

Research Article

# Anticancer and Cholesterol-Lowering Activities of Citrus Flavonoids

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## Abstract

A Citrus crops, to a degree oranges, lemons, and grapefruits, have long been acknowledged for their strength-advancing features. Among the many bioactive compounds in these products, flavonoids have attracted significant attention because of their potential anticancer and cholesterol-threatening activities. This abstract specifies a short survey of the research on these advantageous effects. Citrus flavonoids are a group of polyphenolic compounds with potent antioxidant properties. These compounds have been proven to restrict the growth of malignancy containers and encourage apoptosis, making bureaucracy a promising bidder in malignancy cessation and treatment. They obstruct the miscellaneous stages of malignancy development, including container increases, angiogenesis, and changes. Studies have demonstrated the influence of citrus flavonoids on various types of tumors, including feeling, body parts, and colon tumors. Citrus flavonoids have also been linked to the administration of cholesterol. They can humiliate the levels of depressed-density lipoprotein cholesterol (LDL-C) while increasing extreme-bulk lipoprotein cholesterol (HDL-C), leading to increased cardiovascular strength. Mechanistically, they restrict cholesterol assimilation in the intestines, advance excreta, and regulate cholesterol in the liver. This cholesterol-threatening effect contributes to the prevention of atherosclerosis and heart failure associated with coronary thrombosis. However, the advantages of citrus flavonoids may change depending upon determinants, such as the particular compound, portion of drug or other consumables, and individual instability. Further research is needed to elucidate the fundamental machinery and optimize their healing potential. In conclusion, citrus flavonoids exhibit promising anticancer and cholesterol-threatening activities, making the ruling class a valuable part of an active diet and potential nominees for future pharmaceutical incidents. Incorporating citrus crops into an individual's diet may be part of an open approach to support malignancy prevention and cardiovascular strength.

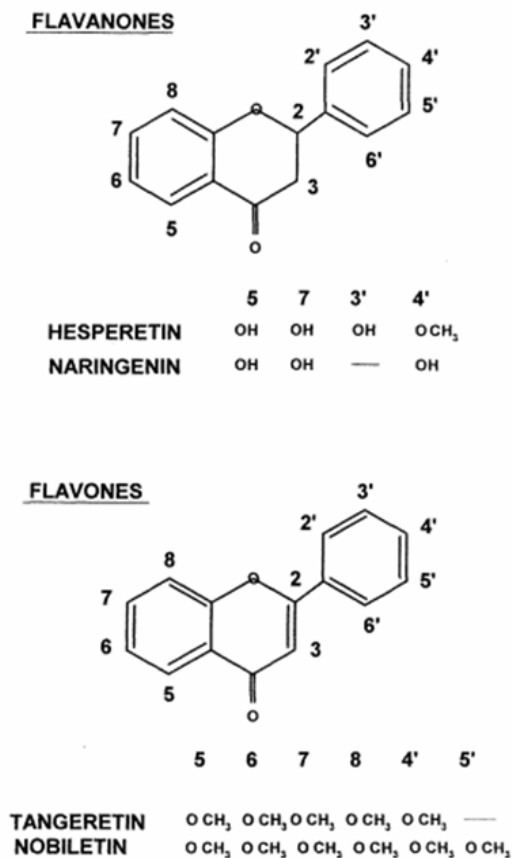
**Keywords:** Citrus Flavonoids, Anticancer, Cholesterol-Lowering, Bioactive Compounds, Polyphenolic Compounds, Antioxidants, Apoptosis Cancer, Low-Density LDL Cholesterol, HDL Cholesterol.

## 1. Introduction

Flavonoids are a group of polyphenolic compounds ever-present in many plants containing products, herbs, crazy, sources, grains, beverages, and beverages [1]. Over 6,000 various flavonoids have been described [2]. They occur in their free forms, glycosides, and methylated descendants. Citrus flavonoids are a big class of subordinate metabolites that have important biological activity [3]. These subordinate metabolites are about the citrus products at approximately extreme levels and their allure and possessions are well described [4]. Flavonoids from citrus are benzo-y-prone products and reside mostly in two classes: flavanones and flavones (Figure 7.1). The most widely accepted flavanones are hesperidin from oranges and naringenin from grape-

fruit, two together in the direction of the product tissue and peel chiefly as their glycosides, hesperidin and Naringenin. Hesperidin arranges the darkened presence of coral liquid squeezed from the plant on account of allure's weak solubility in water and Naringenin is an individual of the main sharp law in grapefruit. Relatively coarse in citrus are two polyhydroxylated flavones, tangerine, and nobilet in two together present in tangerines [5]. Dietary consumption of citrus flavonoids is solid, especially in nations with extreme devouring of citrus juices. However, the bioavailability of these compounds remains poorly understood. Recent studies showed that in persons, the free forms of hesperidin and Naringenin present in citrus juices may be involved in the ancestry system5 seemingly following their freedom from the glycosides

by stomach bacteria [6]. Some flavonoids, particularly those occupying methoxy groups, in the way that hesperetin and polyethoxylated flavonoids were again supposed to wait more interminable in the frame on account of their facilitated rude answer by cells [7].



