

Effect of Arogh, A Herbal Tea on Elevated Lipid Profiles in Rats

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Abstract: Arogh, A polyherbal formulation was found to be effective in treating hypercholesteremia in rats. Increase in body weight, total cholesterol, triglycerides, LDL and VLDL observed in cholesterol fed animals were attenuated by treatment with Arogh. It also reversed the histopathological changes produced in various organs by cholesterol supplementation. Liver and kidney function tests were not altered by either cholesterol or Arogh treatment. Arogh exerts a significant hypolipidemic effect, prevents atherosclerotic changes and reduces obesity in experimental animals.

Key wrds: Cholesterol, arogh, hypolipidemic effect

INTRODUCTION

Atherosclerosis is one of the major diseases causing considerable morbidity and mortality in the present day population^[1]. It is a slow progressive disease that begins in childhood and usually manifests in late middle or old age. It is a documented fact that hyperlipidemia is an important risk factor in atherosclerosis^[2]. A wide variety of therapeutic agents in modern medicine are available for the pharmacotherapy of hyperlipidemic status. However, most of the existing hypolipidemic drugs are not safe and free from side effects^[3]. In time honoured traditional systems of medicine, regular usage of many herbs has been conventionally recommended in the management of hyperlipidemia and other sequelae. Arogh, the herbal tea preparation contains many herbs such as *Hibiscus rosachinensis*, *Eclipta alba*, *Zingiber officinale*, *Nelumbo nucifera*, *Hemidesmus indicus*, *Glycyrrhiza glabra*, *Rosa damascena*, *Terminalia chebula* and *Quercus infectoria* which have been documented to be beneficial in lowering cholesterol level and hypertension, as described in ayurvedic literature.

This herbal tea preparation is being used for many years for the management of obesity and hypertension. However, scientific support to prove the traditional claims remains to be established. Hence, in the present study an endeavour has been made to delineate the edifying effect of Arogh in the changes in lipid profile mediated by cholesterol. The data likely to be generated by this paradigm might be helpful to investigate the claim on this preparation.

MATERIALS AND METHODS

Animals: Male wistar albino rats weighing 150-175 g were used in the study. They were housed in animal experimental laboratory of the Institution under normal housing condition and ambient temperature (28-31 °C). Food and water were allowed *ad libitum*.

Drugs and chemicals used: Arogh was gifted by rumi herbals, chennai, gemfibrosil was purchased from cadila, cholesterol from sigma chemicals and coconut oil from parachute.

Method of inducing hyperlipidemia: The rats were rendered hyperlipidemic using the modified method for^[4]. The rats were orally administered with cholesterol 1500 mg kg⁻¹ body weight in coconut oil (2 mL/rat) with the help of an intragastric tube. The animals received cholesterol continuously for 60 days.

Drug treatment schedule: Thirty animals were chosen for the study and divided into five groups of six each. The control group of animals received normal chow diet throughout the experimental period. Another group of animals received cholesterol supplementation for two months as described earlier.

Arogh treatment: Five gram of Arogh was weighed, soaked in 50 mL of water for 15 min and boiled for 2 min. The decoction was cooled and filtered decoction (30 mL) was considered to represent 5 g of herbal

powder. Two doses of Arogh, viz., 750 mg kg⁻¹ (0.75 mL) and 1.5 g kg⁻¹ (15 mL) were selected for the study. The animals received Arogh at 9 AM everyday, 30 min before cholesterol supplementation.

Gemfibrosil: Another group of animals received gemfibrosil (as suspension in 1% CMC) orally in a dose of 100 mg kg⁻¹ body weight, 30 min prior to cholesterol supplementation. All the treatments were continued for a period of 60 days.

Parameters studied: The blood samples were withdrawn by retro-orbital puncture under light ether anesthesia. The serum was separated and the following lipid profile was estimated. Total Cholesterol (TC), Triglycerides (TGL) and High-Density Lipoprotein (HDL) were estimated by using semi auto analyzer. The level of Low-Density Lipoprotein (LDL) and Very Low-Density Lipoprotein (VLDL) were computed from calculation by using Friedward formula^[9]. The lipid profile was analyzed on days 0 and 60 after completion of different treatments.

