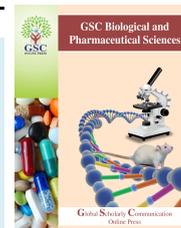


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(RESEARCH ARTICLE)



Biological study on the impact of commonly used commercial fats and oil and threats of atherosclerosis

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Abstract

Poor health due to suboptimal nutrition influences on many diseases including diabetes mellitus, obesity, cardiovascular diseases (CVD), elevated cholesterol and blood pressure (BP). World health organization estimated that mortality rate and disease burden due to CVD was around 17.3 million. The prevalence rate of coronary artery diseases (CAD) is higher in Pakistan that is 30% of people age above 45 years. The objective of study involves evaluation of commonly used fats and oil to investigate its effect on lipid profile through in vivo studies. Hypercholesterolaemia was induced by feeding high cholesterol diet. Thirty Sprague dawdle rats were taken having five rats in each group. Margarine, butter, vanaspati ghee and olive oil were given with a specific dose 10 g/100 g feed given 6 weeks on hypercholesterolaemic rats. Maximum weight gain was observed in G2, G3, G4 fed; margarine, butter, vanaspati ghee respectively. And maximum reduction in weight was observed in G5 fed on olive oil. Feeding cholesterol diet showed elevated level of lipid profile in G1. Coincident giving various fats and oil along with high cholesterol diet caused a highly significant increase in serum total cholesterol, low density lipoprotein (LDL), triglycerides except G5. Maximum increase of cholesterol was observed in groups fed butter, margarine, vanaspati ghee were recorded as 177.6 mg/dL, 185.5 mg/dL, 180.7 mg/dL. Minimum reduction in cholesterol was recorded in olive oil group as 170.2 mg/dL. Results suggested that the addition of fats showed a negative influence on lipid profile in hypercholesterolaemic rats.

Keywords: Health disorders; Atherosclerosis; Hypercholesterolemia; Fats and oil; Diseases; Prevention

1. Introduction

Atherosclerosis is the most prominent root of cardiovascular diseases (CVD). Risk of cardiovascular diseases has multifactor symptoms with combination from both inflammatory and lipid metabolism that affects vascular functions. Hypercholesterolaemia is a disease in which level of flowing low density lipoprotein (LDL) in blood is increased. Usually it is known as main modifiable risk factor of CVD. Although, little particles consider that it is more atherogenic because it is more susceptible to oxidation then it increases the rate of macrophages [1]. Cardiovascular diseases (CVD) are main public health issue and major cause of mortality and morbidity in western world. The major cause of metabolic syndrome, such as hypertension, obesity and diabetes is due to elevated consumption of fats. Several evidence have recognized that cholesterol rich diet including intermediate density lipoprotein (IDL), very low density lipoproteins (VLDL), apolipoprotein B (Apo-B), and their constituent directly related in the formation of atherosclerotic cardiovascular diseases (ASCVD) [2]. In recent years dietary fatty acids have become a central theme in nutrition research. Fat in diet is essential but in limited amount for human body to survive. Energy provided by 1g fat is 9 Kcal while 4 Kcal provided from carbohydrate and protein.

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Fat that taken from diet is required for the absorption of fat soluble vitamins, hormones and major building block of cell membrane. Fat is very important for our survival but its consumption should be limited otherwise it may be linked to many diseases onset like obesity and coronary heart disease (CHD). The edible oils of interest in our study were olive oil which contains monounsaturated fatty acids and also has a higher quantity of antioxidants. This oil is involved in numerous biological functions which have a beneficial effect on our health. Olive oil scavenges free radicals and has enough for inflammation and autoimmune diseases prevention [3]. Olive oil has a greater amount of MUFA than any other oil or fat. Many researches done on different oils and fats used in diet such as butter, olive oil and coconut oil and they increase CVD risk factors in men and women. Butter contains (66% saturated fat) which is about 40% palmitic (16:0) and stearic (18:0) acids, that high in saturated fat and it increases the cholesterol in blood. Consumption of a higher quantity of excessive saturated fatty acids (SFA) increases lipid storage and inflammation that is related with the threat of inflammatory cardiovascular diseases. In a current study, based on hydrogenated oils, trans fatty acid (TFA) may elevate blood cholesterol as compared to non-hydrogenated oils. If TFA intake is increased in blood then it also elevates the level of low-density lipoprotein and increases the risk of coronary diseases and also decreases high density lipoprotein.

