ORIGINAL ARTICLE

Effect of Betulinic Acid Vs Simvastatin on Hypelipidemic Mice Model

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ABSTRACT

Objective: To compare the effect of Betulinic acid and Simvastatin on Triglycerides and Low Density Lipoprotein Cholesterol (LDL-C) in Balb/C Mice.

Study Design: Experimental randomized control trial.

Place and Duration of Study: Study was conducted at Biochemistry department Islamic International Medical College Rawalpindi in collaboration with National Institute of Health Islamabad from November 2017 to April 2018.

Materials and Methods: A total of 40 mice were randomly divided into 4 groups. Excluding one of the 4 groups as negative control, the remaining three were fed with high fat diet for 21 days to achieve raised levels of Triglycerides and Low density lipoprotein. After that out of the three high fat diet fed groups, one group was left untreated considering the positive control group and of the other 2 groups one was treated with simvastatin and 2nd one was given betulinic acid for the next 21 days. Sampling was done by cardiac puncture on 42nd day. Triglycerides and LDL-C levels were measured in serum samples. Data thus obtained was analyzed by one way ANOVA through SPSS 21.

Results: Study showed that positive control group showed a rise in mean triglycerides levels from 130mg/dL to 220mg/dL and mean LDL-C from 23mg/dL to 46mg/dL. Betulinic acid showed significantly better control than simvastatin as mean serum levels of Triglycerides were 146mg/dL as compared to 178mg/dL and mean serum levels of LDL-C were 28mg/dL as compared to 34mg/dL of simvastatin. Considering the $p<0.05$, TGs had a ($p<0.001$) and LDL-C had a ($p<0.001$).

Conclusion: Betulinic acid showed a far better control over the levels of Triglycerides and LDL-C in HFD fed Balb/C mice as compared to simvastatin.

Key Words: *Betulinic Acid, LDL-C; low density lipoprotein cholesterol, Simvastatin.*

Introduction

Atherosclerosis is a “lipid-driven” inflammation of the arterial wall[1], caused by the accumulation of excess lipids into the arterial wall.[2] It has been observed over the years that atherogenesis phenomenon is caused by triglyceride rich lipoproteins (i.e low density lipoproteins LDL).[3] Triglycerides in the serum are derived from fats in our food or from other energy sources. Hypertriglyceridemia is characterized by elevation in triglyceride levels[4] and is independently associated with cardiovascular disease (CVD). High levels of LDL-cholesterol is an indication of extra lipids in blood, which results in increased risk of cardiovascular complications.[5] Globally conducted estimation of deaths because of CVD by 'WHO' revealed 17.5 million deaths in the year 2012.[6] While according to 2013 Global Burden of Disease Study, CVD is responsible for 30% of deaths worldwide.[7] In the last half of the 20th century many epidemiological and experimental studies identified high levels of LDL-C as being atherogenic and advocated a linear relationship with rate of onset of CHD; notable among these are The “Framingham Heart Study”[8], the “Lipid Research Clinics” (LRC) trial by Patsy and Wies in 2014, and the “Multiple Risk Factor Intervention Trial” (MRFIT) in 2013. NLA (National Lipid Association) expert panel advised Lifestyle and drug therapies intended to reduce morbidity and mortality associated with dyslipidemia.[1] Statins are the mainstay of treatment options in management of hyperlipidemia.[1] They block the de-novo synthesis of cholesterol by inhibiting “3-hydroxy-3-methyl-glutaryl- coenzyme A reductase”, simultaneously upregulating the LDL-C receptors and enhancing the clearance of plasma lipoproteins. Thus, statins work in a “self-limiting” manner and manage cholesterol

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levels in blood.[9] Although generally statins are endured well but poor compliance[10] with them may be caused by many of the side effects, including gastrointestinal disturbances, body-aches, respiratory problems, and headaches. Of all these adverse effects Liver and Muscle related problems are remarkably higher in numbers.[11,12,13,14] Recently, in blood, higher levels of glucose and glycosylated hemoglobin Hb-A1C have also been reported in association with administration of Statins.[13] As evidence gap is still there, the American College of Cardiology/American Heart Association (ACC/AHA) Blood Cholesterol Guidelines 2013 included dyslipidemia in the recommendations for high priority research areas.[15]

Keeping the recommendation in view we chose a noble compound named Betulinic acid (BA), a triterpene with a pentacyclic structure. This is found in the in leaves of Ziziphus spina-christi[16] as well as from stem of white bark birch tree[17] , and in various other plants in tropical regions such as Tryphyllumpeltaum, Ancistrocladusheyneaus, Diospyorosleucomelas, Tetracerabolivіana, and Syzygiumformosanum. BA and its derivatives have been the subject of intense study which is primarily focused on their anti-cancer effects, anti-HIV, anti-bacterial, anti-inflammatory, antimalarial, anti-helminthic, and other pharmaceutical properties.[18,19] The direct effect of Betulinic Acid on lipid metabolism is recently being evaluated for the better treatment options available to treat dyslipidemia. As it proved to be beneficial in the treatment of nonalcoholic fatty liver disease (NAFLD).[20]

In this study we compared the effect of Betulinic acid and Simvastatin on Triglycerides and Low Density Lipoprotein Cholesterol in Balb/C Mice.

