Research Article

Efficiency of Purified Statin from *Aspergillus tamarii* GRD119 to Lower Cholesterol Levels *in vivo*

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ABSTRACT

This study aims to evaluate the efficacy of anti-cholesterol activity by statin produced by *Aspergillus tamarii* GRD119. The statin isolated was first screened for antioxidant potential followed by anticholesterol studies *in vivo*. The antioxidant potential shown by the statin indicates it to be a resourceful compound with antioxidant potential. The treated populations showed a significant decrease in the content of lipid profiles. Comparative study showed the purified statin to have equivalent effect as that of the commercial drug induced rats.

Keywords: *A. tamarii*, statin, antioxidant, anti-cholesterol

INTRODUCTION

Cardiovascular diseases are the second largest cause of mortality worldwide. Increase in the cholesterol level is a major risk factor for progression of atherosclerosis, which is usually accompanied by the production of free radicals. Hypercholesterolemia has been considered as a major risk factor for coronary heart disease and atherosclerosis (Romero-Corral).[1] Patients with cardiovascular disease showed significant increases in lipid peroxidation that correlates with severity of hypercholesterolemia (Botto; Plachta).[2,3] Recent scientific research strategies have been focusing on the removal of reactive oxygen species (ROS). It is almost accepted that atherosclerosis is a disorder of lipid transport and metabolism.

The blood lipids (total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides) have been shown to be related to the development of coronary heart disease (CHD), since these risk factors play an important role in determining atherogenesis and the subsequent pace of atherosclerosis. Evidence from clinicopathological and epidemiological studies overwhelmingly confirms that hyperlipidemia is the primary prerequisite for atherosclerosis manifested in premature cardiovascular disability and death. Hyperlipidemia is caused by a diet high in fat, especially saturated fat and cholesterol. The International Atherosclerosis Project, found that the degree of atherosclerosis was directly proportional to the prevalence of CHD and stroke, and that lipid levels were directly related to plaque damage The higher the serum cholesterol, the greater the plaque build up (Temple and Burkitt).[4]

With the etiological preeminence of hyperlipidemia in CHD, various drugs have been utilized to lower the blood lipids, such as clofibrate, niacin, cholestyramine, statin and gemfibrozil. Although these drugs were successful in reducing serum cholesterol levels, they produced unpleasant and distressing side effects (Temple and Burkitt).[4] HMG-CoA reductase inhibitors or statins are clearly the most effective class of drugs that act by lowering LDL cholesterol and beneficially impact the morbidity and mortality on cardiovascular system. This impact has seen its efficiency of the drug being prescribed to many millions worldwide who take them on a regular basis.

Several *in vitro* and *in vivo* studies have been carried out targeting the antioxidant potential of various types of natural and synthetic statins such as atorvastatin (Wassmann), pravastatin (Alanazi), fluvastatin and simvastatin (Franzoni).[5-7] Many of these studies have been further carried out to assess the property and effect of the statin on cholesterol, artheroschlerosis and lipoproteins (Wassmann).[5] Statins from various fungi have been screened for potential antioxidant activity, although, statin was first isolated from *Aspergillus terreus* (lovastatin) and from *Penicillium citrinum* (mevastatin). Statin production from cultivated fungi such as *Aspergillus sp.* (Hassan and Azam), *Collybia marasmioides* (Britz.) *Ganoderma pfeifferi*, *Stropharia semiglobata*, *Crucibulum leave*, *Cyathus stercoreus*, *Hebeloma truncatum*, *Ustilago maydis* have been reported (Columbo and Bianco).[8,9]

In the present study, an attempt is made to assay the antioxidant potential and efficiency of statin extracted and purified from *A. tamarii* GRD119 (JX110981) as a cholesterol lowering agent.

