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Original Article

Effect of Betulinic Acid on Lipid Homeostasis and Atherosclerotic Index

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ABSTRACT

Introduction: Dysregulation in lipid metabolism and inflammatory response triggering lipid oxidation results in hyperlipidemia and induces the progression of atherosclerotic cardiovascular disease. Betulinic acid possesses lipid lowering and anti-inflammatory properties, therefore it may play an important role in athero-sclerosis prevention and Lipid homeostasis.

Material &Methods: An experimental study was conducted at the campus of Northwest school of medicine, Hayatabad for a period of two months from 1st Dec 2021 – 3rd Feb 2022. A sample of 30 male BALB/c mice was divided into three groups. Group I was the negative control that received normal food and water for a period of 42 days. Group II was positive control given a diet high in fat content. Group III was also given diet high in fat content and betulinic acid in a dose of 10mg/kg.

Results: The serum TG level (mg/kg) of group II was 202.3 ± 28, VLDL (mg/kg) was 40.2 ± 5.7, TC (mg/kg) was 205.6 ± 51.8, LD (mg/kg) was 40.5 ± 5.9 and HDL (mg/kg) was 29.4 ± 5.6. AI was calculated to be 0.47 ± 0.09. Betulinic acid group showed a TG level of 120.7 ± 29.3, VLDL of 34.1 ± 9.3, TC of 152.7 ± 40.9, LDL of 33.8 ± 8.7 and HDL of 49.3 ± 3.4. AI for group III was 0.02 ± 0.07. Between group II & III, Paired sample t test showed a significant difference of 0.00 in TG levels, 0.04 for VLDL level, 0.4 for total cholesterol, 0.03 for LDL and 0.00 for HDL. The atherogenic index also showed a statistically significant P value of <0.05 **Conclusion:** Our findings suggest a significant protective effect of BA in cardiovascular and atherosclerotic diseases. It not only reduces LDL, VLDL, TG and cholesterol levels, reducing the atherogenic index, but also raised the serum HDL levels.

Key words; Atherogenic index, Betulinic acid, lipid homeostasis

Introduction

Homeostasis of lipids has been observed to play key role in regulation of proper functioning as well as life span of the cells.[1] Multiple systems including a network of lipid sensors adiponectin and apolipoprteins are in place to maintain this homeostasis which actually regulate lipid metabolism and in turn control the production of various metabolic products essential for optimum cell functioning.[1,2,3,4] Any dysregulation in lipid metabolism results in an imbalance of the biomarkers present in blood and is termed as dyslipidemia or in certain conditions as hyperlipidemia which is the basis of many diseases like Obesity, metabolic syndrome, hypertension as well as atherosclerotic cardiovascular disease .[3,5,6] Homeostatic dysregulation resulting in hyperlipidemia doubles the risk of CVD as compared to other causes.[7]

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It was observed in 2017 that around 17.9 million people died of CVD worldwide.[8,9] while recently in 2018 it has begun to rise in women aged 35-40 with high body mass index in the United States as CVD accounts for 20% of total disease burden in women and 24% of total disease burden in men.[10] Previously CVD was believed to be the leading cause of death in developed countries.[11] Now Over last 10 years global mortality burden has increased by 12.5% and roots to this increase has been in Asia and South East Asia.[12]

The atherogenic lipoproteins has been a very helpful tool in identifying the dyslipidemia.[13]It is strongly associated with Atherosclerotic cardiovascular disease (ASCVD) and has been labeled as a modifiable factor.[14] Evidence shows that the ratio of Triglycerides with high density lipoproteins (HDL-C) is useful in prediction of atherogenesis or ASCVD.[15,16] According to latest ACC/AHA guidelines to cut down the global mortality burden caused by ASCVD due to hyperlipidemia, lifestyle modification stands as the foundation among the choices of treatment.[17,18] In systematic evidence based reviews, it has been

established that use of 3-hydroxy 3-methyle glutaryl co-A reductase inhibitor (statins) as secondary prevention has shown marked reduction in ASCVD.[17] Atherogenic index is a quantifiable biomarker for the assessment of the cardio-vascular disease risk. The level of triglycerides and HDL make up the atherogenic index and the value of -0.3 to 0.1 indicate low risk, 0.1 to 0.24 indicates medium risk and an index of above 0.24 is indicative of the high risk of cardio-vascular disease.

Betulinic acid belonging to Betula species is known for its anti-viral, cytotoxic and anti-metastatic properties[18].Studies revealed the beneficial effects of this plant derivative in maintaining lipid profiles within the normal limits. It not only has lipolytic potential but also reduces the lipo-genesis as well as accumulation of the lipids in the experimental models[19,20]. The current study is aimed at investigating the effects of betulinic acid on the lipid homeostasis as well as the atherogenic index.

