Comparative Study of Betulinic Acid Versus Simvastatin on Total Cholesterol and HDL in Hyperlipidemic Model

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ABSTRACT

Background & Objectives: Hyperlipidemia is a major risk factor for atherosclerotic cardiovascular disease. Simvastatin (SIM), an HMG Co-A reductase inhibitor, is used for its treatment but many unwanted effects have been reported. To combat the limitations of this standard drug, better treatment options are under discussion in the field of research. One of the novel compounds is betulinic acid a triterpene which has showed a wide range of therapeutic effects.

Methodology: In our study on high fat diet fed hyperlipidemic Balb/c mice we compared this standard drug with a novel compound betulinic acid (BA). Forty mice were randomly divided into 4 groups, Group I was used as NC (Negative Control), group II Positive Control (PC), group III (BA) and group IV SIM respectively. Groups III and IV were given orally 10 mg/kg body wt. BA and SIM respectively. Terminal sampling was performed on day 43 for estimation of total cholesterol and High Density Lipoprotein (HDL).

Results: BA showed significantly better control over total cholesterol and HDL as compared with SIM. Total cholesterol of BA treated group showed $p < 0.001$ like SIM treated group ($p < 0.001$), whereas significant ($p < 0.001$) improvements in HDL levels was seen in BA as compared to group SIM ($p < 0.01$).

Conclusion: Our results showed that Betulinic acid is better than simvastatin because of its significantly positive effect on HDL when compared with SIM.

Keywords: Atherosclerotic cardiovascular disease, betulinic acid, simvastatin, high fat diet, high density lipoproteins, total cholesterol.

INTRODUCTION

The global burden of disease in 2013 revealed that about 30% of all deaths in the world result from cardiovascular diseases. During the years of 2012 and 2013 there were about 2.3 million people who suffered from chronic heart diseases in the UK alone.[1] In Europe Cardiovascular events are the primary cause of death. Despite recent decrease in the mortality rate in many countries, it still accounts for more than 4 million deaths in a year i.e. about half of all deaths in Europe.[2] WHO and the World Bank estimated that in South Asian region during 1990's every 4th death was from CVD. CHD mortality among population of South Asian origin has increased about 1.5 to 4.0 times more than the indigenous populations.[3] Clinical studies conducted during last 20 years showed that healthy improvement in blood levels of lipids can lower down the incidence and mortality of CHD significantly. Prevention and treatment of hyperlipidemia has been of great importance in this regard, and is basically achieved by the use of 'lipid lowering therapy' (LLT). Available data from different researches depicts that lowering the levels of lipids in blood can remarkably reduce the incidence of atherosclerosis which in turn decreases the incidence of CVD.[4] High levels of 'low-density lipoprotein' cholesterol (LDL-C) and low levels of 'high-density lipoprotein' cholesterol (HDL-C) in the blood are two main factors leading to CVD.

Drug therapies that decrease LDL-C levels (for example, statins) have shown to decrease the incidence of CVD.[5] While higher levels of HDL have proven to be preventive of CVD hence named as "good cholesterol". Keeping in view the statistics, in the clinical practice drugs that are used to lower down lipid levels mainly include statins, cholesterol absorption inhibitors, niacin etc. While statins provide the main support for treating hyperlipidemia.[4] This group of drugs (Statins)[6] act to decrease the *de novo* synthesis of cholesterol by blocking the rate-limiting enzyme, HMG-CoA reductase of the *de novo* pathway. They are competitive inhibitors of hydroxymethylglutaryl Co-A reductases.