Histopathological studies: After the completion of the study (60 days) the animals were sacrificed by cervical decapitation and liver, kidney and aorta were dissected out. Sections were taken from the specimens and studied for histopathological changes.

Statistical analysis: The results were analyzed statistically by employing Student's paired 't' test and unpaired 't' test wherever appropriate. p < 0.05 was considered as statistically significant.

RESULTS

During the study period, all the animals remained healthy. No mortality was observed in any of the treatment groups.

Body weight changes: An increase in the body weight of all the animals was evident after sixty days of treatment with various drugs. In animals treated with cholesterol, the percentage increase in body weight was two fold when compared to control animals (Table 1). In animals that were treated with gemfibrosil cholesterol administration did not show any appreciable increase in body weight.

Administration of Arogh significantly reduced the cholesterol induced increase in body weight by 50% in both the doses (Table 1). The percentage reduction in the body weight induced by gemfibrosil and Arogh was comparable.

Changes in serum total cholesterol and triglycerides:

Administration of exogenous cholesterol resulted in a significant elevation in various parameters of lipid profile. The serum total cholesterol level did not change appreciably between 0 and 60 days measurement in control group of animals. In animals that were treated only with cholesterol the serum total cholesterol was significantly increased. An apparent decrement in cholesterol level with low dose of Arogh and significant decrease with high dose of Arogh was revealed. A maximum reduction in the serum total cholesterol was observed in the animals that received gemfibrosil (Table 2).

There was a significant increase in serum triglycerides in animals fed with cholesterol compared to control group of animals (Table 2). This elevation in serum triglyceride levels was effectively prevented by treatment with both gemfibrosil and Arogh (in both the doses).

Changes in serum lipoprotein fractions: The level of LDL was significantly increased (≈ 30%) in cholesterol fed animals compared to control animals. This increase was completely antagonized by gemfibrosil treatment. However, Arogh administration attenuated this elevation significantly only in higher dose (Table 3).

Table 1: Effect of gemfibrosil or Arogh on Cholesterol induced increase in body weight in rats

Treatment mg kg ⁻¹ p.o	Body weight in grams		% Increase
	0 day	60 days	
Control	153.3±2.1	170.0±1.8 ***	11.00
Cholesterol 1500	154.2±2.0	189.0±2.4 **	22.7
Gemfibrosil 100	155.0±2.2	172.0±1.7 ** †	11.0
+ Cholesterol			
Arogh 750	175.0±2.2	192.0±2.5 **	9.7
+ Cholesterol			
Arogh 1500	158.3±3.0	174.0±1.5 ** †	10
+ Cholesterol			

Each value represents the Mean±SEM, n=6

** p < 0.001 compared to 0 day value (Student's paired 't' test)

† p < 0.001 compared to cholesterol treatment alone (Day 60)

Table 2: Effect of gemfibrosil and Arogh on cholesterol induced changes in serum total cholesterol and triglycerides

Treatment mg kg ⁻¹ p.o	Cholesterol (mg dL ⁻¹)		Triglycerides (mg dL ⁻¹)	
	0day	60 days	0 day	60 days
Control	72.5±3.3	71.3±3.4	34.0±2.3	35.0±2.1
Cholesterol 1500	75.7±2.4	94.3±2.4 **	34.7±2.2	42.5±2.7 *
Gemfibrosil 100	74.8±2.8	75.8±3.1 †	35.2±1.7	28.2±1.9* †
+ Cholesterol				
Arogh 750	71.7±2.8	87.3±3.0 **	33.7±1.9	35.5±1.6 †
+ Cholesterol				
Arogh 1500	74.1±3.1	79.1± 3.3 †	34.5±2.0	34.8±1.5 †
+ Cholesterol				

Each value represents the Mean±SEM of six observations.