Artherosclerosis is a provocative process that happens due to the gathering of lipid within the arterial wall. It rises when plasma cholesterol level in blood is elevated which changes arterial endothelial cells permeability that store cholesterol containing low density lipoproteins (LDL) bind to the extracellular proteoglycan rich matrix accumulate into the arterial wall. Monocytes circulating also attach to endothelial cells that provide adhesion molecules, like vascular adhesion which provide movements to monocytes via diapedesis between endothelial junctions and reside in sub endothelial space, monocytes converted into macrophages and foamy macrophages [4]. The activated endothelium which has adhesion molecules that is early phase in atherosclerosis, allow lymphocytes such as monocytes and T cells to attach wall of blood vessel and penetrate into inner dendritic cell, B cells, neutrophils and mast cells also locate in lesions. The abundant immune competent cells are present largely in atherosclerotic lesion producing cytokines. When plaque occurs in blood vessel it lowers blood flow and induces CVD. In arterothrombosis when thrombus were damaged, through the impact of chemokines and proinflammatory protein cytokines on the fibrous cap. CVD induced and prothrombotic material is attached to coagulation that inhibits the flow of blood. With the modification of CVD it also lowers the risk of its related diseases like hypertension; and diabetes [5].

Consumption of dietary polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid (MUFA) play an important role in production and oxidation of SFA and also decrease fatty content in hypercholesterolemia. They also have inflammatory properties and are beneficial in managing autoimmune diseases. In insulin resistance, Omega 6 fatty acid is important in controlling and also play an important role in preventing, coronary heart diseases, depression, aging and cancer [6]. The objective of study involves evaluation of commonly used fats and oil to investigate its effect on lipid profile through in vivo studies. So, this research provides useful information regarding the choices of fats and oils for healthy weight gain and disease prevention specifically for hypercholesterolaemic patients in future.

2. Material and methods

The research work was carried out in the National Institute of Food Science and Technology, University of Agriculture, Faisalabad. For that purpose, Margarine, Butter, Hydrogenated ghee and Olive oil were procured from local market.

2.1. Experimental animal model

In this research role of commonly used fats and oil were assessed on hypercholesterolaemia by planning experimental model. For this purpose, 30 rats were attained and housed in animal room of National Institute of Food Science and Technology, University of Agriculture, Faisalabad. Rats were adapted for a period of 1 week by the providing regular diet and water ad libitum.

2.2. Composition of experimental diet and induction of hypercholesterolaemia

Induction of hypercholesterolaemia was done by feeding high cholesterol diet (2% cholesterol) with some modification according to procedure reported by [7]. Normal diet was containing 75% carbohydrates, 15% protein, 10% fat; high cholesterol diet was containing 75% carbohydrates, 15% protein, 8% fat and 2% cholesterol of total Kcal of the diet.

2.3. Experimental protocol

There were six groups G₀, G₁, G₂, G₃, G₄, G₅ in which animal Sprague dawley rat were distributed and each of them consisting of five rats. In experimental study, rats were attained for 1 week, 2 weeks for induction of

hypercholesterolemia and 6 weeks for study period. Normal diet alone was given to G₀ categorized as normal group. For the purpose of inducing hypercholesterolaemia, high cholesterol was given to rest of the groups and after 2 weeks baseline measurements were recorded to observe the level of hypercholesterolaemic induction. High cholesterol diet alone provided to G₁. Along with high cholesterol diet, margarine, butter, vanaspati ghee, olive oil were given to G₂, G₃, G₄, G₅ to evaluate the effect of commonly used fats and oil on hypercholesterolaemic rats.

Table 1 Treatment Plan

Groups	Description	Treatments
G ₀	Normal	Normal Diet
G ₁	Control	High cholesterol Diet
G ₂	Margarine	High cholesterol Diet + Margarine
G ₃	Butter	High cholesterol Diet + Butter
G ₄	Vanaspati ghee	High cholesterol Diet + Vanaspati ghee
G ₅	Olive oil	High cholesterol Diet + Olive oil

2.4. Biological assessment

Diet consumption was evaluated on weekly basis. Body weight gain was measured after 15 days interval. Blood samples were assembled and resultant parameters were considered to evaluate serum lipid profile of hypercholesterolaemic rats [8].

