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ANTI-HYPERLIPIDEMIC ACTIVITY OF NUTRISLIM GREEN TEA (AYU-606) - AN AYURVEDIC FORMULATION IN HIGH FAT DIET INDUCED HYPERLIPIDEMIA IN RATS.

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ABSTRACT
The present study aimed to assess the potential Antihyperlipidemic activity of Nutrislim Green Tea (AYU-606) an Ayurvedic formulation developed by Ayurwin Pharma Pvt Ltd Bengaluru in high fat induced hyperlipidemic rats. The Antihyperlipidemic effects of orally administered Nutrislim Green Tea (AYU-606) powder were evaluated in high cholesterol cocktail diet induced hyperlipidemic rats. The standard atorvastatin (10mg/kg) was administered by oral gavage. The animals of all groups (including group II) except normal group were provided the high cholesterol cocktail diet pellets separately for two hours daily for a period of ten days. The rats of group II receive HCD along with normal diet. This group is used to induce hypercholesterolemia. The animals were provided with normal diet for the remaining 20 days. The group III and IV were administered with AYU-606 by oral gavaging daily for 20 days from 11th to 30th day of the study period. The V group animals were administered atorvastatin (10mg/kg) by oral gavaging for a period of 20 days from 11th to 30th day of the study period. The rats administered with HCD and AYU-606 (group III and IV) has shown a significant reduction in the serum total cholesterol level, and significant increased levels of HDL cholesterol when compared to group II rats (HCD only). The rats administered with HCD and AYU-606 (group III) has shown a significant reduction in the serum LDL, when compared to group II rats. The rats administered with HCD and AYU-606 (group III) hasalso shown reduction in the serum triglyceride level when compared to group II rats. These results validate traditional knowledge and suggest that Nutrislim Green Tea (AYU-606) may be potentially useful for managing hypercholesterolemia and hypertriglyceridemia.

KEYWORDS: Hyperlipidemia, Green tea, hypertriglyceridemia, hypercholesterolemia. atorvastatin.

INTRODUCTION
Obesity is a medical condition in which excess body fat gets accumulated. People are considered obese when their body mass index (BMI) exceeds 30Kg/m^2. As per the recent reports there has been a starling increase in rates of obesity and overweight in both adults (28% increase) and children (up by 47%) in the past 33 years, with the number of overweight and obese people rising from 857 million in 1980 to 2.1 billion in 2013. However, the rates vary widely throughout the world with more than half of the world's obese individuals living in just 10 countries- the USA(more than 13%), China and India (15% combined), Russia, Brazil, Mexico, Egypt, Germany, Pakistan, and Indonesia. Overweight and obesity were first considered a problem of rich countries only, but now they have become a concern in poor countries also. India is not exception to them and there are rising numbers of people in middle-class who are obese.

Obesity increases the risk of developing a number of serious health conditions, including coronary heart disease, high BP, stroke, type 2 diabetes mellitus, cancer, sleep apnea, gallstones, osteoarthritis, infertility, etc. Cardiovascular disease is a major cause of morbidity and a leading contributor to mortality in both developed and developing countries. India is a developing country and has a high level of CVD mortality, accounting for nearly 30-40 percent of all deaths. Coronary artery disease (CAD) is the third leading cause of mortality all over the world. Atherosclerosis is the principle cause of myocardial and cerebral infarction.[1] Obesity is one of the major risk factors for CAD and other risk factors for CAD are diabetes mellitus, hypertension, smoking and dyslipoproteinemias.[2]

Although several factors, such as diet high in saturated fats and cholesterol, age, family history, hypertension and life style play a significant role in causing heart

failure, the high levels of cholesterol particularly Total Cholesterol (TC), Triglycerides (TG) and Low density Lipoprotein (LDL) cholesterol are mainly responsible for the onset of CHDs.[3] Hyperlipidemias are classified according to which types of lipids are elevated, that is hypercholesterolemia, hypertriglyceridemia or both in combined hyperlipidemia. It is a known fact that elevated cholesterol, especially LDL cholesterol, is a risk factor for the development of cardiovascular diseases like atherosclerosis, congestive heart disease and myocardial infarction, which are the common reasons for the mortality and morbidity.

