

Research article

Lowering of lipid composition in aorta of guinea pigs by *Curcuma domestica*

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Abstract

Background: A short-term study was carried out using guinea pigs to determine the effects of *Curcuma domestica* on lipid composition in the serum and aorta.

Methods: Animals were given food pellets containing 4% (w/w) powdered rhizome of *C. domestica* in order to determine its effect on cholesterol, triglyceride and phospholipid levels in the aorta and serum. The animals were fed either a cholesterol free diet or a high cholesterol diet (2% cholesterol, w/w, in food pellet) in order to induce hypercholesterolemia. After five weeks of this diet treatment, blood and aorta were taken for biochemical analysis and histological studies.

Results: *C. domestica* in the diet showed no significant effect on the levels of cholesterol, triglyceride and phospholipid in the serum and aorta of the cholesterol free diet animals. However, addition of *C. domestica* to a high cholesterol diet counteracted increases in the levels of cholesterol, triglyceride and phospholipid in the aorta. Histology studies showed less cholesterol deposits in the aorta of high cholesterol diet animals given *C. domestica* compared to the high cholesterol diet animals not given *C. domestica* supplement. *C. domestica* also had a lowering effect on triglyceride level in the serum of high cholesterol diet animals but showed no effect on serum cholesterol and phospholipid levels.

Conclusion: This study has shown that dietary intake of *C. domestica* decreased all lipid composition levels in the aorta and also the serum triglyceride level. In addition, *C. domestica* also reduced cholesterol deposition in the aorta of high cholesterol diet animals.

Background

Curcuma domestica, Valetton (Zingiberaceae) or tumeric (locally known as *kunyit*) has been widely used in India and other parts of Southeast Asia as a spice and a coloring agent in cooking. It is rich in vitamins, carbohydrates, proteins and also contains an array of oils. In

traditional medicine, the rhizomes are used to treat gum inflammation, rheumatism and diarrhea. The medicinal properties of *C. domestica* have prompted many studies to be carried out to determine its pharmacological activities. Ghatak and Basu (1972) [1] found that curcumin (a bioactive component of *C. domestica*) possesses anti-in-

Table 1: Effect of *C. domestica* on cholesterol, triglyceride and phospholipid levels in the serum and aorta.

Group	Cholesterol		Triglyceride		Phospholipid	
	serum (mg/ml)	aorta (μ g/g protein)	serum (mg/ml)	aorta (mg/g protein)	serum (μ mol/ml)	aorta (mmol/g protein)
untreated (control)	4.32 \pm 0.86	5.28 \pm 1.23	7.31 \pm 2.53	28.00 \pm 6.25	0.12 \pm 0.01	1.06 \pm 0.02
given high cholesterol diet	4.66 \pm 1.20	22.06 \pm 4.63*	22.47 \pm 5.29*	65.34 \pm 11.72*	0.14 \pm 0.01	2.48 \pm 0.50*
given high cholesterol diet and <i>C. domestica</i>	4.40 \pm 0.89	5.26 \pm 1.82**	7.30 \pm 0.46**	28.67 \pm 5.17**	0.13 \pm 0.01	1.07 \pm 0.17**
given <i>C. domestica</i>	2.25 \pm 0.90	5.13 \pm 1.24	6.40 \pm 0.45	23.94 \pm 5.14	0.12 \pm 0.01	1.05 \pm 0.09

The data are presented as mean \pm SEM of 6 animals. * Significantly different when compared to untreated group (control), $p < 0.05$

** Significantly different when compared to group given high cholesterol diet, $p < 0.05$

flammatory activity in the carrageenin-induced edema test in rats with an ED₅₀ of 2.1 mg/kg (*i.p.*). They also showed that 3 mg/kg (body weight) of curcumin, intraperitoneally, inhibited formalin-induced arthritis by 45 to 50% in rats. An *in vitro* study conducted by Shankar and Murthy (1979) showed that turmeric oil suppressed the growth of some intestinal and pathogenic bacteria [2].

It has been shown that ingestion of *C. domestica* extract or curcumin counteracted the increment of liver cholesterol in rats which were given a high cholesterol diet [3]. Ethanolic extract of *C. domestica* has also been reported to lower the levels of cholesterol, phospholipid and triglyceride in the serum of Triton-induced hyperlipidemic rats [4]. The conclusion drawn was that the extract may have a protective effect against atherosclerosis since it reduced the low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels in the serum and increased the high density lipoprotein (HDL)/ total cholesterol ratio [4]. However, studies have not been carried out to examine the effect of *C. domestica* on the aorta itself. In this study, we investigated the effect of *C. domestica* on the aorta by determining the lipid composition in the aorta and examining the structural changes in the aortic wall. As a comparison, the serum lipid levels were also determined.

