

**EVALUATION OF ANTIHYPERCHOLESTEROLEMIC ACTIVITY OF  
ANTICHOL AGAINST CHOLESTEROL COCKTAIL INDUCED  
HYPERCHOLESTEROLEMIA IN RATS**

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**Summary**

In the present study Antichol, a polyherbal formulation was evaluated for its antihypercholesterolemic activity against cholesterol cocktail induced hypercholesterolemia in rats. Antichol, at an oral dose of 0.25 and 1.25 mg/kg, significantly prevented the cholesterol cocktail induced changes in the serum glucose, lipid profile (triglyceride, total cholesterol, LDL cholesterol and VLDL cholesterol) and ALP levels ( $p < 0.05$ ). In addition, it also significantly prevented the cholesterol induced fatty degeneration of the liver and changes in the liver antioxidant enzymes (SOD, GSH and catalase). Based on the above results it may be concluded that, the polyherbal formulation, Antichol, shows a significant protection against cholesterol cocktail induced hypercholesterolemia in rats. The above activity of Antichol may be attributed to its beneficial effects on the *in vivo* antioxidant system.

**Keywords:** Metabolic disorder; Hyperlipidemia; Cholesterol; Antichol; Lovastatin; Lipidperoxidation.

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### Introduction

Atherosclerosis is a leading cause of deaths due to heart attack (CHD) and stroke. This disease is a form of thickening and/or hardening of the arteries, and is characterized by plaque build-up in both large and medium size vessels such as the aorta or carotid arteries. Atherosclerosis actually accounts for 75% of all deaths from cardiovascular diseases (1). Oxidative processes may play an important role in the pathogenesis of many chronic diseases, including atherosclerosis, cancer, arthritis, eye disease, and reperfusion injury during myocardial infarction. Data from *in vitro* and *in vivo* studies suggest that oxidative damage to low-density lipoprotein (LDL) promotes several steps in atherogenesis, including endothelial cell damage, foam cell accumulation, and growth and synthesis of autoantibodies (2-9). In addition, animal studies suggest that free radicals may directly damage arterial endothelium, promote thrombosis, and interfere with normal vasomotor regulation (10-12). Oxidative damage may enhance atherogenesis by a cascade of reactions. *In vitro* data have demonstrated the possible role of these antioxidants in preventing or slowing various steps in atherogenesis by inhibiting the oxidation of LDL or other free radical reactions. These antioxidants have also been shown to prevent experimental atherogenesis in many but not all animal models of atherosclerosis (13,14).

Antichol, a polyherbal formulation manufactured by Bangalore Pharmaceutical & Research Laboratories Pvt. Ltd., Bangalore, India, contains aqueous extracts of *Curcuma longa* (45 %w/w), *Commiphora mukul* (10 %w/w), *Emblica officinalis* (10 %w/w), *Terminalia arjuna* (10 %w/w), *Terminalia belerica* (7 %w/w), *Terminalia chebula* (5 %w/w), *Garcinia cambogia* (8 %w/w) and *Pterocarpus marsupium* (5 %w/w). In the present study the polyherbal formulation, Antichol, was tested for its antihypercholesterolemic activity against cholesterol cocktail induced hypercholesterolemia in rats

### Materials and Methods

#### Animals:

Male Sprague-Dawley rats (150-180g) were obtained from NIMHANS, Bangalore. They were maintained under controlled conditions with free access to standard rodent pellet diet (Amrit feeds Ltd., Bangalore.) and drinking water in plastic bottle with stainless steel sipper tube. All experiments were done according to CPCSEA guidelines after getting the approval of the Institutional Animal Ethics Committee.

#### Drugs and chemicals:

Lovastatin was a gift sample from Micro Labs Ltd., Bangalore, India. Cholesterol was purchased from LOBA chemicals, Mumbai, India. ASAT, ALAT, ALP, triglycerides, total cholesterol, HDL cholesterol, VLDL cholesterol, LDL cholesterol and glucose diagnostic kits were obtained from Merck Ltd., Mumbai, India. The other chemicals and reagents used were of analytical grade.

**Dose selection:**

Based on the clinical oral dose of Antichol (2-6 g/day), it was decided to use two dose levels of 0.25 and 1.25 g/kg, p.o., in rats.