The disturbed balance between reactive oxygen species and antioxidant defenses result in oxidative stress. The onset of diabetes is closely associated with oxidative stress. The oxidative stress induces chronic obstructive pulmonary disease (COPD) by impairing the functioning of several kinds of proteins in the lung tissue. The increased production of oxidative stress also plays a role in such pathologies as hypertension, atherosclerosis, asthma, diabetes and kidney disease. In recent years, there has been an increasing interest in finding natural antioxidants, which can protect the human body from free radicals and retard the progress of many chronic diseases. There is considerable interest in finding safer antioxidants from natural sources to replace synthetic ones.

Several studies have concluded that hypercholesterolemia is also responsible for oxidative stress due to increased lipid peroxidation. Obesity is associated with increased lipid peroxidation and decreased levels of various antioxidants. Oxidative stress is defined as an imbalance between the production of reactive oxygen species or free radicals and antioxidant defense, which may induce tissue injury. Superoxide dismutase (SOD) is a naturally occurring enzyme that protects the body against active oxygen free radicals by scavenging excess superoxide. SOD has particular value as an antioxidant that can help to protect against cell destruction.[2]

The present drugs for the treatment of hyperlipidemia include mainly statins and fibrates. The drugs which reduce the cholesterol level like fibrates and bile acid sequestrants were used for several decades. But their importance is decreased in recent years due to the introduction of statins. Some statins like cerivastatin were withdrawn from the market as kidney toxicity was noticed in some patients due to rhabdomyolysis.[4]

2. MATERIAL AND METHODS

Drugs and chemicals cholesterol, cholic acid and other chemicals were purchased from VASA Scientific suppliers Bengaluru. The biochemical kits used for the estimations were procured from Anjan diagnostics Bengaluru, an authorized distributor for ERBA Diagnostics Mannheim. The standard drug atorvastatin was procured from approved medical store. The sample AYU-606 was supplied by Ayurwin Pharma Pvt Ltd, Bengaluru-560010. The instruments and lab accessories of PG research laboratory of Pharmacology Department, PES College of Pharmacy- Auto analyzer, micro-centrifuge, micropipette.

Animals

Male Wistar rats weighing 180-230 g were used as experimental animals and maintained as per standard guidelines in accordance with Institutional Animal Ethics Committee (IAEC) regulations and approved by CPCSEA (IAEC approval No-PESCP/11/IAEC/2015 Dated- 10-1-2015). Male albino Wistar rats (180- 230 g) were housed for 1 week under a 12h/12h light/dark cycle in a temperature and humidity controlled room. The animals were given free access to food and water. After adaptation to the above conditions for one week, the healthy animals were used for the study.

Experimental design

The rats were divided into five groups of 6 animals each (n=6). Group I rats served as untreated control and received standard normal diet throughout the study period.

The animals of all groups (including group II) except normal group were provided the high cholesterol cocktail diet pellets separately to eat for a period of two hours daily for a period of ten days. The rats of group II receive HCD along with normal diet. This group is used to induce hypercholesterolemia. The animals were provided with normal diet for the remaining 20 days. The group III and IV were administered with medium and high doses of AYU-606 by oral gavaging daily for 20 days from 11th to 30th day of the study period. The V group animals were administered atorvastatin (10mg/kg) by oral gavaging for a period of 20 days from 11th to 30th day of the study period.[5]

HCD

High fat diet cocktail was prepared by mixing cholesterol (100g), cholic acid (50g), 1 liter of coconut oil mixed with boiled eggs (10) and was blended together using wheat as a binding agent, then this was made into small eatable sized balls.

After 24h of the last treatment, the animals were separated and body weight of each rat was determined. Then blood samples were collected by retro-orbital puncture under ether anesthesia. The blood was drawn into vacutainer tubes without containing anticoagulant. The blood drawn was approximately 2.5 times the volume needed for use. The tubes were allowed for blood clotting by incubating in an upright position at room temperature for 30- 45min. These tubes were then centrifuged at 2000 rpm for 10 min. Then the supernatant serum transferred from the centrifuge tube at room temperature using a clean pipette. The serum was used for the estimation of cholesterol, TG, LDL, High density lipoprotein (HDL) and Very low density lipoprotein

(VLDL) by using Erba diagnostic kits and auto-analyzer.