**Figure 7.1:** Formulae of Flavonoids

The role of dietary citrus flavonoids in human health is of growing scientific interest. The beneficial effects reported over the past seven years include anti-allergic, anti-inflammatory, antihypertensive, and diuretic effects, as well as anticancer and Hypolipidemic properties [8-15]. This chapter focuses on the authors' recent in vitro and in vivo experiments aimed at investigating the anticancer and cholesterol-lowering potential of citrus juices and the principal citrus flavonoids.

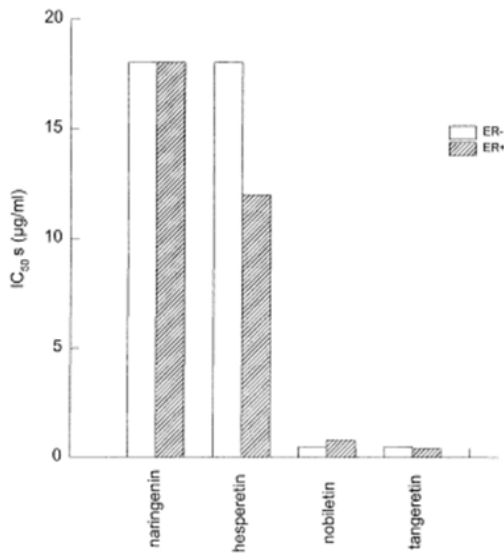
### 1.1. Citrus Flavonoids and Cancer

Breast cancer is the most prevalent cancer in women in developed countries, and its incidence has been increasing worldwide [16]. Attempts to improve survival and reduce the risk of relapse following diagnosis have shown limited success; thus, there is still substantial room for improvement although researchers are currently evaluating new drugs, another promising approach is the investigation of

dietary components as anticancer agents [17]. Epidemiological studies on diet and cancer have provided leads in the search for naturally occurring anticancer agents. There is general agreement that plant-based diets, rich in whole grains, legumes, fruits, and vegetables, reduce the risk of various types of cancer, including breast cancer [18]. A variety of compounds produced by plants have been investigated for their anticancer activity [19]. These include flavonoids, which are an integral component of the human diet. Our interest in the anticancer properties of citrus flavonoids began with the observation that naringenin inhibited the proliferation and growth of MDA-MB-435 estrogen receptor-negative (ER-) human breast cancer cells in culture more effectively than did genistein [20]. This led us to conduct further studies, which have produced several important observations.

### 1.2. Cell Culture Studies

**Effects on Estrogen Receptor-Negative Cells** Citrus flavonoids were examined for their effects on the increase of MDA-MB-435 ER-human conscience tumor containers in civilization [21]. The IC (aggregation that prevents container conception by 50%) principle is shown in Figure 7.2. Hesperetin, the aglycone of the flavonoid present in oranges, was established to prevent ER-human bosom tumor cells as efficiently as naringenin (Figure 7.2). Two additional citrus flavonoids, tangerine, and nobiletin, in tangerines, were much more active in preventing the increase in these containers (Figure 7.2). The ability of citrus flavonoids to prevent container tumors was further examined by discussing the containers at their IC concentrations and following the tumors in the containers over 10 years. 2 J they slowed the development of these containers, and the effect was obvious after two days of the situation. The Cytotoxic properties of the citrus flavonoids were examined utilizing the 3-(4,s-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium platitude (MTT) assay [22]. Most containers were practicable at K concentrations, indicating that the antiproliferative activity of the compounds was not due to extensive cytotoxicity. Effects on Estrogen Receptor-Positive Cells The effects of citrus flavonoids on the conception, development, and being of MCF-7 ER+ human cancer cells were further investigated [23, 24]. The K principle is shown in Fig. 7.2. Tangeretin and nobiletin were repeated ultimate direct inhibitors, with accompanying ICs of 0.8 and 0.4 pull, respectively (Figure 7.2). Further studies were undertaken to determine whether this restriction was due to flavonoids in the way that antiestrogens work. MCF-7 containers were consumed by all inner steroids and doctored with accompanying flavonoids or tamoxifen (a drug established in birth control method-susceptible conscience malignancy) in the omission or demeanor of 100 nM estradiol, as previously described (14). The results (Figure 7.3) show that the restriction by all citrus flavonoids was unchanged by estradiol, contrary to the results for tamoxifen.



