipoprotein regulation by walnuts. Walnut treatment in hypercholesterolemic, postmenopausal females treated with 40 g/day (i.e., 1.6 servings/day; n=15) of walnuts for 4 weeks., elevated α-linolenic acid and its epoxides in all lipoproteins and depleted mid-chain alcohols in VLDL and LDL, but not HDL. Walnuts also reduced TNFα-induced diabetic adipocyte production of IL-6 (-48%, P=.0006) and IL-8 (-30%, P=.01). This study shows that modest walnut consumption can alter lipoprotein lipid profiles and enhance their ability to inhibit TNFα-dependent pro-inflammatory responses in human diabetic primary adipocytes. Moreover, this study suggests the oxylipins mediate LDL action of adipocytes[22].Further studies can be planned to further specify what kind of combination diet can be most suited to a favourable lipid profile. Randomized controlled trials can be designed using variable dietary controls which could be habitual diet or a typical Pakistani diet as well as a variable combination of low to high carbohydrates to fat ratio and vice versa. High Performance Liquid chromatographic studies can also be designed to further evaluate extract on its individual constituents and their efficacy on specific good and bad lipoproteins in blood.

CONCLUSION

This study indicates that ethanolic extracts of walnut leaves have marked hypolipidemic effects. Lowering of serum cholesterol, TGs, LDL, TC/HDL and LDL/HDL ratio and significantly increase HDL levels can correct hypercholesterolemia using walnuts and may play a valuable role in the prevention of cardiovascular illnesses.

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