**Preparation of cholesterol cocktail:**

Cholesterol cocktail was prepared by dissolving 100 g cholesterol, 30 g propylthiouracil, and 100 g cholic acid in 1000 ml of peanut oil.

**Antihypercholesterolemic activity in rats:**

Animals were divided into five groups of six each. Group 1 was administered vehicle (peanut oil 10 ml/kg, p.o.) and served as normal, Group 2 was administered cholesterol cocktail (10 ml/kg, p.o.) and served as control, Group 3 was administered lovastatin (40 mg/kg, p.o.) along with cholesterol cocktail (10 ml/kg, p.o.), Group 4 was administered antichol (0.25 g/kg, p.o.) along with cholesterol cocktail (10 ml/kg, p.o.), and Group 5 was administered antichol (1.25 g/kg, p.o.) along with cholesterol cocktail (10 ml/kg, p.o.). All the animals received their assigned treatments for a period of 50 days. On day 51 the overnight fasted animals were anesthetized with ether and the blood was collected from retro-orbital plexus, the serum was separated and used for the estimation of triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, glucose, ASAT, ALAT and ALP using Merck diagnostic kits. After blood collection the animals were culled by deep ether anesthesia and the liver was excised and part of the liver was used for the estimation of liver SOD, GSH, catalase and lipid peroxide levels using standard procedures and the remaining part was fixed in 10% neutral buffered formalin for histopathological analysis.

**Statistical analysis:**

The data was represented as mean  $\pm$  S.E.M. Results were analyzed statistically by one-way ANOVA followed by Dunnett's multiple comparison test using Prism software (Version 4). The minimum level of significance was set at  $p < 0.05$ .

**Results**

**Effect of antichol on serum biochemical parameters:**

Antichol at a dose of 0.25 and 1.25 g/kg, p.o., significantly prevented the cholesterol cocktail induced elevation in the serum triglycerides, total cholesterol, LDL cholesterol, VLDL cholesterol, glucose and ALP in a dose dependent manner ( $p < 0.05$ ). Antichol at a dose of 1.25 g/kg, shows a non significant protection against cholesterol induced changes in serum HDL cholesterol, ASAT and ALAT levels. The standard, Lovastatin (40 mg/kg, p.o.) shows significant protection on all these parameters except on serum glucose, ASAT, ALAT and ALP levels (Table 1).

**Effect of antichol on liver antioxidant parameters:**

Antichol at a dose of 1.25 g/kg, p.o., significantly prevented the cholesterol cocktail induced decrease in the liver GSH, SOD and catalase levels ( $p < 0.05$ ) and also showed a non significant protection against the cholesterol cocktail induced raise in MDA levels. The standard, lovastatin (40 mg/kg, p.o.), showed no significant protection against cholesterol cocktail induced changes in the liver MDA, GSH, SOD and catalase levels (Table 2).

**Effect on antichol on liver histopathology:**

Antichol at a dose of 1.25 g/kg, p.o., and shows moderate degree of protection against cholesterol induced changes in the liver architecture, such as loss of architecture, swollen hepatocytes and sinusoidal spaces and on the fatty degeneration. The standard, lovastatin (40 mg/kg, p.o.), shows a moderate degree of protection against cholesterol induced histopathological changes (Figure 1).

**Discussion**

In the present study antichol significantly prevented the cholesterol induced changes in the serum glucose, lipid profile and liver antioxidant enzymes and changes in the liver histopathology. It was well established that the oxidative stress plays an important role in the pathogenesis of atherosclerosis (10-14). Preparations with potential antioxidant activity may be useful in the prevention of atherosclerosis. Antichol formulation contains aqueous extracts of *Curcuma longa*, *Commiphora mukul*, *Emblica officinalis*, *Terminalia arjuna*, *Terminalia belerica*, *Terminalia chebula*, *Garcinia cambogia* and *Pterocarpus marsupium*, the antioxidant properties of these plants have been well documented, in addition to their beneficial effects on the lipid metabolism (15-20). The anti-atherosclerotic activity of antichol, therefore, may be attributed to its ability to reduce the oxidative stress *in vivo* and its beneficial effects of the lipid metabolism. In conclusion, the results of the present study indicate that the antichol formulation shows significant anti-atherosclerotic activity against cholesterol cocktail induced atherosclerosis in rats.

