ORIGINAL ARTICLE

Comparative Study of Aescin and Atorvastatin on Lipid Profile of Albino Wistar Rats

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ABSTRACT

Objective: To observe the effects of Aescin and Atorvastatin on the lipid profile of Albino Wistar rats.

Study Design: Quasi-experimental study.

Place and Duration of Study: Postgraduate research laboratory at ISRA University, Hyderabad from 6th June 2018 to 7th October 2018.

Materials and Methods: Fifty albino Wistar rats were divided into five groups: Group A (Control), Group B (High-fat diet), Group C (Aescin + high-fat diet), Group D (Atorvastatin + high-fat diet), Group E (Aescin + Atorvastatin + high-fat diet). Pre and post-experimental body weight and biochemical analysis was done through ANOVA on SPSS version 22. The significance level was $p \leq 0.05$.

Results: Marked reduction in serum Total Cholesterol (71.36 ±10.1), Triglycerides (83 ±25.66), and Low-density lipoprotein-cholesterol (32 ± 3.76) while elevation in levels of High-density lipoprotein-cholesterol (45 ± 11.85) was observed in Group E as compared to Group B. Statistically significant difference in mean post-experimental body weight body was also observed between all study groups ($p \leq 0.05$).

Conclusion: Combination therapy of Aescin and Atorvastatin has significant protective effects on lipid profile when compared with individual therapy of either drug.

Key Words: *Aescin, Atorvastatin, Cholesterol, Hyperlipidemia, Triglycerides.*

Introduction

Cardiovascular diseases (CVDs) are the leading cause of death worldwide, killing more people than any other disease annually.[1] In 2016, around 18 million people were reported to have died from CVDs, representing 31% of all deaths around the globe.[1] Ecological ethnographic studies have reported that South Asian people are comparatively at a higher risk of CVDs than other ethnicities.[2] Alarmingly, CVDs are responsible for more than 25% of deaths in this part of the world.[2] The estimates also show that one in every fifth middle-aged adult in Pakistan may be suffering from subclinical CVDs.[3] This rising toll of CVDs globally is related to the gross incidence of atherosclerotic diseases owing to a sedentary lifestyle and co-morbidities like; Diabetes, Hypertension, dyslipidemia, obesity, etc.

Dyslipidemia is a disorder of lipoprotein metabolism including lipoprotein overproduction or deficiency. It can be aggravated by increased level of Low-Density Lipoprotein-Cholesterol (LDL-C) and triglycerides (TAGs)) or a decrease in High-Density Lipoprotein-Cholesterol (HDL-C) in the blood.[4] The most common form of dyslipidemia is hyperlipidemia. Hyperlipidemias can be classified by specific genetic abnormalities, also termed as familial and alteration in plasma lipoprotein metabolism, which is acquired.[5]

Circulating LDL-C in the blood can invade the artery wall and lead to the development of fatty plaques in a process called atherosclerosis, which is also accompanied by primary endothelial injury.[6]

It has been observed that even 1% decrease in the concentration of plasma lipid levels by lipid-lowering therapies results in a 2% reduction in the prevalence of CVDs[7,8]

There are different classes of drugs that are used to treat hyperlipidemia, which include niacin, fibrates, and cholesterol binding drugs ezetimibe, omega 3 fatty acids and dietary supplements.[9]

Among these, statins are usually the first line lipid-lowering therapy, which primarily targets plasma LDL-C.[10] According to a study, patients who do not

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respond to statin treatment remain at a higher risk of developing CVDs.[11]

Atorvastatin is one of the most efficacious statins having major LDL-C lowering properties. It reduces the production of cholesterol through inhibiting 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA) in the liver.[12]

Similarly, another lipid-lowering agent, Aescin is an important ingredient taken out of Aesculus hippocastanum tree. It is very popular for being anti-inflammatory, anti-edematous and anti-oxidative. It also inhibits the pancreatic lipase in the gastrointestinal tract, preventing the absorption of lipids and increasing the excretion of fat content in feces thus decreasing the total cholesterol, very-low-density lipoprotein cholesterol (VLDL-C), LDL-C and TAGs and an increase in HDL-C levels in serum.[13]

After extensive literature review, it was found no study has been conducted in Pakistan that has demonstrated the comparative effects of aescin and atorvastatin on lipid profile of albino Wistar rats. The current study, therefore, was designed to highlight the potential protective effects of aescin and atorvastatin both individually and in combined form. This will not only provide the baseline for future human studies but also help in designing possible efficacious add-on therapies.