2.5. Serum Lipid Profile

Serum lipid profile of rats include low density lipoprotein (LDL), High density lipoprotein (HDL), cholesterol and triglycerides measured by following methods and their detail is given below:

2.5.1. Cholesterol

Serum cholesterol of rats was measured by using the following protocol of [9].

2.5.2. High and low density lipoprotein

High density lipoprotein (HDL) and low density lipoproteins (LDL) in serum samples were deliberate by following the Dos Santos [10].

2.5.3. Triglycerides

Triglycerides in serum sample were calculated by Dixon and O'brien [11].

2.6. Hematological analysis

Red blood cells (RBC), white blood cell (WBC) and platelets count estimation was carried out following the method of Kamatani [12].

2.7. Statistical analysis

To assess the level of significance all factors of study was measured accurately and statistically via one-way factorial by Tukey test. Set standard for statistical significance in all cases was considered as $p < 0.05$. Statistical analysis was done via analytical software Statistix 8.1 Al-Okbi [13].

2.8. Statistical analysis

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3. Results

Table 1 compares the serum lipid profiles of normal rats (rats fed with normal diet) with rats fed with High cholesterol diet and rats fed with (fats and oil besides HCD) along with different fats and oil. When rats were fed with high-cholesterol diet, there was a significant increase in the serum total cholesterol level and LDL cholesterol levels (both) in all groups. LDL level increased in fats treated group (margarine, butter, vanaspati ghee) and cholesterol fed rats. Further, triglyceride level showed a significant rise in the cholesterol fed rat group and also in (rats fed with normal chow diet versus rats fed with normal chow diet + different oils). Whereas a significant change was observed in margarine, vanaspati ghee, and butter fed group. HDL level decreased in all fats treated group.

Table 2 compares the feed intake body weight gain and hematological parameters of control rats (rats fed with normal diet) with rats fed with HCD and rats fed with high cholesterol diet (HCD) and different fats and oil group. Feed intake in all groups is significant and body weight gain is also significant in all groups elevated weight gain is noticed in vanaspati ghee fed group and lower weight gain is noticed in olive oil feed group. RBC level decreased significantly in all fats and oil treated groups and in high cholesterol diet group. WBC and platelets significantly decrease in all fats and oil treated groups also in high cholesterol group.

Table 2 Lipid profile parameters in control and different treated groups

Groups (n= 5)	TAG (mg/dL)	Total Cholesterol (mg/dl)	HDL Cholesterol (mg/dL)	LDL Cholesterol (mg/dL)
Group 0	79.6±2.71	109.67±3.9	23.5±0.74	29.42±2.81
Group 1	150.5±4.4	173.2±3.88	23.4±0.75	105.4±5.3
Group 2	126.5±0.84	177.6±4.25	23.7±0.7	96.6±18.14
Group 3	130.2±1.24	179.5±5.9	23.6±0.65	95.4±6.12
Group 4	134.86±18.9	180.3±6.92	23.5±0.93	100.5±4.7
Group 5	95.6±4.38	143.44±7.45	23.5±0.78	75.66±5.31

Values are expressed as Mean±SD: n=5 for control and all other group

Table 3 Feed intake, body weight gain and hematological parameters in control and different treated groups

Groups n= 5	Feed intake 20 g/per body weight	Body weight gain g/month	RBC ×10 ⁶ /μL	WBC ×10 ⁹ /L	Platelets ×10 ⁹ /L
Group 0	20.4±3.68	97.6±9.73	7.08±0.015	10220±153.18	150850±134.16
Group 1	22.4±3.68	150.5±3.47	6.48±0.23	43790±101.55	4222960±1137.2
Group 2	18.4±3.08	122.8±2.52	6.56±0.015	10010±77.18	382790±375101.98
Group 3	21.9±3.86	142±1.18	6.17±0.225	34180±527.3	471580±475.34
Group 4	21.7±4.12	142.8±3.69	6.55±0.225	10280±62.705	422190±403.6
Group 5	14.9±1.73	129.2±2.75	6.56±0.015	10310±69.2	470690±2170.6

4. Discussion

Hypercholesterolemia is one of the predominant risk factor for development of atherogenesis and coronary heart diseases. In development and progress of atherogenesis reactive oxygen species (ROS) play a major role causing the conversion of LDL to oxidized LDL, which is consumed by macrophages. If macrophages increase the feed of oxidized LDL causes the formation of atherosclerotic plaques. Excessive uptake of modified macrophages causes the transformation of atherosclerotic plaques. Vulnerability of LDL to oxidation depends both on concentration of pro-oxidant stimuli.