Table 1. Effect of antichol on serum biochemical parameters

Group	Treatment	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	VLDL cholesterol (mg/dl)	Glucose (mg/dl)	ASAT u/l	ALAT u/l	ALP u/l
1 (Normal)	Peanut oil, (10 ml/kg, p.o.)	102.8 ± 0.6	100.4 ± 1.6	54.3 ± 1.2	25.5 ± 1.9	20.6 ± 0.1	76.9 ± 1.0	89.4 ± 0.8	48.4 ± 0.6	215.9 ± 5.6
2 (Control)	Cholesterol cocktail (10 ml/kg, p.o.)	264.9 ± 1.2 <sup>#</sup>	173.1 ± 0.6 <sup>#</sup>	36.2 ± 1.3 <sup>#</sup>	79.5 ± 1.7 <sup>#</sup>	50.5 ± 1.2 <sup>#</sup>	153.8 ± 2.8 <sup>#</sup>	122.9 ± 5.9 <sup>#</sup>	103.1 ± 1.9 <sup>#</sup>	335.8 ± 14.6 <sup>#</sup>
3	Lovastatin (40 mg/kg, p.o.)	211.3 ± 9.6 <sup>*</sup>	151.6 ± 6.7 <sup>*</sup>	45.5 ± 1.7 <sup>*</sup>	68.9 ± 1.7 <sup>*</sup>	42.3 ± 1.6 <sup>*</sup>	141.2 ± 5.5	128.3 ± 5.9	106.7 ± 3.5	318.1 ± 7.1
4	Antichol (0.25 g/kg, p.o.)	206.4 ± 3.9 <sup>*</sup>	135.8 ± 4.7 <sup>*</sup>	38.2 ± 2.9	53.3 ± 3.1 <sup>*</sup>	36.6 ± 2.5 <sup>*</sup>	122.0 ± 2.1 <sup>*</sup>	126.7 ± 4.2	107.9 ± 3.8	271.1 ± 9.6 <sup>*</sup>
5	Antichol (1.25 g/kg, p.o.)	188.7 ± 3.3 <sup>*</sup>	123.9 ± 5.9 <sup>*</sup>	44.4 ± 3.5	41.1 ± 3.0 <sup>*</sup>	35.3 ± 2.5 <sup>*</sup>	101.5 ± 5.2 <sup>*</sup>	108.8 ± 7.2	103.2 ± 4.6	259.6 ± 9.4 <sup>*</sup>