Materials and Methods

This experimental randomized control trial was conducted in department of Biochemistry at Islamic International Medical College of Riphah International University in collaboration with National Institute of Health Islamabad in 6 months duration from November 2017 to April 2018. After getting approval from the Ethical Review Committee (ERC) of Islamic International Medical College, and Regulatory Authority of National Institute of Health Islamabad a total of 40 subjects were randomly selected from a population of male mice bred in the animal house of NIH according to international standards for experimental purposes. For this our inclusion criteria was Balb/c adult mice (6-7 weeks old). Only healthy male mice weighing 30±5gms were selected under the guidance of a veterinary doctor and animal house supervisor appointed by NIH. These 40 subjects were randomly divided into 4 groups where every member of the population had the same chance of being in any of the four groups and all four groups were exposed to 12 hour light/dark cycle while being provided with a temperature of 22±5°C, kept and maintained at NIH with the help of NIH approved animal handlers. Subjects were equally divided into 4 groups. Group I was named NC for negative control and was given normal rodent chow throughout the experiment (42 days). Group II named PC (positive control) was provided with High Fat Diet (HFD consisted of 25% fats, 25% sucrose and 50% standardized Rodent chow[20,21] purchased from NIH) throughout the experiment (42 days). Group III named BA (betulinic acid) was provided with HFD for 21 days. Afterwards they were treated with betulinic acid while being fed with standardized rodent chow from day 22 to day 42 i.e the last day of experiment. Group IV named SIM (simvastatin) was also provided with HFD for 21 days. And from day 22 onwards they were treated with simvastatin while being fed with standardized rodent chow from day 22 to day 42 i.e last day of experiment.

On day 21 after overnight fast serum samples of 2 subjects from HFD groups (i.e PC, BA and SIM) were collected on random selection method to ensure established hyperlipidemia. After the confirmation of established hyperlipidemia, two groups i.e BA and SIM were administered with treatment drugs Betulinic Acid and Simvastatin respectively, at the dosage searched from literature (i.e; Betulinic acid: 10mg/kg/day[20] and Simvastatin: 10mg/kg/day[22]). Considering 30gm weight, their doses were 0.3mg per mouse. Drugs obtained were in powder forms and readily soluble in water so they were administered in dissolved form through oral route. During this time HFD was discontinued for the treatment groups BA and SIM.

On the final day after an overnight fast samples were taken after anaesthetizing the mice in a closed lid glass jar containing cotton wool soaked in

chloroform. Sampling was done through cardiac puncture 1.5±0.5ml of blood was collected from each subject and was stored in previously labeled SST (serum separating tube with clot activating gel) and were placed upright in a stand in a cold storage box. Samples were analyzed within 24 hours of collection. After separating serum by centrifugation at 2500rpm for 10 min, samples were analyzed with reagents purchased from Merck. Protocol of technique used for Triglycerides levels was endpoint direct method and for LDL-C levels was enzymatic direct endpoint method. Semiautomated biochemical analyzer Merck 300 was used for the analysis of Triglycerides and LDL-C.

Collected data was analyzed using SPSS (Statistical Package for Social Sciences) software version 21, used for the analysis of data. Normally distributed quantitative variables were expressed as Means±S.E.M. as it was an analysis between 4 groups so One-way ANOVA (analysis of variance) was applied and the differences among group means was observed while a p-value of <0.05 was set to be significant.

Results

Levels of serum triglycerides were compared in the form of Mean±S.E.M. serum triglyceride in positive control group was 220 ±7.727 mg/dL as compared to negative control group 130.7 ±4.883 mg/dL with a p<0.001. While treatment groups BA with 146.2 ±16.03 mg/dL and SIM with 178.8 ±6.959 mg/dL showed significant reduction with p<0.001 and p<0.05 respectively. This is evident in Fig 1.