The objective of the current study was to observe the effects of Aescin and Atorvastatin on body weight and lipid profile of male Albino Wistar rats as well as to compare the difference of individual versus combination therapy in reduction of hyperlipidemia.

Materials and Methods

This quasi-experimental study was conducted at the Postgraduate center of ISRA University, Hyderabad from 6th June to 7th October 2018. Fifty healthy male albino Wistar rats of weight range of 175-300g were included using non-probability purposive sampling. All rats of female gender and with any sickness were excluded from the study. The study was approved by the Ethical Review Committee of ISRA University, Hyderabad. The rats were kept in a proper hygienic and well-ventilated environment. Room temperature of 25 ±2°C and day and night cycle per 12 hours was maintained. After an acclimatization period of ten days, all rats were equally divided into five different groups. Group A (Control) received standard chow diet and water ad libitum, Group B received a high-fat diet of 400mg/kg, Group C received Aescin 75 mg along with high-fat diet, Group D received Atorvastatin 80 mg along with high fat and Group E received Aescin 50mg + Atorvastatin 40mg along with high-fat diet.[14,15] Aescin was administered in the form of horse chestnut as its extract contains 70% Aescin.[15] Pre and post-experimental body weights of all experimental animals were recorded. All the rats were euthanized by placing them under the inverted glass jar with chloroform soaked cotton swabs. The rats were sacrificed by cervical dislocation. Blood samples were collected by cardiac puncture through a syringe and then transferred to gel-tubes which were kept in a vertical position and then tubes were centrifuged at 5000 rpm for 5 min to separate serum which was used for biochemical analysis. The estimation of random lipid profile (Total cholesterol, LDL-C, TAGs, and HDL-C) was carried out by Roche diagnostic kit method on an automatic modular analyzer at Isra University Diagnostic Laboratory, Hyderabad.

The data was analyzed using SPSS (Statistical Package for Social Sciences) version 22. One-way analysis of variance (ANOVA) was applied to compare the means of various quantitative variables among groups A, B, and C, D, and E.Statistical significance was taken at $p \leq 0.05$.

Results

The Mean±SD post-experimental body weight in group A, B, C, D, and E was noted as 198+35.90, 284+19.71, 218+32.55, 251+55.01 and 202+48.46 grams respectively and a statistically significant difference was noted ($p<0.05$) among all the groups. A marked increase in body weight was observed in Group B. Aescin and Atorvastatin treated hyperlipidemic rats (groups C and D) revealed a decrease in body weight, with the Aescin group (Group C) showing better results. However, Aescin and Atorvastatin combination therapy group (Group E) showed the best results that reveal the combination therapy prevented the body weight gain significantly (Table I).

The post-experimental biochemical analysis (mean±SD) findings of all study groups are reported in Table II. A statistically significant difference ($p<0.05$) in mean levels of serum cholesterol, TAGs, HDL-C and LDL-C was observed among experimental groups. A significant increase in serum levels of

Table: I Mean Bodyweight (Grams) Levels Among Control and Experimental Groups

Groups	Mean(±SD)	P-value
Group A (Control)	**198(±35.90)**	**0.001***
Group B (Experimental control + High-fat diet)	**284(±19.71)**	
Group C (Aescin + High-fat diet)	**218(±32.55)**	
Group D (Atorvastatin + High-fat diet)	**251(±55.01)**	
Group E (Aescin + Atorvastatin + High fat Diet)	**202(±48.46)**	

Significant Findings (<0.05)

cholesterol, TAGs, and LDL-C while a decrease in serum levels of HDL-C was noted in the hyperlipidemic group (Group B). Aescin and Atorvastatin treated hyperlipidemic rats (groups C and D) revealed a decrease in levels of total cholesterol, TAGs, and LDL-C and an increase in HDL-C levels, with Aescin group (Group C) showing comparatively better results. However, Aescin and Atorvastatin combination therapy group (Group E) showed significant results with near-normal levels of all lipid profile parameters.