Methods

Animals and diets

Twenty four, male, Dunkin Hartley guinea pigs (*Cavia porcellus*) weighing 700–1000 g were used in this experiment. They were housed individually and were given commercial rabbit chow pellets (Gold Coin Company, Malaysia) daily and a once weekly, vegetable diet of mustard leaves, cabbage and carrot. The animals were divided equally into four groups, i.e. untreated (control), high cholesterol diet-treated (hypercholesterolemic animal),

C. domestica plus high cholesterol diet-treated and solely *C. domestica* diet-treated group.

The control group was given the commercial rabbit chow and vegetable diet through out the experiment. Hypercholesterolemia was induced in the animals by giving cholesterol (Sigma Chemical Co., St. Louis, USA) mixed with the rabbit chow pellet (2% cholesterol, w/w, in food pellet) [5]. For each 100 g of ground rabbit chow pellet, 2 g of cholesterol were added and mixed with a little sterile distilled water. This mixture was made into pellet form and dried in an oven (50°C), overnight. To study the effect of *C. domestica*, powdered *C. domestica* was mixed in the rabbit chow (4% *C. domestica*, w/w, in food pellet) [6]. *C. domestica* rhizome was purchased from a local market in Kuala Lumpur Malaysia. Powdered *C. domestica* was prepared by drying the sliced rhizome in an oven at 45°C for 12 hours and grinding it into powder with a blender. The powdered *C. domestica* was mixed in the rabbit chow in the same way as described for cholesterol.

Collection of serum and aorta

After five weeks of diet treatment, the animals were fasted overnight in preparation for serum and aorta collection. At 8.00 in the morning, the animals were weighed, anesthetized under chloroform and the thoracic abdominal cavity was opened. Blood was collected by heart puncture and serum was separated by centrifugation of the blood. The heart together with the aorta (2–3 cm length) was excised from each animal. The aorta was cut at the origin and removed from the heart. A 2 mm section of the aorta of each animal was soaked in a 10% (v/v) formal saline solution for H & E staining and another 2 mm section was placed in kriomatrix and kept at -20°C for frozen sections. The remaining aorta was soaked in deionized water and homogenized for biochemical analysis.

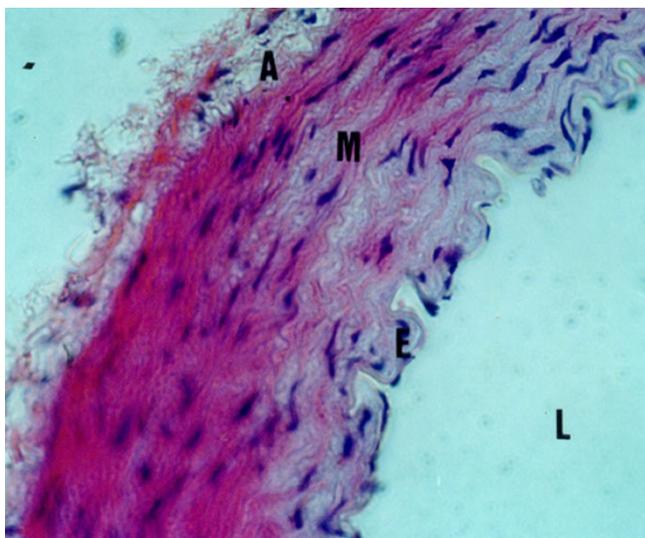


Figure 1
Aorta of untreated (control) animals that was stained with H & E (400 \times). l = lumen, e = endothelium layer (intima tunica), m = media tunica, a = adventitia tunica

Biochemical analyses

Triglyceride level in the serum and aorta were determined using Fletcher's method [7]. Lipid was first extracted from serum and aorta using the method of Folch et al. [8] before the cholesterol and phospholipid levels were determined. The cholesterol level was determined by carrying out gas chromatography (Chemo 8610HT) as previously described [9] and detected by FID 8 61 detector. Estimation of cholesterol levels in both samples were calculated using the internal standard method [10]. Phospholipid level was determined according to the method of Murison et al. [11].

Histology

The aorta sections that were soaked in 10% formal saline solution were processed for normal histological section. The tissue samples were ultrasectioned (5–6 μm thickness), stained with hematoxylin and eosin (H&E) and examined under a light microscope for observation of structural abnormality. For frozen sections, ultra-thin sections of the aorta (8 μm thickness) were stained with Schultz stain [12] and examined for cholesterol deposits.

Statistical analyses

Mean values obtained in the biochemical analyses were analysed for statistical difference with the Student's t-test.

Results

Biochemical Analyses

Table 1 shows that incorporation of 2% cholesterol in the animal diet significantly increased the levels of chole-

sterol, triglyceride and phospholipid in the aorta 4, 2.3 and 2.3 fold respectively compared to the untreated (control) animals. However, serum lipid values show that only the triglyceride level was significantly elevated (3 fold) and no significant change was observed in the other two lipid composition levels in the serum.

Addition of *C. domestica* to the cholesterol free animal diet showed no significant effect on the levels of cholesterol, triglyceride and phospholipid in the serum and aorta compared to the control group (Table 1). Although there appears to be a lowering of serum cholesterol level, this reduction, however, was not significant. On the other hand, addition of *C. domestica* to the high cholesterol animal diet resulted in significantly lowered levels in cholesterol, triglyceride and phospholipid in the aorta compared to the levels of the hypercholesterolemic animals ($p < 0.05$). Reduction of 76%, 56% and 57% in the cholesterol, triglyceride and phospholipid levels respectively, were observed. However, in the serum the lowering effect of *C. domestica* was only observed in the triglyceride level (-68%). The addition of *C. domestica* to the high cholesterol animal diet had no effect on the phospholipid and cholesterol levels in the serum.

Histology

The aorta of animals given mixed diet (cholesterol and *C. domestica*) and *C. domestica* only showed normal histology (Figure 1). However, aorta of high cholesterol diet animals showed spaces within the intima tunica and media tunica. These spaces had originally contained fat droplets which were dissolved during the H & E staining procedure (Figure 2). Observation of tissues that were

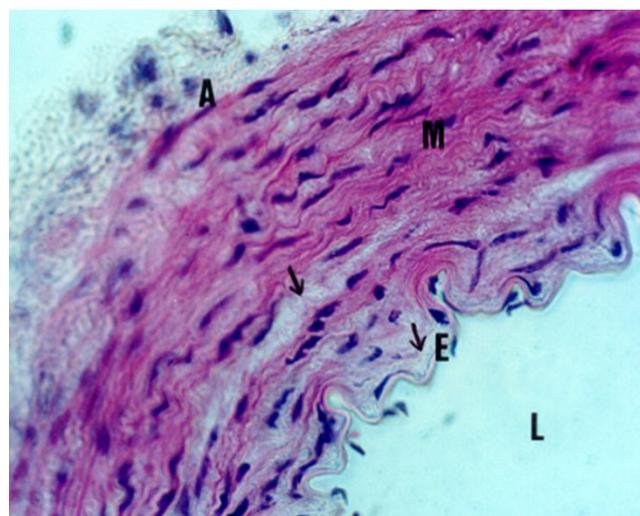


Figure 2
Aorta of a high cholesterol diet animal (400 \times). Arrows indicate spaces within intima tunica and within media tunica.

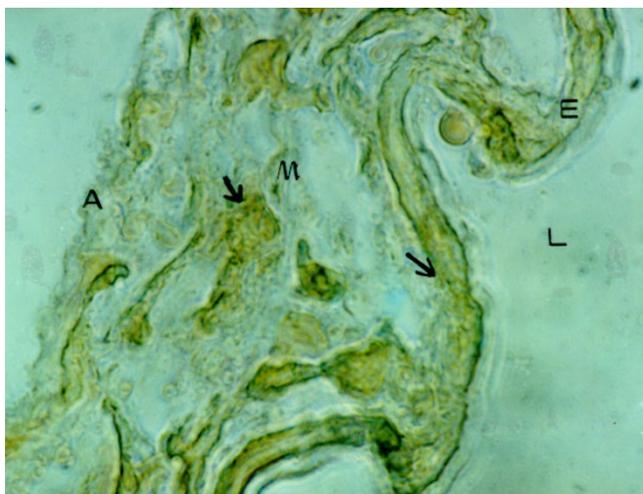


Figure 3
Aorta of a high cholesterol diet animal (400×). Arrows indicate cholesterol deposits in the endothelium layer and media tunica region.

stained with Schultz stain showed cholesterol deposits in the aorta of the high cholesterol diet group and *C. domestica* plus high cholesterol diet group (Figure 3 & 4). In the high cholesterol diet animals, cholesterol deposits were concentrated in the media tunica and intima tunica regions, mainly in the endothelial layer and to a lesser degree in the adventitia tunica region. Less cholesterol deposits were seen in the aorta of the mixed diet animals with very little deposits observed in the intima tunica and media tunica region and no cholesterol deposit seen in the adventitia tunica region.

Discussion

This study found that in spite of the high cholesterol diet given to the animals, there was no corresponding increase in cholesterol and phospholipid levels in the serum. This is contrary to earlier reports which stated that a high cholesterol diet increased serum cholesterol and phospholipid levels in animals given the diet for varying periods of time [13, 14, 15]. However, a study by Srinivasan et al. (1964) [3] found no increase in the serum cholesterol level of rats given 0.5% (0.5 g/100 g basal diet) cholesterol after 6 weeks diet treatment. At the present time, we have no definite explanation for these anomalous results as previous experiments conducted in our lab where the same rabbit chow, vegetable diet and similar percentage of cholesterol were given to guinea pigs for shorter (four weeks) and longer (seven weeks) duration resulted in an increased level of cholesterol in the serum [16]. However, it is not known whether the serum phospholipid level actually increased as it was not recorded.

A possible reason for the lack of increase in the serum cholesterol level in this study could be that more cholesterol was excreted in the bile acid and feces of hypercholesterolemic animals. On the other hand, the lack of increase in the serum phospholipid level could possibly be due to a higher level of phospholipase that metabolised the blood phospholipid in hypercholesterolemic animals.

However, it is clear that the high cholesterol diet significantly increased all lipid composition levels in the aorta. These elevations were lowered when *C. domestica* was mixed in the diet of high cholesterol-diet animals. This is the first study that shows *C. domestica* has this lowering effect on lipid composition in the aorta. In the serum, this lowering effect was seen only in the triglyceride level and not with cholesterol and phospholipid as both these levels were not elevated in the high cholesterol diet group.

Apart from lowering lipid composition in the aorta, *C. domestica* also reduced the cholesterol depositions in the aorta of high cholesterol diet animals. These results indicate that addition of *C. domestica* to the high cholesterol diet of guinea pigs prevented a build up of lipids in the aorta. It is possible that *C. domestica* may project these effects through the reduction of aorta cholesterol level which may simultaneously lower the levels of triglyceride and phospholipid in the aorta. Dixit et al. (1988) have shown that the hypocholesterolemic effect of ethanolic extract of *C. domestica* in the serum was followed by the lowering of triglyceride and phospholipid levels in the serum [4] and it has been repeatedly reported that *C.*

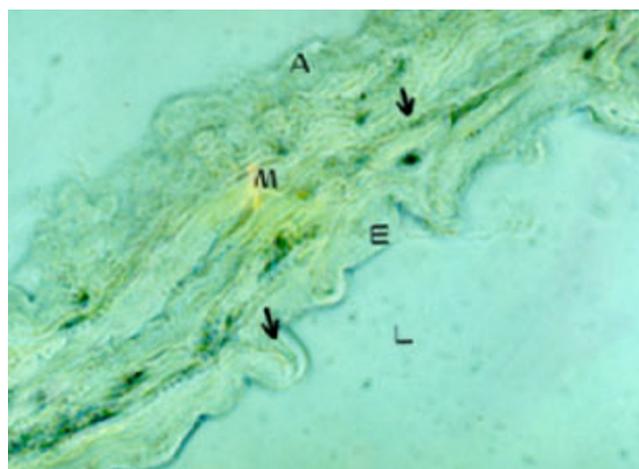


Figure 4
Aorta of an animal on a high cholesterol diet plus *C. domestica* (400×). Arrows indicate very little cholesterol deposits in the endothelium layer and media tunica region.

domestica has cholesterol lowering activity in small and larger animals [15, 17, 18]. Thus the hypocholesterolemic effect of *C. domestica* can probably be explained by its known properties to stimulate bile fluid secretion as well as biliary cholesterol secretion [19, 20] and enhance excretion of bile acids and cholesterol in feces [21]. Hence, these properties of *C. domestica* may explain the lower cholesterol level in tissue such as aorta. Following this, studies ought to be carried out to ascertain whether *C. domestica* has the same effect in human subjects.

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