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Evaluation of Anti--Cholesterolemic activity of Ayur-ns-01 in high Cholesterol Diet Induced Hypercholesterolemia in rats.

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ABSTRACT

Hyper-cholesterolaemia is one of the leading causes of the major diseases like angina pectoris, myocardial infarction, atherosclerosis which increases the mortality rate every year. This study includes the evaluation of sample Ayur NS01 for anti-cholesterolemic effect using two models HCD induced hypercholesterolemia and lipid emulsion tolerance test (LETT) in rats. The evaluation was made in different doses (1.35gm/kg, 1.8gm/kg, 2.25gm/kg) of Ayur NS01 sample. The lipid profile parameters like LDL, total cholesterol, VLDL and lipid emulsion tolerance were studied in the normal, high cholesterol diet induced hypercholesterolemic, AyurNS01 and the standard drug atorvastatin (10mg/kg) administered rats. The results of HCD alone administered rats (group II) were compared with normal rats. The results obtained in the HCD and various doses of rats of AyurNS01, atorvastatin were compared with HCD alone administered hypercholesterolemic rats. From the results of first model of this study it is confirmed that the sample of AYUR NS01 has shown a significant reduction in the total cholesterol, triglycerides, LDL levels when it is compared to the HCD induced hypercholesterolemia rats. From the results of Lipid emulsion tolerance test, it is also confirmed that the sample of has also shown a reduction in the serum triglyceride levels in the AYUR NS01 treated groups as compared to the control rats treated with lipid emulsion.

Keywords- Hyper-cholesterolemia, high cholesterol diet, lipid emulsion, Ayur NS-01.

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INTRODUCTION

According to the lipid hypothesis, abnormal cholesterol levels, actually higher concentrations of LDL particles and lower concentrations of functional HDL particles in blood is called as Hypercholesteremia. This is strongly associated with cardiovascular diseases because these promote atheroma development in arteries (atherosclerosis). This disease process leads to myocardial infarction (heart attack), stroke, and peripheral vascular disease.¹ Since higher blood LDL, especially higher LDL particle concentrations and smaller LDL particle size, contribute to this process more than the cholesterol content of the HDL particles.²

Elevated cholesterol levels are treated with a strict diet consisting of low saturated fat, trans fat-free, low cholesterol foods, then followed by one of various hypo-lipidemic agents such as statins and fibrates, cholesterol absorption inhibitors, nicotinic acid derivatives or bile acid sequestrates.³ Extreme cases have previously been treated with partial ileal bypass surgery, which has now been superseded by medication. Aphaeresis-based treatments are still used for very severe hyperlipidaemias that are either unresponsive to treatment or require rapid lowering of blood lipids.⁴

Statins (or HMG-CoA reductase inhibitors) are the widely used line of drugs for the lowering of blood cholesterol levels, they act on the enzyme HMG CoA reductase which is involved in the synthesis of cholesterol in the liver. But there are many side effects encountered with its use.⁵ Some of the frequent adverse effects seen with statins such as atorvastatin, simvastatin are headache, nausea, bowel upset, rashes, sleep disturbances (probably more with lipophilic drugs), rise in serum transaminase, muscle tenderness and rise in CPK levels, myopathy is the serious effect seen.⁶

Nutraceuticals are products derived from food sources that provide extra health benefits, in addition to the basic nutritional value found in foods.⁷ Depending on the jurisdiction, products may claim to prevent chronic diseases, improve health, delay the aging process, increase life expectancy, or support the structure or function of the body.⁸

The costs of the drugs and conventional healthcare system are dissatisfied by the consumers and now are interested in natural products for the treatment and prevention of obesity related problems. About 40% of Americans use alternative system of medicine, nutraceuticals (herbals/botanicals) account for a significant proportion.⁹

MATERIALS AND METHOD

Sample collection

The sample AyurNS01 was obtained from Neutraceutical Division of Ayurwin Limited, Bangalore.

Drugs and chemicals

High cholesterol diet { cocktail diet (100g cholesterol + 50g cholic acid in 1 litre coconut oil) + egg yolk}, saline, sodium citrate, oral gavage, micro tips, micro pipettes, eppendorf tubes, auto analyser, mortar and pestle, ERBA diagnostic kits for triglycerides, HDL-cholesterol, total Cholesterol.

Evaluation of anticholesterolemic effect of Ayur-NS01 in high cholesterol diet (HCD) induced hyper-cholesterolaemia in rats

Male albino wistar rats (150-200) 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 grouped. Group I was treated with normal feed pellets which serves as the negative control, Group II with HCD which serves as the HCD control and Groups III, IV and V were treated with Ayur-NS01 doses 1.35, 1.8 and 2.25 g/kg along with HCD, Group VI was treated with standard drug atorvastatin (10 mg/kg) + HCD.¹⁰

The treatment schedule for Ayur-NS01 is for 20 days and the remaining is for 30 days. The HCD diet consisting of cholic acid, cholesterol, coconut oil and egg yolk was blended together using wheat as a binding agent, made into small eatable sized balls and the fixed quantity of pellets were kept separately to eat for a period of two hours. The Ayur NS 01 was administered by oral gavage route.

After 24h of the last treatment, the blood samples were collected by retro-orbital puncture under ether anaesthesia. 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 were then centrifuged at 2000 rpm for 10 min. Then the supernatant serum was transferred from the centrifuge tube at room temperature using a clean pipette. The serum was used for the estimation of cholesterol, TG, LDL, HDL and VLDL by using Erba diagnostic kits and auto-analyser.

Evaluation of anticholesterolemic effect of Ayur-NS01 using lipid emulsion tolerance test (LETT) in rats.