RESULTS

Table- Showing the effect on the body weight, HCD, AYU-606 and atorvastatin administered rats.

Group N=6	BW before the study in gms	BW after the study in gms After 30 days	Gain (+) / loss (-) in the weight in gms
I	211.67 ± 8.72	246.66 ± 11.15	+ 34.99
II	211.66±8.72	221.66±6.00	+ 9.99
III	238.33 ± 4.01	215.00 ± 7.18	- 23.33
IV	220 ± 6.83	220.00 ± 6.83	-
V	216.66 ± 2.10	205.00 ± 5.62	- 11.66

The weights of the normal rats (Group I) have shown noticeable increase in their weights after 30days. The Group II (HCD treated) rats also shown little increase in their body weights. But the weights of the rats Group III (AYU-606 dose 630mg/kg + HCD) and Group V (atorvastatin 10mg/kg + HCD), have shown noticeable decrease in their weights after 30days. However no change in the body weights of the rats of Group IV (AYU-606 dose 950mg/kg+ HCD).

Table- Showing the effect on the TC, serum HDL cholesterol, serum LDL cholesterol and serum triglycerides in normal, HCD, AYU-606 and atorvastatin administered rats.

Group N=6	Serum total cholesterol mg/dL	Serum HDL mg/dL	Serum LDL mg/dL	Serum TGs mg/dL
I	160.80 ± 6.02	53.21 ± 2.15	62.65 ± 5.11	224.62 ± 6.58
II	309.19±12.73 ***	50.12 ± 3.05*	211.46 ± 13.95***	238.15 ± 1.00*
III	218.00 ± 7.48**	55.96 ± 5.16*	119.34 ± 5.00**	213.40 ± 2.76*
IV	172.68 ± 8.65***	52.25 ± 8.39*	71.84 ± 5.55***	242.9 ±1.89
V	165.06 ± 2.63***	48.46 ± 5.85	74.16 ± 8.15***	212.18 ±12.05*

Effects on serum total cholesterol

The HCD administered rats (group II) have shown significant increase in the serum total cholesterol level when the results are compared to the normal group (group I). This confirms the development of hypercholesterolemia in group II rats. The rats treated with standard atorvastatin (group V) has shown significant antihypercholesterolemic effect when the results are compared to group II rats. The rats administered with HCD and AYU-606 (group III and IV) has shown a significant reduction in the serum total cholesterol level, when the results are compared to group II rats. The results indicate the total cholesterol lowering effect of AYU-606.

Effects on serum HDL cholesterol

The HCD administered rats (group II) have shown significant decrease in the serum HDL cholesterol level when the results are compared to the normal group (group-I). This confirms the development of hypercholesterolemia in group II rats. The rats administered with HCD and AYU-606 (group III and IV) has shown a significant increase in the serum HDL cholesterol level, when the results are compared to group II rats. The results indicate the antihypercholesterolemia effect of AYU-606 by elevating the level of HDL cholesterol. Hence it may have cardio protective effect.

Effects on serum LDL cholesterol

The HCD administered rats (group II) have shown significant increase in the serum LDL cholesterol level when the results are compared to the normal group (group I). This confirms the development of hypercholesteromia in group II rats. The rats treated with standard atorvastatin have shown significant antihypercholesterolemia effect as there is lowered level of the LDL cholesterol level when compared to group II control rats. The rats administered with HCD and AYU-606 (group III) have shown a significant reduction in the serum LDL, when the results are compared to group II rats. The rats administered with HCD plus high dose of AYU-606 (group IV) have shown highly significant serum LDL cholesterol lowering effect. These results confirm the cholesterol lowering effect of AYU-606.

Effects on serum triglycerides

The HCD administered rats (group II) have shown increase in the serum triglyceride level when the results are compared to the normal group (group I). This confirms the development of hyperlipidemia in group II rats. The rats treated with standard atorvastatin (group V) has shown antihyperlipidemia effect when the results are compared to group II rats. The rats administered with HCD and AYU-606 (group III) have shown antihyperlipidemia effect reduction in the serum triglyceride level, similar to standard atorvastatin when the results are compared to group II rats. The result of these confirms the lipid lowering effect of AYU-606.