MATERIALS AND METHODS

Sample Preparation

Spores of 5 day old culture of *Aspergillus tamarii* GRD119 (JX110981) were inoculated into the fermentation broth (Dextrose-10g, Peptone-10g, KNO_3-2g, $NH_4H_2PO_4$-2g, $MgSO_4.7H_2O$-0.5g, $CaCl_2$-0.1g) and incubated for 7 days in a rotary shaker at 180rpm for seven days. The broth was

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180

acidified to pH 3.0 with 10% HCl. The acidified broth was extracted with equal volume of ethyl acetate under shaking condition for 2 h. The samples were subsequently centrifuged at 1500 rpm for 15 min and the organic phase was collected for further steps of purification by column chromatography and lyophilized.

The samples used in this study include a commercial statin drug (Dr. Reddy's Laboratories, India) and the statin compound obtained from *A.tamarii* GRD119 (JX110981). The commercial statin and the isolated compound was dissolved in ethyl acetate and stored at 4 $^{\circ}$C. 100 µg/ml of the purified statin was subjected to Standard Antioxidant assays (DPPH Radical Scavenging Assay). The Standard Antioxidant Assays was checked for percentage inhibition or scavenging activity by the following formula.

$$Scavenging\ Activity = \frac{(Absorbance\ of\ standard - Absorbance\ of\ Sample)}{Absorbance\ of\ Sample} \times 100$$

Selection and Maintenance of Animals

Male albino rats weighing 150-180 mg were procured from Animal House Maintenance Lab, School of Biotechnology, Dr. G R Damodaran College of Science, Coimbatore. The animals were housed in spacious cages and fed with standard pellet diet supplied by AVM Foods, Coimbatore, Tamilnadu, India. Food and water was provided *ad libitium*. A week's time was allowed for the animals to get acclimatized to the laboratory conditions.

Preparation of Samples of Statin

2mg of commercial statin tablet and 6mg of lyophilized purified fungal extract was dissolved in 1ml of sterile distilled water and administered on all days after the acclimatization period by mixing the entire 1 ml with the feed.

Atherogenic Feeding and Grouping of Rats

The animals were initially maintained using standard commercial feed purchased and acclimatized for a week. The study was performed using a high cholesterol diet where high cholesterol products were supplemented with the commercial feed. All these components were mixed with commercial feed (Table 1) and administered as the high cholesterol diet.

Table 1: Components of the high cholesterol diet administered to Albino Rats

Component	Measured Amount (g)
Wheat flour	15
Roasted Bengal Flour	58
Groundnut Flour	10
Milk Powder	5
Powdered cashew nut	4
Salt	4
Casein	4

The feed for each animal was weighed separately on all days, transferred into a separate container and mixed with sufficient water and steamed to a semisolid

consistency. The feeding was continued for a period of 45 days. After one week adaptation period, the animals were divided into five groups with three animals in each group (Table 2).

Table 2: Grouping of animals for experimental study *in vivo*

Groups	Experimental Design
Group I / Normal	Served as normal control rats where the diet administered was the normal diet without the cholesterol products.
Group II	Rats fed with 10g of high cholesterol diet per kg Body Weight (BW) of the rat for 45 days.
Group III	Rats fed with 10g of high cholesterol diet containing per kg of BW of the rat for 45 days along with 2mg of commercial statin per kg BW
Group IV	Rats fed with 6mg purified fungal statin in addition to 10g of high cholesterol diet per kg BW of the rat for 45 days

Collection of Serum

Through the entire course of experimental regimen, at interval of 15 days the body weight was observed. The weight after 7 days of acclimatization served as the initial weight for the experiment. After 45 days, the rats were deprived of food overnight and the blood was collected by cardiac puncture under mild chloroform anaesthesia. Care was taken not to sacrifice the mice. Serum was prepared from the collected blood. The blood samples were made to stand for 20 mins under room temperature and centrifuged at 2500 rpm for 10 mins to separate the blood cells from serum. The serum was transferred and screened for cholesterol (CHO) levels and liver marker enzymes.