Statins decrease the levels of cholesterol in the

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situations of decreased food intake of cholesterol. The cholesterol *de novo* synthesis is also responsible for provision of isoprenoids which provide lipid anchorage for many proteins making them able to take part in transduction of signals and metabolism. Thus, apart from lowering lipid levels in blood, statins have many other effects.[7] There are many pleiotropic effects of statins. They increase nitric oxide (NO) formation in the endothelium and thus inhibit vascular inflammation. They are also known for reducing inflammatory biomarkers[7] like CRP in the circulation.[6] Irrespective of the mechanical reason, decreasing the inflammatory process is taken as the second choice (after lowering lipid levels) in inhibiting the advancement of atherosclerotic phenomenon. So, 'anti-inflammatory compounds' which do not have lipid lowering activity are currently being developed to treat atherosclerosis.[6] Though statins demonstrate agreeable result in treating hyperlipidemia, only 40% – 60% of the patients using statins adhere to it appropriately.[8] Although statins usually show good tolerance, but they are accompanied with many side effects such as digestive system disturbances, myalgia, respiratory problems, and headaches. Recently statins are also being linked with raised levels of glucose and glycosylated hemoglobin (A1C) in blood.[9] Moreover a rise in liver enzymes specifically transaminases have also been observed rather infrequently, so it is advised not to recommend statins in acute liver diseases, as well as in cases of chronic ailments with unexplained elevation of liver transaminases.

Recently researchers in food sciences are focusing on developing more treatment options for diseases like hyperlipidemia by identifying active ingredients of food extracts that inhibit lipid accumulation in the body.[4]

Triterpenoid is a group of chemicals containing many compounds, which are recently attracting attention from researchers in the medical field all over the world due to their favorable bioactivity against different disease. Betulinic acid (3-hydroxy-lup-20 (29)-en-28-oic acid)[10] structurally speaking is a pentacyclic lupine type compound belonging to a class named triterpenoid which is distributed broadly in the plant kingdom. Betulin is the precursor of betulinic acid shown in figure 1.

The considerable interest in BA is due to a variety of activities including biological and pharmacological effects like the anti-inflammatory,[11,12] anti-bacterial, anti-viral, anti-malarial, anti-HIV, and anti-tumor effects.[13] It is believed that BA has a prospective potential in anticancer therapeutic regimens because of its cytotoxic property specifically upon tumor cells.[14] Other reported biological and pharmacological activities of BA include hepatoprotective effect, anti-AIDS and anti-depression effects.[15,16] As hyperlipidemia leads to cardiovascular disease by inducing inflammatory mechanism, Betulinic Acid may have a potential therapeutic role because of its proved anti-inflammatory effect. So, the role of BA in hyperlipidemia is recently under discussion.[15]

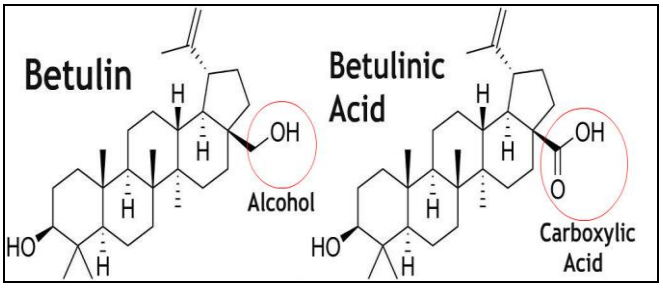

Fig. 1: *Structure of Betulinic Acid and its precursor.* Adopted from: *https://wildalaskachaga.com/benefits/sterols/betulin-and-betulinic-acid/*

MATERIAL AND METHOD

This was an experimental randomized control trial of 6 months duration (Nov 2017 – Apr 2018) conducted at Biochemistry Department of Islamic International Medical College, Riphah International University in collaboration with National Institute of Health Islamabad. In this study we included 40 healthy adults (6 – 7 weeks old) male Balb/c mice weighing 30 ± 5 gms and randomly divided them into 4 groups and keeping in 12 hour light/dark cycle with a temperature of 22 ± 5°C. These standard conditions were provided at National Institute of Health Islamabad with the help of NIH approved animal handlers.

Group I was named as NC for negative control and throughout the trial of 42 days it was provided with normal standardized rodent chow (approved and purchased from National Institute of Health Islamabad). Group II labeled PC (positive control) was given high fat diet (HFD)[17,18] all along the experiment. HFD, constituted of 25% fats, 25% sucrose and 50% standardized Rodent chow,[19,20] was prepared at NIH.