Graph representation of the results

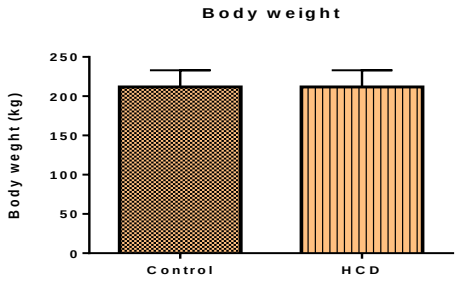

Fig No 1.

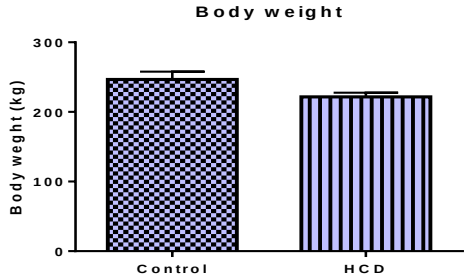

Fig No 2.

The graph (Fig No 1) showing no difference in the body weights of rats of the normal (group I) and HCD treated (group II) before the study. But after the study period insignificant decrease in the body weights have been observed in HCD treated rats (group II) when compared to normal rats (gp I).Graph (Fig No2) showing the differences in the body weights of rats in group II (HCD control) and group III, IV, V (HCD + AYU-606 and atorvastatin) before and after the study.

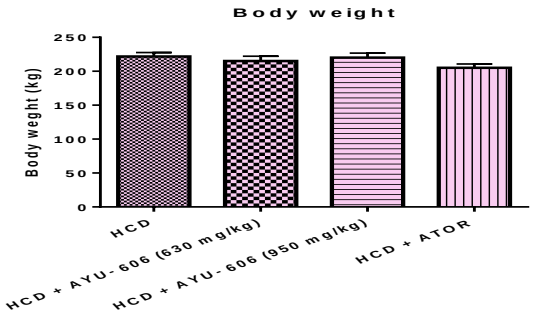

Fig No 3.

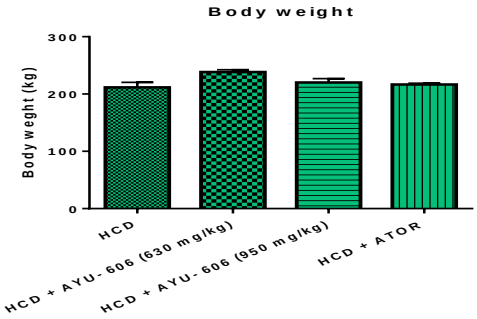

Fig No 4.

The graph (Fig 3,4) showing decrease in the body weights of the rats of group III (AYU-606 dose 630mg/kg + HCD), group IV (AYU-606 dose 950mg/kg+ HCD), and group V (atorvastatin 10mg/kg+ HCD) when compared to the body weights of group II (HCD treated) rats.

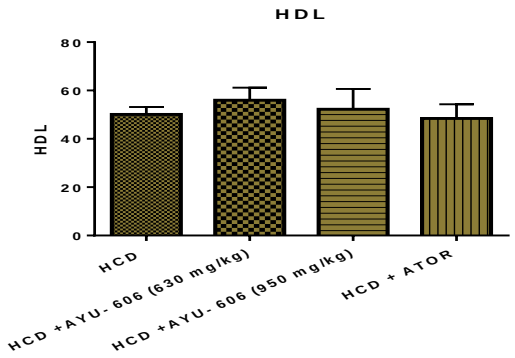

Fig No 5.

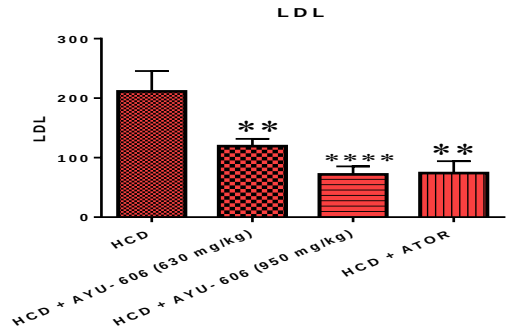

Fig No 6.