Administration of different fats and oil in rat daily for 2 months found a significant change in lipid profile as evident from the results of this study.

Lipid profile is main indicator of atherosclerosis. Our study is first to investigate the effects of commonly used fats and oil in sprague dawley rats.

Rats fed on high cholesterol diet and vegetable oils show a significant increase in lipid profile compared to the control. There is a significant increase in serum total cholesterol, LDL and triglycerides in all groups. Butter increases the LDL cholesterol significantly more than other group treated. A previous study evaluated different oils such as butter, olive oil and coconut oil. Results from review study indicate that butter consumption was significantly increased LDL-C concentration as compared with olive oil and coconut oil [14].

Margarine and vanaspati ghee also increased LDL significantly; but olive oil did not change its level. Furthermore, total cholesterol level and LDL cholesterol found to be increased in all fats and oil treated groups. The increase in TAG found in the olive oil group. Addition of commonly used fats and oil fed with high cholesterol diets has a positive influence on lipid metabolism. In the present study, effect of commonly used fats and oil on lipid profile was observed. Increased level of lipid profile was found in hypercholesterolaemic rats. Simultaneous administration of commonly used fats and oil with high cholesterol diet caused a significant increase in serum total cholesterol, LDL and Triglycerides.

In butter saturated fatty acid (SFA) and Trans fatty acids (TFA) are atherothrombic and associated with myocardial infarction MI and CHD more than other CVD outcomes [15], so butter increases the triglyceride (TC), LDL cholesterol and triglyceride treated group.

Margarine have high level of trans fatty acid so trans fatty acids and saturated fat produce similarly high levels of LDL cholesterol in plasma, but trans fatty acids also lower HDL cholesterol. Another study inspected to determine oxidation of vanaspati ghee while heating free radicals formed and examine its effect on lipid profile in vitro studies. It was indicated that vanaspati ghee have significant effect on lipid profile and peroxidation effect on tissue level [16].

Vegetable oils, such as olive and soya oils, are recommended for consumption due to their high content of monounsaturated and polyunsaturated fatty acids (MUFA) and (PUFA) [17]. A study comparing the impact of sunflower, fish, and virgin olive oils on the progression of experimental atherosclerosis in rabbits found that extra virgin olive oil; and to a lesser extent, fish oil, stops its progression. In another study, the aorta and coronary arteries of albino rats administered olive oil showed less atheromatous lesions compared with animals fed on corn oil or peanut oil.

At the end of study haematological analysis was done in which RBC, WBC and Platelets were determined by Kamatani [12]. The external structure of cholesterol constitutes the non-polar, hydrophobic lipid of the the enveloping layer of RBC membrane. When cholesterol level increases in blood it affects the fluidity of the membrane decreases and its outer lipid shell stiffens. Previous studies have shown that if blood cholesterol increases in blood that reduced the O₂ transport: in addition the haemoglobin curve also shifted to the left. Early studies demonstrated that high blood cholesterol concentrations were associated with reduced blood O₂ transport; in essence, the hemoglobin dissociation curve was shifted to the left. Present studies have shown that the cholesterol also affects hematological membrane barrier to O₂ diffusion delayed O₂ entry into the RBC during saturation and delayed O₂ release from the RBC during desaturation. Results indicated that consumption of fats significantly increase the RBC, WBC and Platelets [18]. Enrichment of food with complementary antioxidants would be a best option to provide additional protective effects [19]. Olive oil scavenges free radicals and has enough for inflammation and auto immune diseases prevention [20].

5. Conclusion

Rat's food intake and growth with both levels of added oil was satisfactory. Overall it is summarized that ingestion of commonly used fats and oil elevated cholesterol level and it increased the risk of atherosclerosis. Butter increases the lipid profile of hypercholesterolaemic rats than any other group. Vanaspati ghee also increased total cholesterol (TC), triglyceride and little impact on HDL. Olive oil that does not increased cholesterol so its use is beneficial for health because it contain high content of MUFA and PUFA and also have antioxidant property to prevent atherosclerosis, but further research should be conducted in order to assess the effect of others fats and oil that used in everyday life.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare that they have no conflict of interest.

Statement of ethical approval

This study was ethically approved by ORIC, University of Agriculture, Faisalabad, Pakistan.

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