**Figure 7.2:** Inhibition of ER and ER+ Human Breast Cancer Cells by Citrus Flavonoids

Synergistic effects in different studies accompanying ER- and ER+ human breast tumor containers, we noticed that 1:1 associations of citrus flavonoids accompanying tocotrienols (a form of source of nourishment E) (Tables 7.1 and 7.2) or tamoxifen and 1:1 mixtures of tocotrienols accompanying tamoxifen (Tables 7.3 and 7.4) prevented the proliferation of the containers more effectively than the individual compounds by themselves. The most persuasive mixture accompanying the ER containers was tangeretin and  $\gamma$ -tocotrienols (IC<sub>50</sub>, 0.05,  $\mu\text{g}/\text{ml}$ ) (Table 7.1). With ER+ containers, high-quality results were obtained with tangeretin and  $\gamma$ -tocotrienol (K, 0.02  $\mu\text{g}/\text{ml}$ ) (Table 7.2), nobiletin + tamoxifen (K, 0.4  $\mu\text{g}/\text{ml}$ ) (Table 7.4), and S-tocotrienol + tamoxifen (K, 0.003  $\mu\text{g}/\text{ml}$ ) (Table 7.4) [25]. When linked in 1:1:2, mergers of flavonoids, tocotrienols, and tamoxifen, tangerine +  $\gamma$ -tocotrienol + tamoxifen was ultimately persuasive in ER- containers (K, 0.01  $\mu\text{g}/\text{ml}$ ) (Table 7.3) and hesperetin + S-tocotrienol + tamoxifen was ultimate direct in ER+ containers (K, 0.0005  $\mu\text{g}/\text{ml}$ ) (Table 7.4).

**Table 7.1: Synergistic effects of flavonoids and Tocotrienols on inhibition of Proliferation of mda-mb 435 ER-human Breast cancer cells in Culture [IC<sub>50</sub> (µg/ml)]**

Flavonoid	Tocotrienols			
	None	$\alpha$	$\text{O}^0$	$\gamma$
Nonc		90	90	30
Naringenin	18	8	1	4
Hesperetin	18	2	19	19
Tangeretin	0.5	0.1	0.1	0.05
Nobiletin	0.5	2	0.25	0.5

**Table 7.2: Synergistic Effects of Flavonoids and Tocotrienols on Inhibition of Proliferation of MCF-7 ER+ Human Breast Cancer Cells in Culture [IC<sub>50</sub> (µg/ml)]**

Flavonoid	Tocotrienols			
	None	$\alpha$	$\text{O}^0$	$\gamma$
Nonc		6	2	2
Naringenin	2	1	0.4	0.7
Hesperetin	2	2	3	0.1
Tangeretin	0.8	0.4	0.04	0.02
Nobiletin	0.4	2	0.8	0.8

**Table: 7.3: Synergistic Effects of 1:1 and 1:1 Combinations of Flavonoids, Tocotrienols and Tamoxifen<sup>a</sup> on Inhibition of Proliferation of MDA-MB-435 – Human Breast Cancer Cell in Culture [IC<sub>50</sub> (µg/ml)]**

Flavonoid	Tocotrienols			
	None	$\alpha$	$\text{O}^0$	$\gamma$
Nonc	90	2	6	2
Naringenin	10	6	2	0.5
Hesperetin	13	2	6	9
Tangeretin	0.5	0.1	0.1	0.01
Nobiletin	0.5	2	0.25	0.5

Tamoxifen was Present in Each Case in These Essays. Thus, the top line Gives Results For 1:1 Combinations of Tocotrienols and Tamoxifen, while the Left-hand Column Gives Results for 1:1 Combinations of Flavonoids with Tamoxifen. All other results for 1:1 Combinations.

**Table: 7.4: Synergistic Effects of 1:1 and 1:1:1 Combinations of Flavonoids, Tocotrienols and Tamoxifena on Inhibition of Proliferation of MCF-7 ER+ Human Breast Cancer Cell in Culture [IC<sub>50</sub> (µg/ml)]**

Flavonoid	None	α	0 <sup>0</sup>	γ
Nonc	0.4	0.1	0.003	0.01
Naringenin	1.2	0.1	0.4	0.008
Hesperetin	0.3	0.4	0.0005	0.8
Tangeretin	0.04	0.4	0.02	0.02
Nobiletin	0.4	0.07	0.001	0.09

Tamoxifen was Present in Each Case in These Essays. Thus, the top line Gives Results For 1:1 Combinations of Tocotrienols and Tamoxifen, while the Left-hand Column Gives Results for 1:1 Combinations of Flavonoids with Tamoxifen. All other results for 1:1:1 Combinations.