Graph (Fig No 5) showing the significant increase in the serum HDL cholesterol levels in Group III (AYU-606 dose 630mg/kg + HCD) and Group IV (AYU-606 dose 630mg/kg + HCD) when compared to Group II (HCD treated) rats.

Graph(Fig No 6) showing the significant decrease in the serum LDL cholesterol levels in Group III (AYU-606 dose 630mg/kg + HCD), Group IV (AYU-606 dose 950mg/kg + HCD) and Group V (atorvastatin dose 10mg/kg + HCD) when compared to Group II (HCD treated) rats.

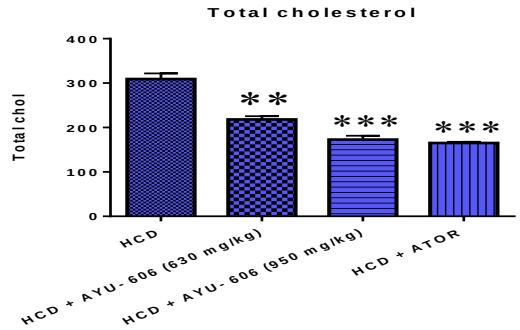

Fig No 7.

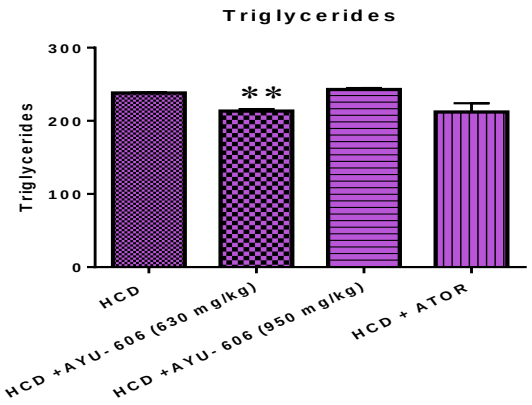

Fig No 8.

Graph (Fig No 7) showing the significant decrease in the serum total cholesterol levels in Group III (AYU-606 dose 630mg/kg+ HCD), Group IV (AYU-606 dose 950mg/kg + HCD) and Group V (atorvastatin dose 10mg/kg + HCD) when compared to Group II (HCD treated) rats.

Graph (Fig No-8) showing the significant decrease in the serum triglycerides levels in Group III (AYU-606 dose 630mg/kg + HCD) and Group V (atorvastatin dose 10mg/kg + HCD) when compared to Group II (HCD treated) rats.

DISCUSSION

Hyperlipidemia is a major contributor for health problems worldwide and leads especially to atherosclerosis, resulting in coronary heart diseases (CHD). According to WHO by 2020, 60% of the cardiovascular cases will be of Indian origin. Hyperlipidemia causes the damage of various tissue cells and also changes the cellular physiological functions leads to the apoptosis of cells. This also leads to the development of various pathological conditions. The administration of high fat cocktail diet (HCD) induces obesity by increasing both serum cholesterol and serum triglyceride levels. The elevated cholesterol levels particularly LDL and VLDL increases the risk of cardiovascular diseases like coronary artery disease. The increase in HDL cholesterol reduces the risk of CAD.[6]

It has been well established that nutrition plays an important role in the etiology of hyperlipidemias and atherosclerosis. Several animal and human studies have confirmed the hypercholesterolemic properties of saturated fatty acids and cholesterol which include increasing total cholesterol and altering lipoprotein pattern and whose mechanisms remain under study. Cholesterol feeding has been often used to elevate serum or tissue cholesterol levels to assess hypercholesterolemia-related metabolic disturbances in different animal models.[7]

The importance of Ayurvedic drugs in the treatment of hyperlipidemia was studied in recent years. Medicinal plants have been used to control the elevated lipid levels in the blood and also used as an inhibitor to trigger oxidative stress which reduces adipose tissue apoptosis.