* p < 0.01, ** p < 0.001 compared to 0 day value (Student's paired 't' test), † P < 0.01 compared to cholesterol treatment alone (60 days)

Table 3: Effect of gemfibrosil or Arogh on the cholesterol induced changes in serum LDL, HDL and VLDL levels in different treatment groups

Treatment (mg kg ⁻¹ p.o.)	LDL (mg dL ⁻¹)		HDL (mg dL ⁻¹)		VLDL (mg dL ⁻¹)	
	0 day	60 days	0 day	60 days	0 days	60 days
Contro	45.1±2.0	44.1±2.7	20.7±1.5	20.3±1.2	6.7±0.4	7.0±0.4
Cholesterol 1500	46.0±1.1	*59.8±1.3	22.8±1.6	*26.0±1.4	6.9±0.5	8.5±0.5
Gemfibrosil 100 + Cholesterol	44.6±0.8	†44.7±1.7	23.2±1.8	25.3±1.5	7.0±0.3	*‡5.8±0.3
Arogh 750 + Cholesterol	46.9±1.2	*56.6±2.1	23.0±1.7	23.8±1.5	6.7±0.94	†7.1±0.4
Arogh 1500 + Cholesterol	45.1±2.0	†48.3±2.4	22.2±1.7	24.3±1.5	6.9±0.4	†6.9±0.3

Each value represents the Mean±SEM of six observations., *P<0.01 compared to 0 day value (Student's paired 't' test), †P<0.05, ‡P<0.01 and †P < 0.001 compared to cholesterol treatment alone (60 days)

Similar to LDL, a significant increase in VLDL was noticed after 60 days in cholesterol fed animals. This increase was effectively attenuated by Arogh (both doses) and gemfibrosil treatment (Table 3).

When compared to control animals there was a mild but significant increase in HDL after cholesterol supplementation for 60 days (Table 3). In animals that received either Arogh or gemfibrosil, a similar increasing trend in HDL levels was evident though the values were not statistically significant.

Serum samples analysed for liver and kidney functions viz., serum AST, ALT, urea and creatinine were not significantly altered when compared to control group after 60 days treatment.

Histopathology: Histology of heart revealed thickening of vessels with inflammatory infiltrate and hyalinization of fibres in cholesterol fed animals. In gemfibrosil treated animals, though perivascular inflammatory infiltrate was revealed. Reversal of other atheromatous changes was observed in cholesterol treatment. Prominent reversal of the changes that were observed in cholesterol treatment was observed in Arogh treated animals.

The blood vessels of animals fed with cholesterol alone revealed presence of lipid laden intimal smooth muscle cells, accumulation of macrophages, elastic fibres and intracellular and extracellular lipid deposits. These changes were conspicuously absent in sections taken from animals receiving Arogh or Gemfibrosil.

In Cholesterol fed animals sections of liver revealed prominent fatty changes of hepatocytes. Gemfibrosil treated animals showed focal fatty changes with nuclear condensation of hepatocytes. Similarly in Arogh, lower dose treated animals, liver section showed mild focal fatty changes. However in animals treated with high dose of Arogh, no fatty changes were evident and only diffuse feathery change of hepatocytes was evident.

Section of kidney taken from cholesterol fed animals did not reveal any changes in architecture compared to control animals. However in animals that received

Table 4: Effect of gemfibrosil or Arogh treatment on serum AST, ALT, creatinine and urea in cholesterol fed animals at the end of treatment

Treatment mg kg ⁻¹ p.o	AST (IU L ⁻¹)	ALT (IU L ⁻¹)	Urea (mg dL ⁻¹)	Creatinine (mg dL ⁻¹)
Control	25.1±3.5	25.3±1.8	32.3±1.8	1.27±0.1
Cholesterol 1500	29.0±2.1	28.3±1.7	34.7±2.2	2.5±2.7
Gemfibrosil 100 + Cholesterol	28.0±2.7	26.3±1.7	30.3±2.7	1.3±0.1
Arogh 750 + Cholesterol	26.2±1.8	28.1±3.0	31.8±1.7	1.25±0.1
Arogh 1500 + Cholesterol	27.2±2.4	26.9±1.4	29.2±2.8	1.27±0.05

Each value represents the Mean±SEM of six observations

Gemfibrosil and cholesterol there was focal tubular dilatation with inflammatory infiltrate. The histology of kidney was not altered in any way after Arogh treatment and the sections showed normal architecture as observed in control animals.

Thus the present experimental evidences support the time tested traditional claim for the usefulness of this polyherbal formulation in the management of hyperlipidemic status.