The lipid emulsion was prepared as follows; corn oil was triturated with cholic acid in a mortar. In a separate beaker cholesterol was dissolved in oleic acid, added to the contents in mortar and

trituated until crackling sound was obtained. Finally saline was mixed to the contents in the mortar to obtain lipid emulsion.

After adaptation for one week, the healthy animals were grouped. After rats had been deprived of food overnight, they were only administered 1 ml of lipid emulsion. Group I was treated with saline, Group II with lipid emulsion and Groups III, IV and V were treated with Ayurwin doses 1st, 2nd, 3rd, Group VI was treated with atorvastatin (10 mg/kg), 30 min before giving LE. Blood samples were collected from lateral tail vein at 0, 0.5, 1, 2, 3, 4 and 5 h after administration of LE. Blood sample was transferred to a tube containing anticoagulant (3.8% sodium citrate or 38mg/ml blood). The volume of the blood in the container was adjusted to the correct volume (blood and anticoagulant ration). The blood and anticoagulant were mixed by inverting the tube about ten times. The blood samples were then centrifuged at 5500 rpm for 10 min at room temperature. Among the three layers developed (from top to bottom): plasma, leucocytes (buffy coat) and erythrocytes, the supernatant plasma is transferred into tubes and used for the measurement of biochemical parameters.

RESULTS AND DISCUSSION

Table 1- The effects on TGs, TC, and HDLc in normal, HCD, Ayur-NS01 and atorvastatin administered rats.

Groups	Treatment	TGs mg/dL	TC mg/dL	HDLc mg/dL
I	Normal diet	85.18±4.45	174.1±4.70	34.15±3.60
II	HCD	97.69±2.52	316.6±12.97 ^x	47.65±1.21 ^y
III	HCD + Ayur –NS 01 (dose 1.35g/kg)	66.77±4.45 ^a	159±7.40 ^a	37.75±2.86
IV	HCD + Ayur –NS 01 (dose 1.8g/kg)	69.86±2.87 ^a	210.9±10.71 ^a	37.67±1.44
V	HCD + Ayur –NS 01 (dose 2.25 g/kg)	43.11±1.97 ^a	229.4±11.72 ^a	46.73±2.52
VI	HCD + Atorvastatin (10mg/kg)	32.92±2.18 ^a	154.9±9.94 ^a	32.12±1.64 ^a

Table 2- The effects on LDLc, VLDLc and AI in normal, HCD, Ayur-NS01 and Atorvastatin administered rats.

Groups	Treatments	LDLc mg/dL	VLDLc mg/dL	AI
I	Normal diet	157±5.66	17.04±0.89	4.40±0.62
II	HCD	288.5±13.45 ^x	19.54±0.50	5.66±0.33
III	HCD + Ayur –NS 01 (dose 1.35g/kg)	134.7±7.60 ^a	13.45±0.866 ^a	3.32±0.36 ^b
IV	HCD + Ayur –NS 01 (dose 1.8g/kg)	187.2±10.31 ^a	13.97±0.57 ^a	4.62±0.29
V	HCD + Ayur –NS 01 (dose 2.25 g/kg)	191.3±12.35 ^a	8.62±0.39 ^a	3.97±0.35
VI	HCD + Atorvastatin (10mg/kg)	129.2±10.43 ^a	6.58±0.43 ^a	3.90±0.44

Each value are expressed as mean ± SEM (n = 6), ^aP < 0.001, ^bP < 0.01, ^cP < 0.05 when compared to HCD alone treated rats. ^xP < 0.001, ^yP < 0.01, ^zP < 0.05 when compared to Normal diet treated rats. One-way ANOVA followed by Tukey's post test.

Table-3 Effect on TG in normal, HCD, Ayur-NS01 and Atorvastatin administered rats.

Groups	Treatment	TG (mg/dL)
I	Normal diet	72.917±18.353
II	HCD	78.267±16.741*
III	HCD + Ayur –NS 01 (dose 1.35g/kg)	68.115±15.180*
IV	HCD + Ayur –NS 01 (dose 1.8g/kg)	62.052±15.005*
V	HCD + Ayur –NS 01 (dose 2.25 g/kg)	64.055±16.266*
VI	HCD + Atorvastatin (10mg/kg)	71.365±16.947

Each value are expressed as mean \pm SEM (n = 6), P<0.0001 when compared to HCD alone treated rats. P<0.0001 when compared to Normal diet treated rats. One-way ANOVA followed by Tukey's post test.

The high cholesterol diet significantly increased the triglyceride, LDLc, total cholesterol, VLDLc and atherosclerosis index. The result reveals that it has no much impact on the HDLc. The values of these were compared with the normal diet administered rats. After administration with various doses of Ayur-NS 01 (1.35, 1.8 and 2.25g/kg gastric lavage) and atorvastatin (10mg/kg gavage route), the effects on total cholesterol, triglyceride, and HDLc were shown in the table No-1,3 and 3. Insignificant increase in the TGs, VLDLc significant increase in the total cholesterol and LDLc was observed in the HCD alone administered group when it is compared to the normal group. The groups treated with various doses of Ayur-NS 01 and atorvastatin along with HCD were shown significant decrease in the TGs, VLDLc, LDLc and total cholesterol when compared to group II rats. The group treated with 2.25g/kg has shown more effect than other doses of the sample.

CONCLUSION

From the results of first and second model of this study it is confirmed that the sample of AYUR NS01 has shown a significant reduction in the total cholesterol, triglycerides, LDL levels when it is compared to the HCD induced hypercholesterolemia rats. But it has no much impact on HDLc level. It is also concluded that the dose 2.25g/kg bw of Ayur-NS-01 has shown good effect in lowering the cholesterol than the other doses. The results of this study are encouraging enough for further studies aimed at targeting its mechanism involved in the reduction of cholesterol level.

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