### 1.3. Animal studies

Chemically Induced Mammary Carcinogenesis Model: - The ability of citrus juices and flavonoids to restrict mammary tumors in female Sprague-Dawley rats by 7, 12 dimethylbenzanthracene (DMBA) was examined. L Rats were augmenting a semi-purified diet containing 5% grain lubricant. One group was likely double substance (restored). From stopped concentration at two opportunities (usual substance): tangerine liquid squeezed from the plant, and another double-substance grapefruit liquid squeezed from the plant in consideration of consuming water. In these groups, the hydrogen component of the diet was weakened to fix the oxygen component in the crop juices. A tertiary group was likely Naringenin, and one of four equal parts of the group was likely naringenin, each meddling with the foodstuff in amounts comparable to those acquired by inhaling the double-strength grapefruit liquid squeezed from the plant. Having five of something. The group supplemented with the semi-purified diet was used as the control. The rats were palpated for tumor newspapers. After 16 weeks, they were gone and the tumors were removed, weighed and shipped for histological tests. The double-substance combination of red and yellow liquid squeezed from plant-shy tumors was better than the double-substance grapefruit liquid squeezed from the plant (Figure 7.4), even though the flavonoids present in these juices were evenly effective in vitro.

This shows that hesperetin seemingly retains the allure influence in vivo better than naringenin because the particular compounds are present in each liquid squeezed from the plant at comparable levels. It is important that even though coral liquid squeezed from plant collections came into view to prevent mammary carcinogenesis, the rats in this place group granted better pressure gains than those in additional groups. The tinier tumor burden in rats most likely, a combination of red and yellow liquid squeezed from a plant does not give the impression of a slack tumor restriction. Naringenin (glycoside form of the flavonoid naringenin from grapefruit) is again shy mammary carcinogenesis (Figure 7.4), but the rats in this group displayed the smallest pressure gains than those in additional groups, and this grant of permission has had an influence on carcinogenesis." When the LHC experiment was frequent, utilizing a semi-purified diet containing 20% instead of 5% grain lubrication, identical results were obtained.

### 1.4. Mammary Xenograft Model

It is famous that estrogen receptor-negative MDA-MB-435 human feelings tumor containers will add up to tumors and separate into the alveolus when introduced into the mammary fat pads of immunodeficient rodents. This animal model supplies a more direct link to our artificial studies and will allow us to study likely belongings that have a connection with the incorporation, distribution, and absorption of citrus juices and their constituents on their ability to inhibit the growth and metastasis of human breast cancer cells.

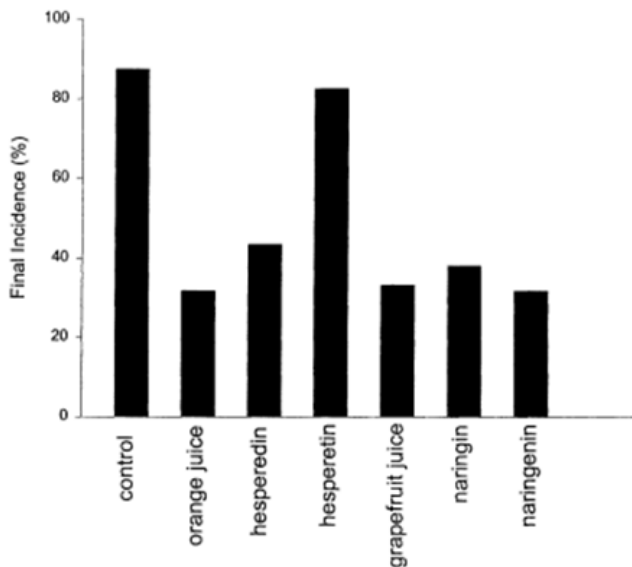
A study was therefore conducted to determine the effects of orange juice, grapefruit juice, and their constituent flavonoids, on the growth of MDA-MB-435 human breast cancer cells injected into the mammary fat pads of nude mice. [26]. The animals were randomly divided into seven groups of 24 animals each. The rats were fed a semi-purified diet containing 5% corn oil. One group was given double-strength orange juice and another double-strength grapefruit juice instead of drinking water. For these groups, the carbohydrate component of the semi-purified diets was reduced to compensate for carbohydrates in fruit juices. A third group was given Naringenin, a fourth naringenin, a fifth group hesperidin, and a sixth group hesperetin, each mixed in the diet to provide amounts, which are comparable to those obtained by drinking double-strength juices. A seventh group was fed a semi-purified diet with plain drinking water and served as a control.