Group III named as BA (betulinic acid) was given HFD only for 21 days to generate the HFD model and then treatment with betulinic acid was started while their feed consisted of standardized rodent chow from day 22 to day 42 i.e. the last day of experiment. Group IV was given the name as SIM (after simvastatin). This was also given HFD for 21 days like group III (BA). And from day 22 onwards their treatment of 21 days with simvastatin was started while they were fed with standardized rodent chow from day 22 to day 42 i.e. last day of experiment.

On day 21 when HFD was discontinued a serum sample of two randomly selected subjects from HFD fed groups were taken after an overnight fast. Through this we ensured that hyperlipidemia has been established. After that the two drugs at the same dosage were

administered to both of the treatment groups i.e. BA and SIM. Calculated dosage for 30 gm weight subject was 0.3mg per mouse considering Simvastatin: 10 mg/kg/day[21] Betulinic acid: 10 mg/kg/day[17] which was given in 1 ml of distal water through oral route.

On the last day of the experiment, fasting serum samples were taken after keeping all standardized aseptic measures into account and anaesthetizing the subjects. Samples were collected through cardiac puncture and 1.5 ± 0.5 ml of blood was taken from each subject and carefully stored in SST (serum separating tube) which were labeled and placed upright in a stand in a cold storage box. After separating serum by centrifugation at 2500 rpm for 10 min, samples were tested for total cholesterol and HDL levels using reagents purchased from Merck by semi-automated biochemical analyzer Merck 300.

SPSS (Statistical Package for Social Sciences software) version 21 was used to analyze the data collected from results of total cholesterol and HDL levels of the subjects' samples. Data analysis showed that it was normally distributed and as the quantitative variables were expressed using Means ± S.E.M. Analysis of Variance (one-way ANOVA) was applied to compare 4 groups and p-value of < 0.05 was determined as significant.

RESULTS

Significant rise ($p < 0.001$) in serum TC was seen in positive control group with a Mean ± SEM of 229.4 ± 12.01 units as compared to negative control where Mean ± SEM was 127.5 ± 5.457 units. BA with Mean ± SEM 150.7 ± 6.975 and SIM 7.2 ± 4.895 showed significant reduction ($p < 0.001$) in serum TC respectively. For comparative review these results are shown in table 1 and they are graphically represented in figure 2.

Table 1: *Results of total Cholesterol levels in Serum.*

Group	*Mean ± SEM of TC*	*Value of Significance*
NC	127.5 ± 5.457	
PC	229.4±12.01	p < 0.001
SIM	127.2 ± 4.895	
BA	150.7 ± 6.975	p < 0.001

Table 2: *Results of HDL levels in Serum.*

Group	*Mean ± SEM of HDL*	*Value of significance*
NC	33.00 ± 3.235	
PC	22.17 ± 0.9458	p < 0.01
SIM	31.00 ± 1.483	
BA	48.67 ± 1.116	p < a0.001

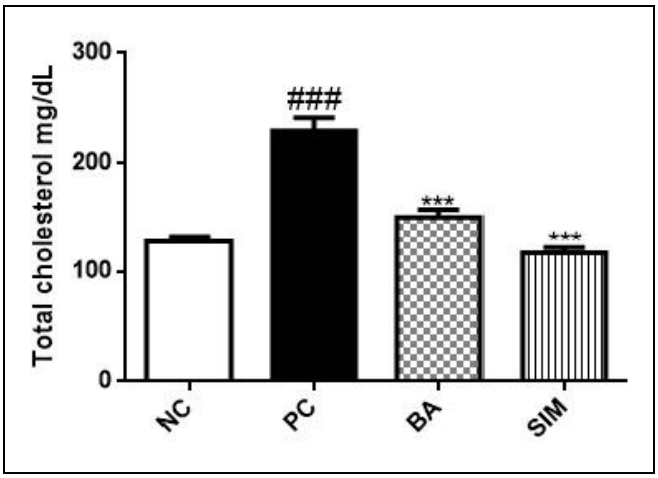

Fig. 2: *Graphical presentation of results of serum TC (mg/dL) in all four groups.*
*# denotes comparison between NC and PC, results showed ### p < 0.001 comparison of NC and PC. *denotes comparison of BA and SIM with PC, our results showed *** p < 0.001 when compared with PC.*