Material & Methods

An experimental study was conducted at the campus of Northwest school of medicine, Hayatabad for a period of two months from 1st Dec 2021 – 3rd Feb 2022. A sample of 30 male BALB/c mice of ages between 6 to 7 weeks and average weight of 30g were chosen and randomly allocated to three groups. Group I (negative control group) was containing 10 mice. They were given normal food and water for a period of 42 days. Group II (positive control) with 10 mice. They were given a diet high in fat content that contained 25% of fats and 25% of sucrose, mixed in the normal rodent diet for a period of 42 days. Group III was also given diet high in fat content for a period of 42 days. From the 22nd day the mice in group III were started on betulinic acid solution prepared in water. It was orally administered in a dose of 10mg/kg per day along with the high fat diet from day 22 - day 42.The mice were put on a fast overnight before the final day (day 42). Mice were sacrificed via a cardiac puncture after being anesthetized. Blood samples were collected and levels of triglycerides, total cholesterol, serum LDL and serum HDL were determined. Results were inserted in SPSS, values were represented as means ± SD, an independent T test was applied with p-value of <0.05 significant.

Results

Table 1 showed our results of negative control group(Group 1) that received normal food and water showing a mean serum triglyceride (TG) value of 136.4 ± 22.5 (mg/dl), VLDL of 37.7 ± 8 (mg/dl), total cholesterol (TC) of 186.5 ± 40.6 mg/dl, LDL of 38.4 ± 10 mg/dl and HDL of 39.7 ± 3.5 mg/dl. The atherogenic index (AI) was 0.15 ± 0.06 mg/dl. Group II was administered high fat diet and was taken as the positive control (PC) group. The mean serum TG level of group II was 202.3 ± 28, VLDL was 40.2 ± 5.7, TC was 205.6 ± 51.8, LDL was 40.5 ± 5.9 and HDL was 29.4 ± 5.6. AI was calculated to be 0.47 ± 0.09. Betulinic acid was administered to group III in addition to the high fat diet. The lipid profile in this group showed a mean TG level of 120.7 ± 29.3, VLDL of 34.1 ± 9.3, TC of 152.7 ± 40.9, LDL of 33.8 ± 8.7 and HDL of 49.3 ± 3.4. AI for group III was 0.02 ± 0.07 (Table 1).

Table 1 – Lipid Profile & Atherogenic Index of Group I (Negative Control/NC),
Group II (Positive Control/PC) and Group III (Betulinic Acid/BA)

Group I (Negative Control)	TG mg/dl	VLDL mg/dl	TC mg/dl	LDL mg/dl	HDL mg/dl	AI
1	149	29.8	143	30	38	0.23
2	180	45.4	146	45	40	0.20
3	130	46	227	46	35	0.20
4	135	47	238	47	37	0.20
5	123	24.6	244	24	46	0.06
6	155	32	183	30	41	0.21
7	127	41.5	176	45	45	0.09
8	150	36	210	46	39	0.22
9	110	44	138	47	40	0.07
10	105	31	160	24	36	0.10

Group II (Positive Control)	TG mg/dl	VLDL mg/dl	TC mg/dl	LDL mg/dl	HDL mg/dl	AI
1	229	45.8	256	47	25	25
2	161	32.2	235	33	31	31
3	231	46.2	241	47	22	22
4	191	38.2	238	38	35	35
5	167	33.4	230	33	22	22
6	233	46.6	132	47	39	39
7	236	47.2	265	47	33	33
8	184	36.8	150	37	31	31
9	189	35.8	172	36	26	26
10	202	40.4	137	40	30	30

Group II (Positive Control)	TG mg/dl	VLDL mg/dl	TC mg/dl	LDL mg/dl	HDL mg/dl	AI
1	229	45.8	256	47	25	25
2	161	32.2	235	33	31	31
3	231	46.2	241	47	22	22
4	191	38.2	238	38	35	35
5	167	33.4	230	33	22	22
6	233	46.6	132	47	39	39
7	236	47.2	265	47	33	33
8	184	36.8	150	37	31	31
9	189	35.8	172	36	26	26
10	202	40.4	137	40	30	30

Comparative analysis of the lipid profiles showed an increased TG, TC, VLDL and LDL in the positive control group these values were normal in the negative control and BA group. In addition HDL levels were highest for the BA group as compared to the other two groups (Fig 1).

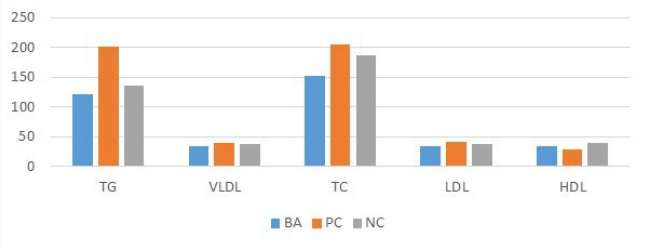

Fig 1 – Comparison of serum Lipid Profiles of Group I (NC), II (PC) & III (BA)

The atherogenic index moved toward moderate to high risk in the positive control group. In the negative control group it was in the no to medium risk category. However, betulinic acid not only lowered the TG values but also raised the HDL values, hence bringing the atherogenic index in the no risk category (Fig 2).