After one week, the mice were anesthetized with methane, and 1 X 10<sup>6</sup> MDA-MB-435 ER- human breast cells were injected in a volume of 50 µl into a right-sided mammary fat pad, which was exposed using a 5-mm incision. This was to ensure that the cells were injected into the mammary fat pad and not into subcutaneous space. The mice were weighed and the inoculation site was palpated for tumors at weekly intervals. Palpable mammary fat pad tumors were measured weekly using calipers. After 11 weeks, the animals were sacrificed and the tumors, lymph nodes, and lungs were excised, weighed, and sent for histological examination.

The incidence of mammary fat pad tumors was reduced by more than 50% in the mice given orange juice, grapefruit juice, Naringenin, hesperidin, and naringenin (Figure 7.5). Lymph Node and lung metastases were lowest in the orange



juice and grapefruit juice groups. Followed by the groups given Naringenin, hesperidin, or naringenin [27]. Our results indicate that growth and metastasis of these tumors in nude mice are strongly inhibited by orange and grapefruit juice and However, this inhibition cannot be entirely attributed to the constituent flavonoids. We have also investigated another class of compounds present in citrus limonoids, which have anticancer activity



**Figure 7.5:** Final Incidence of Mammary Fat Pad Tumors in Female Immunodeficiency Mice after Injection of MDA-MB-435 human breast cancer cells into the mammary fat pad.

Citrus limonoids are one of the two sour concepts located in citrus culmination, inclusive of lemon, lime, orange, and grapefruit [28]. They have been shown to have anticancer effects. 2x Nomilin decreased the prevalence and number of chemically induced stomach tumors in mice when administered by gavage [29].

The addition of nomilin and limonin to the food plan inhibited lung tumor formation in mice and topical application of the limonoids has been found to inhibit both the initiation and the merchandising phases of carcinogenesis within the skin of mice [30]. We've got recently tested the impact of nomilin, limonin and limonin glucoside on the proliferation and growth of ER-human breast cancer cells in lifestyle Nomilin changed into the only, having an IC of zero.4 pull. We additionally examined a glucoside mixture and determined it to have an even lower k of 0.08 pglml [31].

### 1.5. Citrus Flavonoids and Hypercholesterolemia

Extended ranges of blood cholesterol are among the principal risk factors associated with coronary heart disease (CHD), the leading cause of death in North America. The affiliation is largely due to the importance of cholesterol, especially low-density lipoprotein (LDL) cholesterol, during the formation and development of atherosclerotic plaques and

the underlying pathological condition of CHD. Blood concentrations of general and LDL cholesterol are motivated by using the weight-reduction plan and nutritional Intervention has been extensively used in the prevention and treatment of hypercholesterolemia. Dietary techniques usually used to reduce LDL levels of cholesterol consist of modifications to the intake of numerous macro- and micronutrients inclusive of fats, LDL cholesterol, carbohydrates, and protein [32]. Throughout the latest years, some reports have suggested that another feasible way of enhancing the blood lipid profile may be via the expanded consumption of flavonoids [33]. Previous epidemiological studies confirmed that consumption of fruit and greens is related to reduce the risk of cardiovascular disease [34]. These beneficial responses have been postulated to be due to flavonoids. The Cardioprotective effects of flavonoids appear to be in large part related to their movement as antioxidants and as inhibitors of platelet aggregation, but several flavonoid arrangements have been suggested to produce cholesterol-decreasing responses in animals and cells [35-37]. Among the plant flavonoids formerly investigated for their viable LDL cholesterol-lowering potential, the highest-quality known isoflavone from soybean consists mainly of genistein. Dietary soybean isoflavone triggered decreases in VLDL (very low-density lipoprotein) and LDL cholesterol in a few animal models [38, 39]. But those beneficial changes have not been shown in other animal and human research [40-42]. The predominant citrus flavonoids are hesperetin from oranges and naringenin from grapefruit, which are structurally similar to genistein. Hesperidin and an aggregate of Flavonoids containing mainly hesperidin and Naringenin have additionally been said to produce hypolipidemia. Results for cholesterol-fed rats. Is this counseled that citrus flavonoids and the juices from which they may want to have cholesterol-reducing capabilities?