Estimation of Cholesterol Levels and various Liver Marker Enzymes

Total Cholesterol (TC), Triglycerides (TG), HDL, LDL, VLDL and ratios of CHO/ HDL and HDL/LDL were estimated. Screening for liver marker enzymes such as alkaline phosphatase, bilirubin, SGOT, SGPT were carried out. All the analysis carried using specified kits from Agappe Diagnostics Limited (India) using Bioanalyser (Robonik) at Dr. G R Damodaran College, Coimbatore and was completed within 24 h of sample collection.

Statistical Analysis

The data was analysed using Statistical Package for Social Sciences (SPSS) 17.0. All the data are shown as Mean ± Standard Error Mean (SEM). Mann Whitney U test was performed to find the significance of the test results obtained as the variables were independent and the sample size was less than 10 (n=3). The significance value was considered at two measure of 95% (p<0.05) and 99% (p<0.01) to be statistically significant.

RESULTS AND DISCUSSION

There has been an increased global interest to identify antioxidant compounds which are pharmologically potent with less or no side effects and are from biological sources (Yen).[10] Although the source of antioxidants have largely been phyto-constituents, an increase in the

interest of antioxidants from microbial sources is fast gaining interest as a potential source for new compounds with radical scavenging activity. Free radicals that are often generated as byproducts from biological reactions or other factors have shown their involvement in various diseases (Sen).[11]

A potential scavenger of free radicals may serve in intervening many diseases. Therefore it is highly important to increase the range of sources for obtaining antioxidants by elucidating the pro-oxidant effect by standard tests.

The presence of the potential free radical scavenging activity of the compound is concentration dependent, as the concentration of the test compounds increases, the radical scavenging activity increases. No antioxidant activity was seen in the concentrations <400µl. The present studies were performed to assess the cholesterol lowering activity of statin from the purified extract of *A. tamarii* GRD119 (JX110981) in rats.

It is well documented that statin commercially has been widely used and confirmed to be cholesterol lowering by *in vivo* and *in vitro* techniques.

Reactive oxygen species (ROS) produced include superoxide radical (O^2-), hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl).

H_2O_2 and O_2 can interact in the presence of certain transition metal ions to yield a highly-reactive oxidizing species, the hydroxyl radical (OH).

Phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals (Rice-Evans; Jorgensen).[12,13]

At the end of 45 days of treatment, the body weight of all the groups treated and normal were compared and measured by Mean ± Standard Error Mean (SEM) (Table 3). A gradual increase in the body weight of the normal population was seen on comparison with a spiked increase in the body weight in Group II where the diet was treated with additional fat products such as casein and powdered cashew nuts.

Among the normal population, the weight of the rats increased significantly at $(p<0.05)$. At the 15 - 30 days of treatment, the body weight of normal rats in groups treated with varied diet, commercial tablet and purified fungal statin increased significantly $(p<0.01)$. At the end of 45 days, the increase in the body weight increased significantly upto $p<0.05$. Therefore, the body weight of the animals grouped clearly indicated all the groups to be statistically significant.

Hyperlipidemia is one of the greatest risk factors contributing to prevalence and severity of cardiovascular diseases. The epidemiologic data shows that the prevalence of dyslipidaemia in Chinese adults aged 18 and above is 18.6%, which is to say the number of dyslipidemic patients has reached 160 million (Zhao).[14] It is also reported that almost 12 million people die of cardiovascular diseases and cerebral apoplexy each year all over the world.

Therefore, it is very important to pay attention to early stage prevention and control of hyperlipidemia in a comprehensive way. However, the risk of hyperlipidemia would be reduced by consumption of flavonoids and their glycosides (Kris-Etherton).[15] The levels of serum lipid profile, TC, TG, LDL-C, VLDL-C and HDL-C in normal and drug treated rats were presented in Table 4.