Table: II Mean Levels of Lipid Profile Parameters among Control and Experimental Groups

	Groups	Mean± SD	p-value
Serum Cholesterol (mg/dl)	A	78 (±16.7)	0.01
	B	158 (±30.91)	
	C	87 (±25.90)	
	D	96.83 (±27.79)	
	E	71.36 (±10.1)	
Serum Triglycerides (mg/dl)	A	81(±21.20)	0.02
	B	130(±26.42)	
	C	92.6(±36.88)	
	D	110(±28.79)	
	E	83(±25.66)	
Serum HDL-C (mg/dl)	A	40(±9.91)	0.01
	B	27(±10.32)	
	C	42(±12.81)	
	D	30(±9.67)	
	E	45(±11.85)	
Serum LDL-C (mg/dl)	A	38(±6.45)	0.01
	B	88(±8.82)	
	C	37(±2.30)	
	D	50(±5.75)	
	E	32(±3.76)	

Significant Findings (<0.05)

Discussion

The present study is based on comparing the individual and combined effects of Aescin and Atorvastatin respectively. There are few studies that have been conducted on Aescin and its role as a lipid-lowering agent but literature is scarce in terms of finding a research article on combination therapy of Aescin and Atorvastatin.[16] The present study showed that both aescin and atorvastatin have lipid-lowering effects, however, combination therapy of both the drugs is a more potent and efficacious lipid-lowering regimen.

Zhang et al. observed in their experiment that bodyweight of albino rats decreased when Aescin was used in high-fat diet groups. These effects were due to their enzyme inhibition and antioxidant activity. These results are consistent with the present study.[17] In our study, we found Aescin to be effective in improving the lipid profile of Wistar rats. The findings of our study are consistent with the study of Sood S et al. which concluded that Aescin derived from hippocastanum plants is effective in preventing the rise of total cholesterol level.[16]

Lella M et al. and Prasad A et al. reported about combined therapy of Atorvastatin and cholesterol binding drug (Ezetimibe) the studies are consistent with our study that Atorvastatin shows better results in combination therapy but in our study, we used Aescin instead of ezetimibe.[18,19] In this study, we observed that Aescin has significant protective effects on lipid profile of albino rats. However, these protective effects were more pronounced when Aescin was used at a comparatively lower dose in combination with Atorvastatin than Aescin alone.

Avci G et al. conducted a similar study on Aescin and high fed diet rat models, according to their findings, total cholesterol and TAGs didn't show any significant decrease in experimental groups.[20] This particular finding is inconsistent with our study. This difference could be due to the short duration of their study (2 weeks) as compared to the duration of this study being 5 weeks. However, the results are consistent with the present study in terms of HDL-C and LDL-C levels as in both studies HDL levels have increased and LDL-C levels decreased with treatment of Aescin both on low and high doses respectively.[20]

Sood S et al. reported in a very similar study on Aescin and its effects on hypercholesteremia as a lipid-lowering agent, their results in terms of HDL-C and LDL-C are similar to the results of the present study as in both HDL-C levels are being increased and LDL-C levels are decreasing.[16] Chatley P et al. conducted an experiment in which he evaluated that the low dose of Atorvastatin (5mg/day) and Finofibrats (160mg/day) in combination therapy was equally effective as compared to high dose of

Atorvastatin (10-40mg) and fenofibrate (160mg-200mg) when given individually.[21] These findings were consistent with the present study. However, they also observed that the combination therapy not only decrease the lipid profile but cause side effects related to high dose. However, the side effects were not observed in the present study, but can be recommended for further studies to strengthen the present study.

With strengths, our study had certain limitations. We could not see the effects of the drugs on other parameters such as high-fat diet-induced cardiovascular toxicity and oxidative stress due to monetary limitations and time constraints. Therefore, further work should be carried out to see the effects of these drugs on other organ systems as well as to compare the side effects of statins and Aescin. Aescin can be used as an add on therapy to conventional treatment of hyperlipidemia. However, this can be made available by conducting maximum experimental and clinical trial to further prove its significance.

Conclusion

This study concludes that both Aescin and Atorvastatin are efficacious in lowering lipid levels. However, Aescin showed significant results as compared to Atorvastatin, whereas combination therapy is most effective in reducing hyperlipidemia.