The histological sections obtained from different organs in various treatment groups have corroborated with the results of the biochemical estimation. Evidence for the accumulation of fat predominantly triglycerides and atherosclerotic changes were prominent in heart, aorta and liver of cholesterol fed animals. These changes were reverted to major extent by gemfibrosil treatment as well as by Arogh.

These observations provide concrete evidences for the safety and efficacy of the herbal tea Arogh in preventing the pathological changes occurring in various organs due to hyperlipidemia.

DISCUSSION

Administration of cholesterol resulted in elevation of the various parameters of lipid profile. Serum total cholesterol, triglycerides, LDL, HDL and VLDL levels were significantly increased in cholesterol fed animals compared to those animals that did not receive cholesterol supplementation (control).

A significant increase in body weight was noted in all the animals after 60 days of study period. The gain in body weight of cholesterol fed animals was nearly 23% compared to 11% increase in control animals. Treatment with gemfibrosil and Arogh in both doses prevented the excessive increase in body weight found in cholesterol fed animals. This observation supports the traditional claim for the usefulness of this preparation in obesity.

The benefits of hypolipidemic therapy may be effected through alteration in one or more parameters of lipid profile. A reduction in total cholesterol, triglycerides, LDL and VLDL or an increase in HDL may be effected by hypolipidemic agents. In this respect, Arogh treatment has significantly lowered all the parameters of lipid profile except modifying HDL levels. The level of HDL was not significantly altered after 60 days of treatment with Arogh indicating that it may have only a minimum role in modulating the metabolism of HDL.

H. indicus present in the combination prevents hyperlipidemia in albino rats^[6] by increasing the liver LDL receptor activity and decreasing hepatic triglyceride synthesis. The phytosterols including β -sitosterol present might inhibit cholesterol absorption^[7]. Sodium glycyrrhethinate obtained from *G. glabra* exhibited hypolipidemic and anti-atherosclerotic effects in rabbits^[8]. Ginger oleoresin present in *Z. officinale* reduces serum and hepatic cholesterol and increases fecal cholesterol excretion^[9].

Inhibiting the oxidation of LDL will also decrease or prevent atherosclerotic changes^[9]. Oxidative modification of LDL or other lipoprotein is important and possibly obligatory in the pathogenesis of atherosclerotic lesion. Many of the herbs used in Arogh have been shown to exhibit potent antioxidant properties. Ether extract of *T. chebula* possess antioxidant properties^[8]. Antioxidant activity of *Z. officinale* is also reported^[9].

Another method by interfering the cellular oxidative damage by its ability to quench free radicals or by increasing the antioxidant defence mechanism may also be responsible for the desired effect. *H. rosa-chinensis*, *E. alba*^[10], *Q. infectoria*^[11], *R. damascena*^[12], *T. chebula*^[13], *Z. officinale*^[14], *N. nucifera*, *H. indicus*^[15] and *G. glabra*^[16,17] present in the combination have been proved to have antiperoxidative activity.

Hence, Arogh may exert its hypolipidemic effect by multidimensional manner of the following mechanisms. It may interfere with the intestinal absorption of cholesterol, alter the lipid metabolism in liver and other tissues or might have a pivotal role in preventing the oxidative damage of LDL and other lipoproteins.

It was also interesting to note that continuous administration of Arogh for two months did not result in any adverse effect. No abnormal autonomic or behavioral responses were noticed in the experimental animals during the study period. The animals in all the groups remained healthy and no mortality was detected in the tenure of the study. Further the liver function and the kidney as analyzed by AST, ALT, urea and creatinine remained unaffected by treatment with Arogh. Histopathological, evidences obtained from liver, kidney, heart and aorta further corroborated with biochemical investigation. These observations confirm the safety profile of Arogh. This is not unexpected because all the herbs used in Arogh are safely employed in traditional system of medicine since time immemorial.

In conclusion, the result of the present study provides a scientific support to the hypolipidemic effect of the herbal tea, Arogh. The biochemical results are corroborated by the histopathological, observations of different tissues. It can be expected that regular usage of polyherbal formulation may help to lower the lipid levels and protect against atherosclerosis and consequent cardiovascular, cerebrovascular and peripheral vascular complications. This study also provides evidence for the safety of this preparation.

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