Table 3: Comparison of body weight through the period of 45 days at intervals of 15 days

Treatment (mg/kg of BW)	Changes in Body Weight (mg)			
	0day (Initial)	15 days	30 days	45 days
Normal	155±1.73	159.33 ± 0.57	163.33 ± 0.57	165.66± 0.57
Group II (High Fat Diet)	156 ± 3.46[b]	179 ± 4.58[a]	186.33 ± 2.51[a]	190.33 ± 1.52[a]
Group III (High Fat Diet + Commercial Statin)	156.66 ± 2.08[b]	184 ± 4.58[a]	174.66 ± 1.52[a]	171.33 ± 1.52[a]
Group IV (High Fat diet + Purified fungal statin)	158.33 ± 2.08[b]	185.33 ± 2.51[a]	170.33 ± 4.5[a]	165.33 ± 2.08[b]

**Table 3: All values have been mentioned in mean ± SEM; [a] – n=3 observations at $p<0.01$ on comparision with normal group, [b] – n=3 observations at $p<0.05$ on comparison with normal group

Table 4: Effect of purified statin and commercial statin on serum lipid profile of normal and induced adult male albino rats

Groups	TC	TG	HDL-C	LDL-C	VLDL-C	CHO/HDL	LDL/HDL
I	62.3 ± 1.1	56.8 ± 3.20	26.6 ± 1.52	24.9 ± 1.4	11.2 ± 0.6	2.3 ± 0.644	0.9 ± 0.1
II	85.8 ± 4.7[a]	116.6 ± 1.2[a]	32.6 ± 1.5[b]	29.5 ± 1.44[b]	23.5 ± 2.6[a]	2.62 ± 0.03[a]	0.9 ± 0.5[b]
III	68.7 ± 1.5[d]	46 ± 8.71[e]	22.6 ± 2.51[e]	36.8 ± 3.40[e]	9.2 ± 1.74[d]	3.05 ± 0.38[d]	1.6 ± 0.3[e]
IV	63.5 ± 3.04[ec]	34 ± 2.64[ec]	12.3 ± 2.5[dc]	39.7 ± 1.16[dc]	6.8 ± 5.2[dc]	5.26 ± 0.87[dc]	3.7 ± 0.7[dc]

Table 4: Each value is given in Mean ± SEM; [a] – n=3 observations at $p<0.01$ on comparison with group I, [b] – n=3 observations at $p<0.05$ on comparison with group I, [c] – observations with $p<0.05$ on comparison with group III, [d] – n=3 observations at $p<0.01$ on comparison with group II, [e] – n=3 observations at $p<0.05$ on comparison with group II.

Statin induced rats showed significant decrease in serum lipid profiles including HDL-C when compared with normal rats. The treated populations (Group III and IV) showed a significant decrease in the content of lipid profiles. When compared to the purified extract, equivalent effect as that of the commercial drug induced rats was observed. Similarly LDL-C level marginally decreased in statin induced rats when compared to rats on high fat diet.

Observed accumulation of lipids is also found by many changes in the regulatory and metabolic mechanisms that arise due to insulin deficiency (Rajalingam).[16] The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma. Further it has been reported that rats treated with statin shows normalized lipid levels (Pathak).[17] Thus the results indicate that statin from *A.tamarii* shows effective cholesterol lowering action by virtue of its lipid lower levels. The increase in the activities of SGOT and SGPT found in rats has correlations with a high concentration of glucose. Therefore, the gluconeogenic action of SGOT and SGPT plays the role of providing new supplies of glucose from other sources such as amino acids (Scott).[18] The activities of specific liver markers such as bilirubin, SGOT, SGPT and alkaline phosphatase in serum showed an increase in its respective concentration among all treated groups. The values on comparison of the Group II with the normal population showed a spiked increase mainly due to the high amount of cholesterol administered to the rats *ad libitum* along with the regular diet. The liver marker enzyme readings obtained from the treated groups (both of commercial statin and fungal statin) showed no major difference with the group treated only with the high fat diet. These results indicate a slight frequency decrease in liver enzyme marker levels (Kiderman).[19]
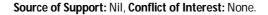
CONCLUSION

Statins have contributed to inhibit the oxidative products by atherogenic feeding. On the basis of the present investigation, it can be noted that statin from *A. tamarii* GRD119 (JX110981) acts in a similar fashion of commercial statin with respect to its cholesterol lowering potency.

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183