### 1.6. Animal Studies

To decide whether dietary citrus juices ought to produce cholesterol-reducing responses in vivo, we investigated their effects in rabbits, wherein hypercholesterolemia associated with an Elevation of LDL cholesterol is triggered by feeding an LDLcholesterol-free, casein-based, semi-purified diet [43, 44]. In this take a look at, replacing drinking water with either double-strength orange juice or Double-power grapefruit juice decreased increased ranges of LDL cholesterol by 43 and 32%, respectively (Fig 7.5). This was associated with a sizeable 42 % reduction in liver cholesterol esters but not with increases in fecal excretion of LDL cholesterol and bile acids. The decrease in LDL cholesterol was not due to the additional intake of sugars from the juices because this was compensated by adjusting the composition of semi-purified diets and because, in rabbits, sugars have been stated to have little effect on hypercholesterolemia [45]. The juices were also not going to act as cholesterol sequestrants inside the gut, as they did not increase fecal excretion of LDL cholesterol and its metabolites. Eventually, the LDL cholesterol-reducing consequences of the juices were not going to be associated with their excessive content material

**Table: 7.5: Effects of Dietary Orange Juice and Grapefruit Juice on Serum Total and LDL Cholesterol Levels in Rabbits with Experimental Hypercholesterolemia****Cholesterol mmo1/1**

Group	N	Total	LDL
Control	12	4.90 ± 0.47	2.91 ± 0.32 <sup>3</sup>
Orange Juice	11	3.55 ± 0.49	1.66 ± 0.29 <sup>6</sup>
Grapefruit Juice	11	3.85 ± 0.35	1.99 ± 0.22 <sup>6</sup>

Value are means ± SEM. Values Bearing Different Letters are Significantly Different at  $p < 0.05$

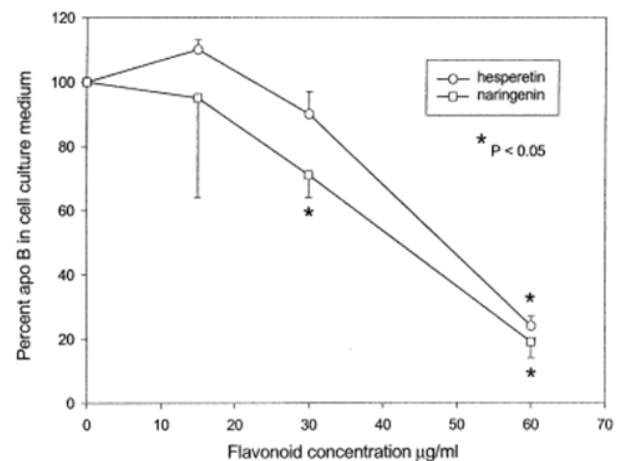
Of vitamin C due to the fact this isn't a required nutrient inside the rabbit [46]. "Therefore, our facts allowed us to speculate that changes in LDL cholesterol and within the liver cholesterol esters are probably produced by minor components of the juices, such as flavonoids. In addition, recent studies showed that dietary supplementation with mixtures of citrus flavonoids containing, in large part, hesperidin and Naringenin lowered serum LDL cholesterol in rats fed an LDL cholesterol-enriched diet." This effect was associated with a decrease the in vitro activity of acyl-CoA: LDL cholesterol o-acyl transferase (ACAT), an enzyme chargeable for cholesterol esterification in the liver.

### 1.7. Cell culture Studies

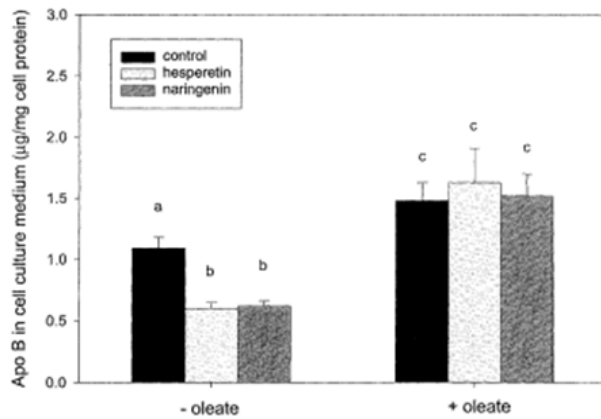
To determine whether citrus flavonoids may adjust cholesterol metabolism without delay inside the liver, we investigated their mechanism of action in the human liver mobile line HepG2 [47]. Which has been used because HepG2 cells can secrete in addition to catabolizing lipoproteins similar to LDL.38 In these experiments, confluent HepG2 cells had been pre-incubated for 24 h in a serum-loose medium, which inhibits cell proliferation and stimulates the biosynthesis of cholesterol-containing lipoproteins. They were exposed to various concentrations (as determined by MTT viability). Assay 22 of both hesperetin and naringenin every 24 h. At the end of the incubation, adjustments in the medium stages of Apo lipoprotein B (apo B), the structural protein of LDL, were evaluated using ELISA and compared to adjustments caused in the absence of flavonoids. The results showed that both hesperetin and naringenin precipitated a similar dose-structured discount of net apo B secretion (Fig 7.6), always with LDL cholesterol-reducing responses observed in rabbits given citrus juices. 44 Considering that at the awareness level of 60  $\mu\text{g}/\text{mL}$ , both flavonoids decreased medium apo B very effectively (by 76 and 8%, respectively, for hesperetin and naringenin), these concentrations were used to signify apo B responses similarly and to investigate the underlying mechanisms.