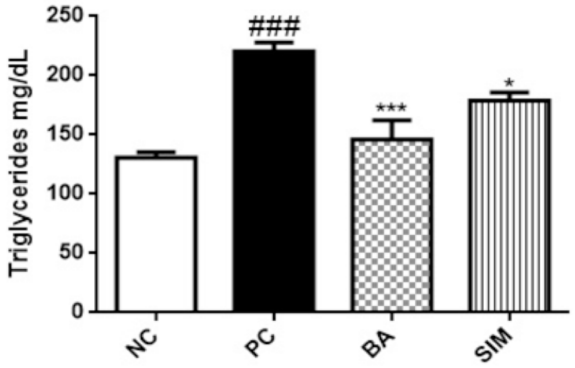

Fig 1: Graphical Presentation of the Results of Serum Triglycerides (Mg/Dl) In All 4 Groups.

Hash (#) denotes comparison between NC and PC, our results showed P<0.001 comparison of NC and PC. Steric (*) denotes comparison of BA and SIM with PC, our results showed *** P<0.001 when compared with PC.

Mean±S.E.M of serum LDL-C levels of positive control group was 46.86±1.844 mg/dL with a p<0.001 as compared to negative control group 23.17±2.088 mg/dL. While treatment group BA had 28.00±3.033 mg/dL with p<0.001 and SIM had 34.83±1.537 mg/dL with p<0.01. The comparative analysis is depicted in Fig 2.

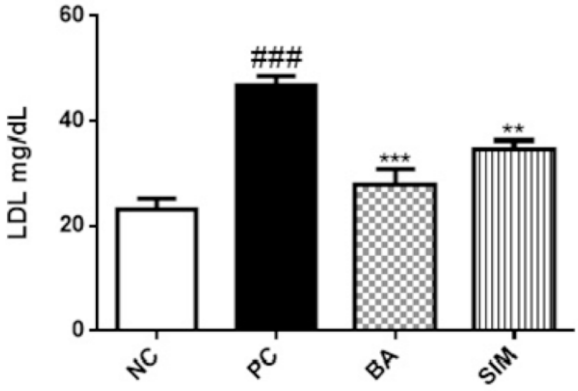

Fig 2: Graphical Presentation of the Results of Serum LDL-C (Mg/Dl) In All 4 Groups.

Hash (#) denotes comparison between NC and PC, our results showed P<0.001 comparison of NC and PC. Steric (*) denotes comparison of BA and SIM with PC, our results showed *** P<0.001 when compared with PC.

Discussion

Dyslipidemia has been responsible for a considerable proportion of Atherosclerotic CVD in the world.[23] For its management a four step approach has been recommended to lower ASCVD which include modification of life style, lowering blood cholesterol levels by the use of drugs (statins). However over the course of decades only approach that has shown overwhelming body of evidence is the drug (statins) therapy approach.[15] Therefore in the guidelines for management of Hyperlipidemia issued by American College of Cardiology / American Heart Association (ACC/AHA), research in this field to the develop better treatment options for management of hyperlipidemia has been recommended.[15] So, we observed effects of betulinic acid on Triglycerides and LDL levels in serum of hyperlipidemic Balb/c mice.

Our study demonstrated that BA treated group kept lipid levels in blood near normal which is in accordance with Quan HY et al. who studied that betulinic acid alleviates non alcoholic fatty liver disease (2013)[20] and Ahangarpour et al. who studied the effect of betulinic acid on leptin, adiponectin, hepatic enzyme levels and lipid profiles in streptozotocin–nicotinamide-induced diabetic mice (2018).[24]

Triglycerides are absorbed from intestines.[25] High levels of Triglyceridess result in increased LDL formation by liver giving rise to ox-LDL and vascular inflammation.[26] Betulinic Acid was determined to effectively lower down Triglycerides.[27] And in this study, at same dosage of 10mg/kg body wt, BA showed a better control of serum Triglycerides ($p<0.001$) as compared to SIM ($p<0.05$) in hyperlipidemic mice as evident in Fig. 1. Additional studies which were conducted by Hai Yan Quan et al[20,27] and Ahangarpour et al.,[24] backed our findings in their animal based researches.

With HFD, LDLc level rise because of excess Triglycerides absorption from intestines and their further accumulation in lipoprotein particle giving rise to LDLc.[25] It was observed that reducing LDLc levels decreased endothelial inflammation.[26] Present study determined that Serum LDL-c levels in BA and SIM were found to be lowered when compared with PC (hyperlipidemic) group and BA showed significantly ($p<0.001$) better results as compared with SIM ($p<0.01$). This is depicted in Fig.2 and is in agreement with the findings of studies on mice by Ahangpour et al. [24]and Juan Peng et al.[28]

Conclusion

It is concluded that, Betulinic Acid and simvastatin both have comparable effects in the rectification of hyperlipidemia in Balb/C mice. Simvastatin efficiently treats hypertriglyceridemia and lowers down LDL-C but betulinic acid shows an overall better control. Hence it may be a good alternative to statins but further researches in this field are needed because Betulinic Acid is a novel compound whose safety profile is yet to be evaluated by the researchers.

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