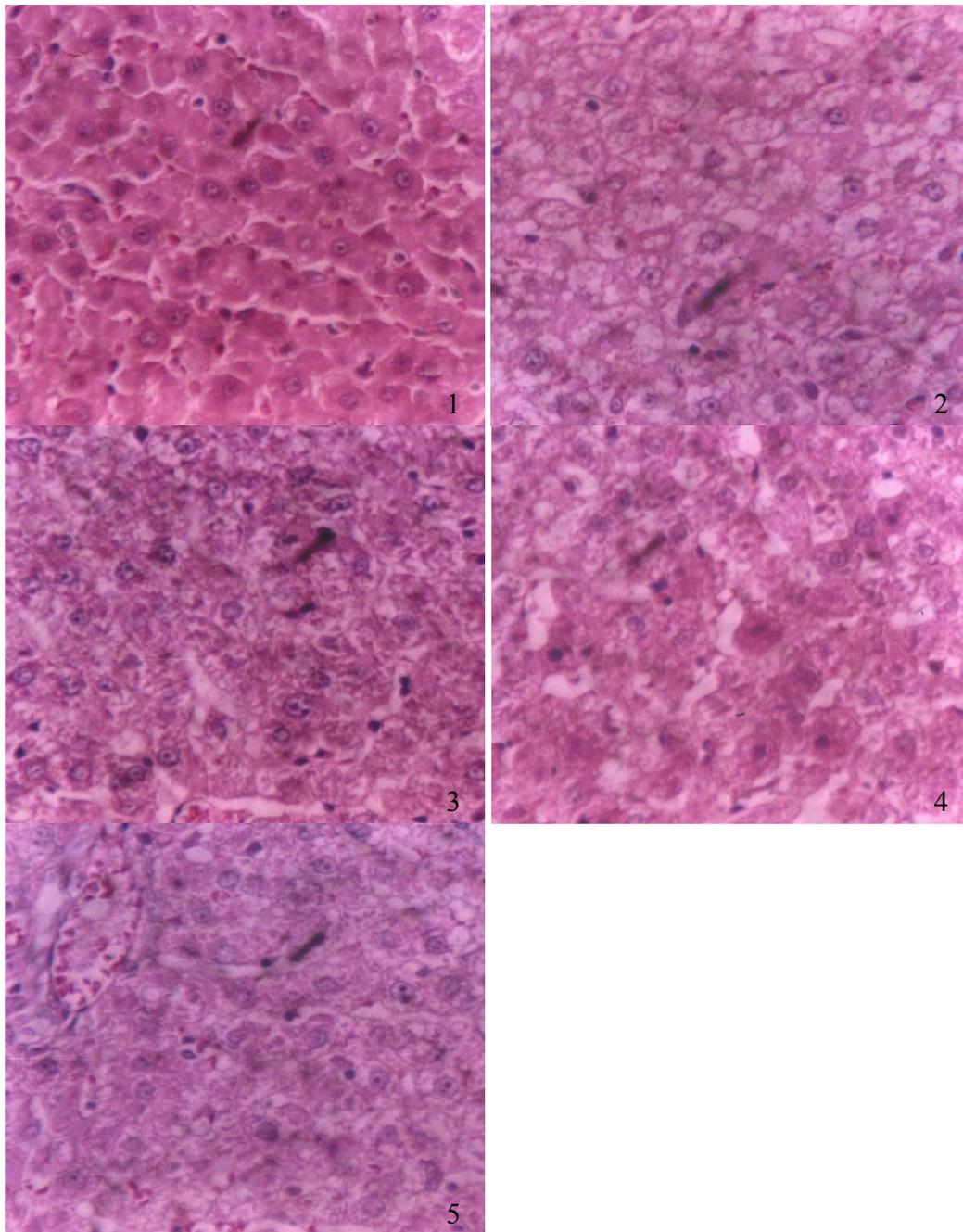
Values are mean ± SEM, n=6, #: p<0.05 when compared to group 1, \*: p<0.05 when compared to group 2.

Table 2. Effect of antichol on liver antioxidant parameters

Group	Treatment	MDA ( $\mu\text{M}/\text{mg}$ tissue)	GSH ( $\mu\text{M}/\text{mg}$ tissue)	SOD (u/mg tissue)	Catalase (u/mg tissue)
1 Normal	Peanut oil, (10 ml/kg, p.o.)	$0.81 \pm 0.05$	$5.99 \pm 0.44$	$12.20 \pm 0.33$	$8.57 \pm 0.35$
2 Control	Cholesterol cocktail (10 ml/kg, p.o.)	$1.20 \pm 0.14^{\#}$	$2.32 \pm 0.33^{\#}$	$6.81 \pm 0.35^{\#}$	$3.76 \pm 0.50^{\#}$
3	Lovastatin (40 mg/kg, p.o.)	$1.27 \pm 0.18$	$2.88 \pm 0.51$	$8.04 \pm 0.40$	$3.00 \pm 0.33$
4	Antichol (0.25 g/kg, p.o.)	$1.27 \pm 0.11$	$2.84 \pm 0.34$	$7.19 \pm 0.37$	$3.08 \pm 0.34$
5	Antichol (1.25 g/kg, p.o.)	$0.93 \pm 0.08$	$4.57 \pm 0.25^*$	$11.06 \pm 0.45^*$	$5.97 \pm 0.38^*$

Values are mean  $\pm$  SEM, n=6, #:  $p < 0.05$  when compared to group 1, \*:  $p < 0.05$  when compared to group 2

Figure 1. Effect of antichol on liver histopathology



1: Group 1(Normal) showing normal appearance of the liver; 2: Group 2 (Control) showing severe degenerative changes such as loss of architecture, swollen hepatocytes and sinusoidal spaces and fatty degeneration; 3: Group 3 (Lovastatin 40 mg/kg, p.o.) showing no protection against cholesterol induced degenerative changes; 4: Group 4 (Antichol 0.25 g/kg, p.o.) showing no protection against cholesterol induced degenerative changes; 5: Group 5 (Antichol 1.25 g/kg, p.o.) showing moderate degree of protection (H&E, 500x).

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