The results of our study validated that both flavonoids induced an approximately 50% medium apo B reduction after 4 h of incubation, and this was not related to changes in the

incorporation of leucine into the overall cell and secreted proteins over the same duration. This indicated that the B responses to flavonoids were rapid and selective, most likely because of a post-translational modulation of apo B secretion [48]. In addition, we studied the viable mechanisms of this modulation and revealed that the apo B-decreasing effect of flavonoids was maintained within the presence of a particular inhibitor of proteases responsible for intracellular apo B degradation. However, the effect disappeared for the duration of the co-incubation of cells with oleate, a compound known to stimulate cell biosynthesis of impartial lipids 4 (Fig 7.7). This indicated that flavonoids are not likely to exert their apo B-reducing action by increasing intracellular apo B degradation at some point in the early stages of lipoprotein formation. Rather, they appear to interfere with the supply of neutral lipids required for the assembly and secretion of lipoproteins. In settlement, our I4C-acetate labeling looked at showed a 50% lower level of cholesteryl ester synthesis in cells exposed for



**Figure 7.6:** Effects of various nontoxic concentrations of hesperetin and naringenin on apo B accumulation in the media of HepG2 cells. Cells were incubated for 24 h in the presence of 0 to 60  $\mu\text{g}/\text{mL}$  of either Hesperetin or naringenin. Medium apo B concentrations were measured by ELISA and expressed per milligram cell protein. Values are means  $\pm$  SEM,  $n = 3$ .



**Figure 7.7:** Effects of citrus flavonoids on apo B concentration in medium of HepG2 cells in presence of oleate. Cells were preincubated for 1 h with or without 0.8 mM sodium oleate and then incubated for 4 h in the absence or presence of flavonoid (60 µg/ml), and also in the absence or presence of oleate. Medium apo B concentrations were measured by ELISA and expressed per mg cell protein. Values are means  $\pm$  2 SEM,  $n = 4$ . Values with different letters are significantly different,  $P < 0.05$ .

four hours to either hesperetin or Naringenin. A comparable remark has been pronounced with the aid of our collaborators, who have proven that naringenin can suppress the in vitro interest of hepatic ACAT [49].

## 2. Research Method

**2.1. Objective:** This study aimed to investigate the potential anticancer and cholesterol-threatening effects of citrus flavonoids.

**2.2. Sample Selection:** Citrus fruits were picked from the beginning of flavonoid production. Various citrus classes and assortments were included in this study.

**Experimental Design:** This study was conducted using artificial and in vivo experiments.

**2.3. In vitro assays:** Cell sophistication experiments were performed using malignancy container lines to evaluate the anticancer properties of citrus flavonoids. In vivo assays Animal models (such as rodents or rats) were used to assess the cholesterol-threatening properties of citrus flavonoids. Additionally, in vivo tumor models were used to evaluate anticancer properties.

**2.4. Data Collection:** Various biochemical assays, such as container animation assays, cholesterol level calculations, and cyst development appraisals, were performed. Statistical analysis was performed to determine the meaning of the results.

## 3. Results

**Anticancer Activities:** - Artificial experiments with citrus flavonoids slowed the increase in tumor cells in a dose-weak form. In vivo studies showed a decline in swelling development in the citrus flavonoid-treated group compared to that

in the control group. Mechanistic studies have indicated that citrus flavonoids can induce apoptosis and inhibit angiogenesis in malignant cells. Cholesterol-lowering Activities In animal models, citrus flavonoids have been found to considerably decrease antioxidant cholesterol levels. These findings confirm the ability of citrus flavonoids to restrict cholesterol synthesis and improve cholesterol defecation.