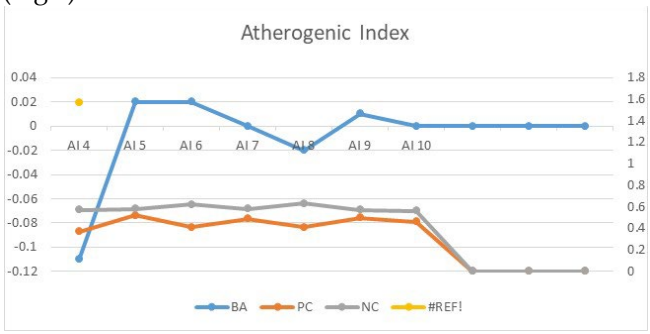

Fig 2 – Comparison of the atherogenic indices of Group I (NC), II (PC) & III (BA), showing a decreased index on administration of the betulinic acid

Paired sample t test was applied to evaluate the significance of the lipid profile parameters and atherogenic index in Group II and III. The t test showed a significant difference of 0.00 in TG levels, 0.04 for VLDL level, 0.4 for total cholesterol, 0.03 for LDL and 0.00 for HDL. The atherogenic index also showed a statistically significant P value of 0.00. (Table-2)

Table 2 – Paired Sample t test of Group II and III

		95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
		Lower	Upper			
Pair 1	Triglycerides-2 - Triglycerides-3	56.43859	106.76141	7.336	9	.000
Pair 2	VLDL-2 - VLDL-3	.09288	12.18712	2.297	9	.047
Pair 3	Total Cholestrol-2 - Total Cholestrol-3	-103.46364	-2.33636	-2.367	9	.042
Pair 4	LDL-2 - LDL-3	.70493	12.69507	2.528	9	.032
Pair 5	HDL-2 - HDL-3	-24.00872	-15.79128	-10.956	9	.000
Pair 6	Atherogenic index-2 - Atherogenic index-3	25.37943	33.38857	16.599	9	.000

Discussion

Atherosclerosis follows an inflammatory response that is triggered by the oxidation of the lipids in the vessels especially LDL.[21] This oxidative modification of the lipids follows a cascade of reactions starting from the suppression of mRNA via activated AMPK and leading to the activation of PPAR-Alpha. PPAR-Alpha has a vital role in lipid homeostasis and is involved in the cellular uptake of the lipids, lipid activation as well as oxidation which forms atherosclerotic plague.[22] Oxidized form of LDL is hence one of the major atherosclerosis inducing factors. Inflammatory response is not only induced by the PPAR activation but also involves the activation of the toll like receptor type 2 (TLR2). The oxidized LDL is internalized first under the influence of CD36 and then binds of the TLR2. It is activated and triggers an inflammatory

response through the enhanced release of the NFkB and cytokines, producing atherosclerosis progression.[23, 24]

Currently available lipid lowering therapeutics, do offer beneficial effects, however, the adverse effects of these agents are a major limiting factor to their use. Statins are also known to increase the serum glucose levels, raises the concern about their use in diabetic patients at a risk of cardiovascular disease. Considering that inflammation forms the basis of lipid oxidation and atherosclerosis progression, therapeutic agents targeting the inflammatory cascade and LDL oxidation may prove to be of therapeutic benefit. Betulinic acid (BA) is structurally a tri-terpenoid possessing anti-inflammatory properties. It not only regulates the activation of a number of receptors involved in the inflammatory cascade but also takes part in the homeostasis of the serum lipids.

Animals receiving BA showed a reduction in the levels of total cholesterol, LDL, VLDL and triglycerides. Our results are in accordance to the studies identifying a reduction in the accumulation of abdominal fat following BA administration with a reduction in the TG and cholesterol levels.[25] A part of these homeostatic effects of BA has been attributed to the inhibition of the pancreatic lipase enzyme and decreased reabsorption of lipid in the small intestine. In this study, an increase in the concentration of total cholesterol, triglycerides, LDL pointed towards the development of atherosclerotic changes during supplementation of high fat diet mice that was remarkably reversed in the animals being administered BA.[25]

Moreover, BA raised the levels of HDL in the experimental animals in our study. These results are comparable to the increase in the HDL levels significantly, even higher as compared to simvastatin for which the levels of rise in HDL were insignificant statistically. Hence it was proposed that in comparison to simvastatin, BA had a better efficacy as a lipid lowering and atherosclerosis protective agent as it not only lowers TG, LDL, VLDL and cholesterol but also raises the HDL levels adding further protective benefit.[25]

Conclusion

Our findings suggest a significant protective effect of BA in cardiovascular and atherosclerotic diseases. It not only reduces LDL, VLDL, TG and cholesterol levels, reducing the atherogenic index, but also raised the serum HDL levels.

Recommendations

Further studies need to be conducted to explore the mechanisms involved in the anti-atherogenic properties of BA. The efficacy should also be compared with other agents currently being utilized as lipid lowering agents.

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Conflict of Interest: Authors declare no conflict of interest

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B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion