Chemopreventive Effect of Quercetin in Sarcoma-180-Bearing Mice

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Summary The flavonoid quercetin was assessed for its chemopreventive property in Sarcoma-180(S-180)-induced ascites tumors. The flavonoid was administered in the diet at 2% level to S-180-bearing mice, and the lipid and lipoprotein profile was studied after the treatment. The plasma cholesterol levels increased in the S-180-bearing mice, with the increase attributed to LDL and VLDL; whereas HDL showed a decrease in the S-180-bearing animals. However on administration of quercetin there was a significant decrease in the cholesterol (free, total and ester) and lipoprotein levels (LDL and VLDL) with a corresponding increase in HDL cholesterol. The cholesterol levels of S-180 ascites fluid were also decreased in the quercetin-treated S-180 tumor.

Key Words: Sarcoma-180, ascites tumor, quercetin, cholesterol, lipoprotein

In recent times a number of natural products have been gaining importance in cancer therapy. Cancer chemoprevention can be defined as prevention of cancer by one or more chemical entities, either as natural drugs or as naturally occurring constituents of the diet [1]. Humans are continuously exposed to a variety of carcinogenic chemicals in their day-to-day life. Chemoprevention has been projected to be the most desirable way of eliminating the impact of human cancer [2]. It has been stated that increased vegetable consumption prevents cancer development [3, 4]. Epidemiological studies show a relationship between diet and cancer; i.e., there is a protective effect of the consumption of vegetables and fruits on various types of cancer [5].

Flavonoids are diphenyl propanoids that are ubiquitous in plant foods and

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form important constituents of the human diet [6]. Such compounds were found to inhibit the metabolism of carcinogens in isolated liver microsomes [7]. Dietary supplementation with the flavonol quercetin inhibited 7,12-dimethylbenz(*a*)anthracene (DMBA) and nitrosomethyl urea-induced mammary cancer in female rats [8]. Hertog *et al.* reported the anticancer activity of five dietary flavonoids [9].

Recently Elangovan *et al.* reported the chemopreventive potential of flavonoids against 20-methylcholanthrene-induced tumorigenesis [10, 11]. Hence in the present study, the lipid profile of Sarcoma-180(S-180)-carrying mice treated with the flavonol quercetin was assessed.

MATERIALS AND METHODS

Materials. The flavonoid quercetin was purchased from Bioorganics India, Ltd., Madras, India. The S-180 cell line was obtained from National Facility for Animal Tissue Culture Centre, Pune, India.

Animals and diet. Males of the inbred strain of Swiss albino mice, 7–8 weeks of age and weighing 18–22 g, were obtained from the King Institute of Preventive Medicine (Madras, India). They were kept in well-ventilated polypropylene cages with 12 h of light and 12 h of darkness. The animals had free access to food (Hindustan Lever Ltd., Bangalore, India), and water was provided *ad libitum*.

The diet was powdered, and a 2% diet containing quercetin was prepared and stored in a sealed container at 4°C.

Antitumor test. Ascites tumors were produced by i.p. injection of 1×10^6 S-180 cells into Swiss albino mice. After 15 days, the animals were grouped as a control, S-180-bearing mice, and quercetin-treated S-180-bearing mice.

Quercetin at a 2% concentration was mixed in the basal diet and administered for 15 days. At the end of this period, the mice were sacrificed by decapitation and blood was collected with EDTA used as an anticoagulant; and the plasma was separated. Plasma and ascites fluid cholesterol [12], total cholesterol, free and esterified cholesterol [13], plasma lipoproteins [14], phospholipids, triglycerides [15] and free fatty acids [16] were estimated. The statistical significance between different groups was compared by Student's *t*-test.

RESULTS

Body weight changes and ascites tumor volume of the three groups of animals are shown in Table 1. Quercetin at a 2% concentration caused a decrease in body weight and ascites fluid volume, when these parameters were compared with those of S-180-bearing mice not receiving the flavonoid.

Table 2 shows that the levels of VLDL and LDL were decreased in the quercetin-treated S-180-bearing mice when compared with those of S-180-bearing mice. However, HDL cholesterol was decreased in the S-180-bearing mice, and on

Table 1. Body weights and ascites fluid volume changes in normal, S-180-bearing, and quercetin-treated S-180-bearing mice.

	Average body weight (g)				Ascites fluid volume (ml)		
	0 day	5th day	10th day	15th day	5th day	10th day	15th day
Control	20±1.3	21±2.3	22±2.2	22±2.3			
S-180-bearing mice	20±1.2	24±2.2*	26±3.1*	30±2.8***	1.2±0.5	7.2±1.2	12±1.2
Quercetin-treated S-180-bearing mice	20±1.4	21±2.1	23±2.6	26±2.5 ⁺	0.8±0.03	5.8±0.2 ⁺	9.3±1.0 ⁺

Values are expressed as the mean±SD for 6 animals in each group. As compared with normal * $p < 0.05$, *** $p < 0.001$. As compared with S-180-bearing mice ⁺ $p < 0.05$.

Table 2. Levels of plasma total cholesterol, free cholesterol, esterified cholesterol, and HDL, LDL, and VLDL cholesterol.

	Total cholesterol	Free cholesterol	Esterified cholesterol	HDL	LDL	VLDL
Control	62±7.1	20.2±2.1	42±3.2	35.1±4.5	18±1.3	7±0.78
S-180-bearing mice	70±7.4	21.8±2.5	48.2±4.2*	28.2±3.1*	23±1.5***	12.5±0.95***
Quercetin treated S-180-bearing mice	67±5.8	21.1±2.1	45.3±4.1	31.1±2.8	20±1.8 ⁺	10.7±0.63 ⁺⁺

Values (mg/dl) are expressed as the mean±SD for 6 animals in each group. As compared with normal * $p < 0.05$, *** $p < 0.001$. As compared with S-180-bearing mice ⁺ $p < 0.05$, ⁺⁺ $p < 0.01$.

Table 3. Levels of triglycerides (mg/dl), phospholipids (mg/dl), and free fatty acids (mg/dl) in plasma of normal, S-180-bearing and quercetin-treated S-180-bearing mice.

	Triglycerides	Phospholipids	Free fatty acids
Control	50±6.4	42±4.8	25±2.2
S-180-bearing mice	68±6.7***	58±3.2***	34±3.1***
Quercetin-treated S-180-bearing mice	56±6.5 ⁺	51±5.6 ⁺	29±2.4 ⁺

Values are expressed as the mean±SD for 6 animals in each group. As compared with normal *** $p < 0.001$. As compared with S-180-bearing mice ⁺ $p < 0.05$.

Table 4. Levels of total cholesterol, free cholesterol, and esterified cholesterol contents in the ascites fluid of S-180-bearing mice and quercetin-treated S-180-bearing mice.

	S-180-bearing mice	Quercetin-treated S-180-bearing mice
Total cholesterol	52.1±4.1	48.6±3.7
Free cholesterol	36 ±3.4	34.1±3.1
Ester cholesterol	18.4±1.6	16.1±1.3 ⁺

Values (mg/dl) are expressed as the mean±SD for 6 animals in each group. As compared with S-180-bearing mice ⁺ $p < 0.05$.

treatment with quercetin showed a moderate increase when compared with that for S-180-bearing mice.

Table 3 shows the plasma levels of triglycerides, phospholipids, and free fatty acids of the three groups of animals. In the S-180-bearing animals all the lipid fractions, namely, triglycerides, phospholipids, and free fatty acids, were increased, and quercetin treatment resulted in a moderate decrease in those levels.

The cholesterol levels of the S-180 ascites fluid were significantly higher than those in the fluid from quercetin-treated S-180-bearing mice, as shown in Table 4.

DISCUSSION

Anticarcinogens should have a profound effect on the expression of tumor in experimental animals and ideally have a similar effect on human cancer. Dietary factors play a significant role in both cancer promotion and prevention [17]. In the present study, quercetin administration was found to reduce the lipid and lipoprotein profile in tumor-bearing mice.

Ray *et al.* reported that S-180 ascites tumor cell proliferation was rapid and that the increase in body weight of the animals was directly proportional to the number of S-180 cells in the ascites fluid [18]. Quercetin treatment of S-180-bearing mice significantly blocked the increase in body weight and ascitic fluid volume, thus indicating the antiproliferative effect of this flavonoid. Tumor weight or volume measurement is commonly used to assess the anticancer capacity of a desired drug [19]. Elangovan *et al.* reported a decreased tumor volume after 15 weeks of quercetin administration in 20-methylcholanthrene-induced fibrosarcoma [10].

Lipid and lipoprotein profiles are gaining importance due to the essential role of these molecules in maintaining the membrane architecture and also in the regulation of physiological activity. The present investigation dealt with the levels of plasma (triglycerides, cholesterol free, and esterified), HDL, VLDL, and LDL in tumor-bearing mice and the effect of quercetin on them. Quercetin-administered S-180-bearing mice were found to have decreased plasma cholesterol levels, and VLDL was significantly decreased, whereas their HDL cholesterol was increased, compared with the levels in S-180-bearing mice. The flavonoids are reported to have hypoglycemic and hypocholesterolemic activities. Quercetin and (+)catechin reduced the elevated cholesterol levels significantly in the serum and liver of cholesterol-fed rats [20, 21].

It has been proposed that regulation of lipid metabolism might be affected in neoplastic cells [22]. Epidemiological studies indicate an increased risk of cancer death in subjects with low plasma cholesterol levels [23]. Since cholesterol homeostasis depends on the presence and function of specific receptors on the cell surface, hypocholesterolemia in neoplastic disease may be produced due to altered activity of these receptors.

The triglyceride pool size was increased by 50% in tumor-bearing mice when

compared with that in controls (Ehrlich Ascites Carcinoma) [24]. The levels of triglycerides, phospholipids, and free fatty acids in the plasma of S-180-bearing mice were found to be significantly decreased by the treatment with quercetin. Tumor excision was demonstrated to return triglyceride levels to normal levels, suggesting that tumor cells may be responsible for hyperglyceridemia [25].

The elevated levels of total, free, and esterified cholesterol in the ascites fluid of S-180-bearing mice were found to be normalized by the flavonoid treatment. It is therefore evident that the flavonol quercetin plays a versatile role in correcting the alteration in lipid and lipoprotein metabolism in S-180-bearing mice.

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