REFERENCES

1. World Health Organization. Cardiovascular diseases (CVDs) Fs, May 2019.
2. Tillin T, Hughes AD, Mayet J, Whincup P, Sattar N, Forouhi NG, et al. The relationship between metabolic risk factors and incident cardiovascular disease in Europeans, South Asians, and African Caribbeans: SABRE (Southall and Brent Revisited)—a prospective population-based study. Journal of the American College of Cardiology. 2013;61(17):1777-86.
3. Shakeel M, Irfan M, Khan IA. Estimating the mutational load for cardiovascular diseases in Pakistani population. PloS one. 2018;13(2):e0192446.
4. Bisht A. An huge updated review on dyslipidemia etiology with various approaches for its treatment. therapy. 2012;4:5.
5. Ibrahim SR, Mohamed GA, Banjar ZM, Kamal HK. Natural antihyperlipidemic agents: Current status and future perspectives. Phytopharmacology. 2013;4(3):492-531.
6. Krauss RM. Lipids and lipoproteins in patients with type 2 diabetes. Diabetes care. 2004;27(6):1496-504.
7. Santos MJ, Fonseca JE. METABOLIC SYNDROME. INFLAMMATION AND ATHEROSCLEROSIS-THE ROLE OF ADIPOKINES IN HEALTH AND IN SYSTEMIC INFLAMMATORY RHEUMATIC DISEASES. Acta reumatologica portuguesa. 2009;34(4).
8. Onyeneke EC, Adebisi KE, Eriyamremu GE, Ojeaburu SI, Asagba SO, Oluba OM. Effect of lipid-based diet on some lipid-metabolizing enzymes. J Med Sci. 2007;7(8):1283-9.
9. Liu J-C, Yang T-Y, Hsu Y-P, Hao W-R, Kao P-F, Sung L-C, et al. Statins dose-dependently exert a chemopreventive effect against lung cancer in COPD patients: a population-based cohort study. Oncotarget. 2016;7(37):59618.
10. Flink L, Underberg JA, Newman JD, Gianos E. The recent national lipid association recommendations: how do they compare to other established dyslipidemia guidelines? Current atherosclerosis reports. 2015;17(4):15.
11. Sirimarco G, Labreuche J, Bruckert E, Goldstein LB, Fox KM, Rothwell PM, et al. Atherogenic dyslipidemia and residual cardiovascular risk in statin-treated patients. Stroke. 2014;45(5):1429-36.
12. Sarabi ZS, Saeidi MG, Khodashahi M, Rezaie AE, Hashemzadeh K, Khodashahi R, et al. Evaluation of the anti-inflammatory effects of atorvastatin on patients with rheumatoid arthritis: a randomized clinical trial. Electronic physician. 2016;8(8):2700.
13. Jiang N, Xin W, Wang T, Zhang L, Fan H, Du Y, et al. Protective effect of aescin from the seeds of Aesculus hippocastanum on liver injury induced by endotoxin in mice. Phytomedicine. 2011;18(14):1276-84.
14. Rajyalakshmi G, Reddy A, Rajesham V. A comparative antihyperlipidemic activity of atorvastatin with simvastatin in rats. Internet J Pharmacol. 2009;6.
15. Guillaume M, Padioleau F. Veinotonic effect, vascular protection, antiinflammatory and free radical scavenging properties of horse chestnut extract. Drug Research. 1994.
16. Sood S, Mishra M, Sood A, Thakur V. Hypoglycaemic and hypocholesterolimic efficacy of horse chestnut (Aesculus indica) using rat model. Journal of Clinical Nutrition and Dietetics. 2015;1(1):1-8.
17. Zhang Z, Li S, Lian X-Y. An overview of genus Aesculus L.: ethnobotany, phytochemistry, and pharmacological activities. Pharm Crops. 2010;1:24-51.
18. Lella M, Indira K. A comparative study of efficacy of atorvastatin alone and its combination with fenofibrate on lipid profile in type 2 diabetes mellitus patients with hyperlipidemia. Journal of advanced pharmaceutical technology & research. 2013;4(3):166.
19. Prasad A, Datta PP, Roy R, Pattanayak C, Panda P. Comparative study of ezetimibe and atorvastatin alone and in combination on lipid profile in rats. Materia socio-medica. 2013;25(3):192.
20. Avci G, Küçükkurt I, Küpeli Akkol E, Yeşilada E. Effects of escin mixture from the seeds of Aesculus hippocastanum on obesity in mice fed a high fat diet. Pharmaceutical biology. 2010;48(3):247-52.
21. Chatley P, Badyal D, Calton R, Khosla P. Combination therapy of low-dose atorvastatin and fenofibrate in mixed hyperlipidemia. Methods and findings in experimental and clinical pharmacology. 2007;29(3):217-22.