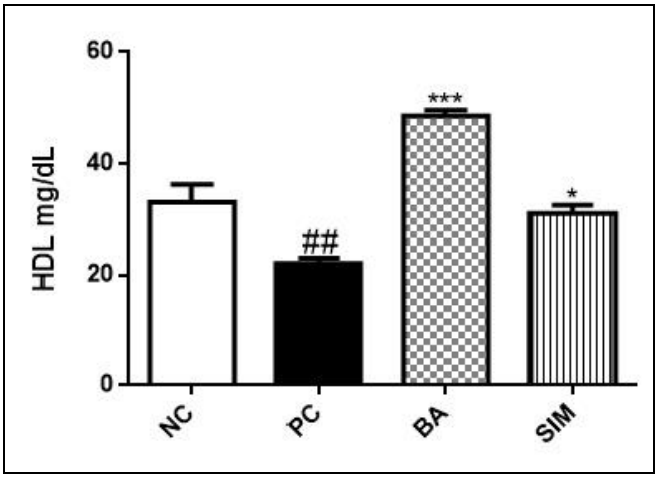

Fig. 3: *Graphical presentation of the results of serum HDL (mg/dL) in all 4 groups.*
*# denotes comparison between NC and PC, our results showed *** p < 0.001 comparison of NC and PC. * denotes comparison of BA and SIM with PC, our results showed ***p < 0.001 when compared with PC.*

Significantly lower values of Mean ± SEM of serum HDL 22.17 ± 0.9458 ($p < 0.01$) was seen in positive control group when matched with negative control 33.00 ± 3.235. Mean ± SEM of BA 48.67 ± 1.116 and SIM 31.00 ± 1.483 showed significant rise ($p < 0.001$) and ($p < 0.05$) in serum HDL individually.

DISCUSSION

Dyslipidemia has been accountable for an estimated 4.4 million deaths every year. Although it is respon-

sible for a considerable portion of atherosclerotic cardiovascular disease, it is a manageable problem.[22] For the management of atherosclerosis CVD a four step approach including life style modification, use of drugs to lower down blood cholesterol levels (for example statins) and excess body weight[23] Despite the promising results, shown by statins in lowering blood cholesterol multiple reports recently have shown that they are associated with many side effects.[24,9,25] Therefore as per the guidelines issued by ACC/AHA further research to evaluate better treatment options for management of hyperlipidemia is needed.[23]

So keeping these recommendations in view in our study we focused on a noble compound, a triterpenoid named betulinic acid and observed its effect on Balb/c mice model of hyperlipidemia.

In our study HFD model we gave a trial of BA in comparison with simvastatin, it was observed that TC levels in serum were found to be lowered in hyper-lipidemic mice treated with BA at a dose of 10 mg/kg/ day as compared with SIM (as shown in the Fig. 2). This is abridged by the claims of Ahangpour *et al.*[31] and Peng *et al.*[32]

Conversely a decrease in the levels of highly dense lipoproteins is observed in hyperlipidemic state.[30] Current study showed BA raised serum HDL levels in a remarkably significant manner ($p < 0.001$) as compared with simvastatin ($p<0.05$) when compared with PC group. This is depicted in Fig. 3 and is in conformity with the discoveries of Ahangpour *et a*[31] and Peng *et al.*[32]

It is concluded that, BA efficiently decreased total cholesterol levels like simvastatin but it has improved the HDL levels more efficiently than simvastatin. Considering the cardio-protective effect of HDL BA will be a better choice of treatment than simvastatin. More research to evaluate this nobel compound's side effect are recommended.

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Author's Contribution

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Conflict of Interest

All authors declare no conflict of interest.

REFERENCES

1. Bhatnagar P, Wickramasinghe K, Williams J, Rayner M, Townsend N. The epidemiology of cardiovascular disease in the UK, 2014. Heart, 2015;
2. Nichols M, Townsend N, Scarborough P, Rayner M. Cardiovascular disease in Europe 2014: epidemiological update. Eur Heart J. 2014; 35: 2950–9.
3. Yusuf S, Reddy S, Ounpuu S, Anand S. Global Burden of Cardiovascular Diseases: Part II: Variations in Cardiovascular Disease by Specific Ethnic Groups and Geographic Regions and Prevention Strategies. Circulation, 2001;
4. Song D-X, Jiang J-G. Hypolipidemic Components from Medicine Food Homology Species Used in China: Pharmacological and Health Effects. Arch Med Res. 2018; 1.
5. Zhang M, Lei D, Peng B, Yang M, Zhang L, Charles MA, et al. BBA-Molecular and Cell Biology of Lipids Assessing the mechanisms of cholesteryl ester transfer protein inhibitors. Biochim Biophys Acta - Mol Cell Biol Lipids. 2017; 1862 (12).
6. Babelova A, Sedding DG, Brandes RP. Anti-atherosclerotic mechanisms of statin therapy. Curr Opin Pharmacol [Internet] 2013; 13 (2): 260–4.
7. Kido T, Kondo K, Kurata H, Fujiwara Y, Urata T, Itakura H, et al. ApoA-I/A-II-HDL positively associates with apoB-lipoproteins as a potential atherogenic indicator. Lipids Health Dis. 2017; 16 (1).
8. Javad MT, Mehrpooya M, Larki-Harchegani A, Ahmadi-moghaddam D, Kalvandi M, Mohammadi Y, et al. Evaluation of the Effect of Education Provided by Pharmacists on Hyperlipidemic Patient's Adherence to Medications and Blood Level of Lipids Article Info Abstract. J Appl Pharm Sci. 2018; 8 (01): 29–33.
9. Park Y, Rha S-W, Choi BG, Choi SY, Goud A, Lee S, et al. Impact of Simvastatin on Development of New-Onset Diabetes Mellitus in Asian Population: Three-Year Clinical Follow Up Results. J Am Coll Cardiol., 2014; 63 (12): A2128.
10. Cichewicz RH, Kouzi SA. Chemistry, Biological Activity, and Chemotherapeutic Potential of Betulinic Acid for the Prevention and Treatment of Cancer and HIV Infection. Med Res Rev. 2004; 24 (1): 90–114.
11. Nader MA, Baraka HN. Effect of betulinic acid on neutrophil recruitment and inflammatory mediator expression in lipopolysaccharide-induced lung inflammation in rats. Eur J Pharm Sci. 2012;
12. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes, 2008; 57 (6): 1470–81.
13. Lee SY, Kim HH, Park SU, Science C. Recent Studies on Betulinic Acid and its Biological and Pharmacological Activity Regional Innovation Center for Dental Science & Engineering , Chosun University, Dear Editor, was a prominent outer-bark constituent in white-barked birch trees (Bag and D. 2015; (Table 1): 199–203.
14. Wu J, Niu Y, Bakur A, Li H, Chen Q. Cell-free production of pentacyclic triterpenoid compound betulinic acid