The high fat diet administration in the present study includes HCD for effective hyperlipidemia induction in rats. There was marked increase in the level of serum total cholesterol, triglycerides, LDL, VLDL and decrease in the level of good cholesterol carrier HDL in the rats (group II) treated with HCD. The elevated LDLs transport cholesterol from the liver to the peripheral blood vessels. The oxidative stress causes the oxidation of LDL and oxidized LDLs are taken by the macrophages. Thus the increased serum LDL level in the blood causes the elevation of altered macrophages (foam cells) which are rich in cholesterol in the intima of arteries and veins and leads to atherosclerosis. The antioxidant activity prevents the oxidation of LDLs and this prevents the triggering of atherosclerosis.

In recent years, flavonoids have been recognized as compounds with potent biological activities that may be active in the prevention of chronic diseases including cardiovascular disease. Their antioxidant activity is well established and epidemiologic studies have suggested associations between flavonoid intake and a lower risk of cardiovascular disease. Flavonoids may prevent oxidative damage and the oxidation of low-density lipoproteins (LDL). Flavonoids may have anti-inflammatory, cholesterol-lowering, antihypertensive and antiplatelet activities. The flavonoid content in AYU-606 was found to be 38.33 mg/g dry powder. This result supports its anti-lipidemic effect.

CONCLUSION

The rats, Group III (AYU-606 dose 630mg/kg + HCD) and Group V (atorvastatin 10mg/kg + HCD), have shown noticeable decrease in their weights after 30days. The rats administered with HCD and AYU-606 (group III and IV) have shown a significant reduction in the serum total cholesterol level, when the results are compared to group II rats (HCD only). The rats administered with HCD and AYU-606 (group III and IV) haves shown a significant increase in the serum HDL cholesterol level, when the results are compared to group II rats. Hence, the formulation may have cardio-protective effect. The rats administered with HCD and AYU-606 (group III) have shown a significant reduction in the serum LDL, when the results are compared to group II rats. The rats administered with HCD and AYU-606 (group III) have shown anti-hyperlipidemia effect reduction in the serum triglyceride level when compared to group II rats.

The findings of this study concluded that Nutrislim Green tea (AYU-606) -an Ayurvedic formulation developed by Ayurwin Pharma Pvt Ltd Bangalore, exhibited antihyperlipidemic activity. This could be useful for prevention or early treatment of complications caused by hyperlipidemia. Further studies would be needed in time to the exact mechanism of action of the

bioactive molecules present in this formulation which are responsible for antihyperlipidemic activity.

REFERENCES

1. Dadhania Sagar S, Shah Nirzarini N, Sachdeva Punam D, Patel Nikunj B, Jani Dilip K. A study of anti-hyperlipidemic activity of polyherbal formulation using various experimental animal models. Inventi Rapid: Ethnopharmacology., 2011; 2(1): 1-4.
2. Mahendra D.Bikkad, Sandeep H. Ghuge, Sachin D. Somwanshi& M. B. Madole. Oxidative stress in obesity. Journal of Medical Education and Research., 2013; 3(1).
3. Dhulasavant V, Shinde S, Pawar M, Naikwade NS. Antihyperlipidemic activity of *Cinnamomum tamala* Nees on high cholesterol diet induced. Int J PharmTech Res., 2010; 2: 2517–21.
4. Oluwatosin A Adaramoye, Olajumoke Akintayo, Jonah Achem and Mihael A Fafunso. Lipid-lowering effects of methanolic extract of *Vernonia amygdalina* leaves in rats fed on high cholesterol rats. Vasc Health Risk Manaq, 2008; 4(1): 235-41.
5. Rao BSD, Saileela CH. Anti-hyperlipidemic activity of methanolic extract of *Rhinacanthus nasutus.* IJRPC., 2013; 3(3): 708-711.
6. Wolf PL, Williams D, Coplon N, Coulson AS. Clin Chem., 1973; 19: 92.
7. Guo-Zhe Sun, Zhao Li, Liang Guo, Ying Zhou, Hong-Mei Yang and Ying-Xian Sun. High prevalence of dyslipidemia and associated risk factors among rural Chinese adults. Lipids in Health and Disease, 2014; 13: 189.