from betulin by the engineered Saccharomyces cerevisiae. Molecules, 2017; 22 (7).
15. Quan HY, Kim DY, Kim SJ, Jo HK, Kim GW, Chung SH. Betulinic acid alleviates non-alcoholic fatty liver by inhibiting SREBP1 activity via the AMPK-mTOR-SREBP signaling pathway. Biochem Pharmacol. 2013; 85 (9): 1330–40.
16. Lee SY, Kim HH, Un S, 3 P. Letter to the editor: Recent Studies on Betulinic Acid and its Biological and Pharmacological Activity. Excli J [Internet], 2015; 14: 199–203.
17. Quan HY, Kim DY, Kim SJ, Jo HK, Kim GW, Chung SH. Betulinic acid alleviates non-alcoholic fatty liver by inhibiting SREBP1 activity via the AMPK-mTOR-SREBP signaling pathway. Biochem Pharmacol., 2013; 85 (9): 1330–40.
18. Del Pozo R, Mardones L, Villagrán M, Muñoz K, Roa S, Rozas F, et al. Efecto de una dieta alta en grasas en el proceso de formación de cálculos biliares de colesterol. Rev Med Chil. 2017; 145 (9): 1099–105.
19. Kirn-E-Muneera K, Majeed A, Naveed AK. Comparative evaluation of Nigella sativa (Kalonji) and simvastatin for the treatment of hyperlipidemia and in the induction of hepatotoxicity. Pak J Pharm Sci. 2015; 28 (2): 493–8.
20. Stanhope KL, Medici V, Bremer AA, Lee V, Lam HD, Nunez M V., et al. A dose-response study of consuming high-fructose corn syrup-sweetened beverages on lipid/lipoprotein risk factors for cardiovascular disease in young adults. Am J Clin Nutr. 2015; 101 (6): 1144–54.
21. Song X, Wang J, Wang P, Tian N, Yang M, Kong L. 1H NMR-based metabolomics approach to evaluate the effect of Xue-Fu-Zhu-Yu decoction on hyperlipidemia rats induced by high-fat diet. J Pharm Biomed Anal. 2013; 78–79: 202–10.
22. Venkitachalam L, Wang K, Porath A, Corbalan R, Hirsch AT, Cohen DJ, et al. Global variation in the prevalence of elevated cholesterol in outpatients with established vascular disease or 3 cardiovascular risk factors according to national indices of economic development and health system performance. Circulation, 2012; 125 (15): 1858–69.
23. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: A report of the American college of cardiology/American heart association task force on practice guidelines. J. Am. Coll. Cardiol.2014; 63 (25 PART B): 2889–934.
24. Schachter M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: An update. Fundam Clin Pharmacol. 2005; 19 (1): 117–25.
25. Aghasadeghi K, Nabavi S, Amirmoezi F, Attar A. Vitamin E supplementation for treatment of statin induced hepatocellular damage: A randomized, double-blind, placebo-controlled trial. Int Cardiovasc Res J. 2018; 12 (1): 29–33.
26. Adiels M, Olofsson SO, Taskinen MR, Borén J. Overproduction of very low-density lipoproteins is the hallmark of the dyslipidemia in the metabolic syndrome. Arterioscler Thromb Vasc Biol. 2008; 28 (7): 1225–36.
27. Altadill T, Dowdy TM, Gill K, Reques A, Menon SS, Moiola CP, et al. Metabolomic and Lipidomic Profiling Identifies the Role of the RNA Editing Pathway in Endometrial Carcinogenesis. Sci Rep. 2017; 7 (1).
28. Palomeras Soler E, Casado Ruiz V. Epidemiology and Risk Factors of Cerebral Ischemia and Ischemic Heart Diseases: Similarities and Differences. Curr Cardiol Rev. 2010; 6 (3): 138–49.
29. Jacobson TA, Ito MK, Maki KC, Orringer CE, Bays HE, Jones PH, et al. National Lipid Association Recommendations for Patient-Centered Management of Dyslipidemia: Part 1—Full Report. J Clin Lipidol. 2015; 9 (2).
30. Taskinen MR, Borén J. New insights into the pathophysiology of dyslipidemia in type 2 diabetes. Atherosclerosis, 2015; 239 (2): 483–95.
31. Ahangarpour A, Shabani R, Farbood Y. The effect of betulinic acid on leptin, adiponectin, hepatic enzyme levels and lipid profiles in streptozotocin-nicotinamide-induced diabetic mice. Res Pharm Sci. 2018; 13 (2): 142–8.
32. Peng J, Lv YC, He PP, Tang YY, Xie W, Liu XY, et al. Betulinic acid downregulates expression of oxidative stress-induced lipoprotein lipase via the PKC/ERK/c-Fos pathway in RAW264.7 macrophages. Biochimie [Internet], 2015; 119: 192–203.