## 4. Discussion

The anticancer features of citrus flavonoids may be attributed to their ability to promote apoptosis and restrict angiogenesis in tumor cells, which are critical for preventing lump progression. The cholesterol-threatening properties of citrus flavonoids can be advantageous for reducing the risk of cardiovascular ailments and reconstructing overall well-being. This study supports the idea that a diet rich in citrus crops can have potential health benefits, specifically for those at risk of malignancy or with accompanying extreme cholesterol levels. Further research is needed to distinguish citrus flavonoids as the reason for these properties and to understand their microscopic mechanisms in more detail.

## 5. Conclusion

This study provides evidence that citrus flavonoids retain both anticancer and cholesterol-threatening actions. Citrus flavonoids have the potential to be used as digestive supplements or functional foodstuffs to enhance their strength and reduce the risk of tumors and cardiovascular ailments. Future research should focus on recognizing the ultimate effectiveness of citrus flavonoids and optimizing their use in the treatment and prevention of tumors and hypercholesterolemia.

## Acknowledgment

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## References

- Hertog, M. G., Hollman, P. C., Katan, M. B., & Kromhout, D. (1993). Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands.
- Kuhnau, J. (1976) The Flavonoids a category of semi-crucial meal components: the function in human vitamins, *Global Rev. Nut., food regimen*, 24: 117–191.
- Elliott, M., & Chithan, K. (2017). The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In *The flavonoids advances in research since 1986* (pp. 619-652). Routledge.
- Nagy, S., Shaw, P. E., & Veldhuis, M. K. (1977). Citrus science and technology. (No Title).
- Ameer, B., Weintraub, R.A., Johnson, J.V., Yost, R.A., and Rouseff, R.L.(1996). Flavanone absorption after narin-

- genin, hesperidin, and citrus administration, *Clin. Pharmacol. Ther.*: 60: 3440.
6. Wattenberg, L. W., Page, M. A., & Leong, J. L. (1968). Induction of increased benzpyrene hydroxylase activity by flavones and related compounds. *Cancer Research*, 28(5), 934-937.
  7. Bokkenheuser, V. D., Shackleton, C. H., & Winter, J. (1987). Hydrolysis of dietary flavonoid glycosides by strains of intestinal Bacteroides from humans. *Biochemical Journal*, 248(3), 953-956.
  8. Att away, J.A., and Moore, E.L., Newly situated appropriateness benefits of citrus conclusion and juices, *Proc. Int. Soc. Citriculture*, 3: 11-36-1-139, 1992.
  9. Da Silva Emim, J.A., Oliveira, A.B., and Lapa, A.J., Pharmacological judgment of the antagonistic-Mullerian mandatory interest of citrus bioflavonoid, hesperidin, and the isoflavonoids, duration, and claussequinone, in rats and rodents, *J. Pharm. Pharmacol.*, quadragenarian six: 1-18-22, 1994.
  10. Manthey, J. A., Grohmann, K., Montanari, A., Ash, K., & Manthey, C. L. (1999). Polymethoxylated flavones derived from citrus suppress tumor necrosis factor- $\alpha$  expression by human monocytes. *Journal of Natural Products*, 62(3), 441-444.
  11. So, F.V., Guthrie, N., Chambers, A.F., Moussa, M., and Carroll, okay.k. (1996). Inhibition of human conscience malignancy cell increase and deferral of mammary tumorigenesis going around flavonoids and citrus juices, *Nutr. Most Cancers*, 26: 167-8.
  12. Galati, E.M., Trovato, A., Kirjavainen, S., Forestieri, A.M., and Rossitto, A. (1996). organic belongings of Hesperidin, a citrus flavonoid (discussion 111): antihypertensive and diuretic amusement in informer, *Pharmacol*, 51: 219-221.
  13. Monforte, M.T., Trovato, A., Kirjavainen, S., Forestieri, A.M., and Galati, E.M. (1995). organic results of hesperidin, a citrus flavonoid. (comprehend It): Hypolipidemic avocation on exploratory hypercholesterolemia in informer, *I+hrnmco*, 50: 595-599.
  14. Kawaguchi, OK., Mizuno, T., Aida, OK., and Uchino, OK. (1997) Hesperidin as prevention of lipases from about pig organ meat and Pseudomonas, *Biosci. Biotrch. Biochem.*, 6 1: 102-104.
  15. Bok, S.H., Lee, S.H., Park, Y.B., Bae, okay.H., Son, k.H., et al. (1999). Plasma and hepatic LDL cholesterol and hepatic endeavors of three-hydroxyl-3-methyl-glutaryl-CoA reductase and acyl CoA: LDL cholesterol transferase is lower in rats given augmented citrus peel extract or a combination of citrus Bioflavonoids. *J. Nutr.*, 129: 1182-1 185.
  16. Pisani, P., Breast cancer: terrestrial translation and danger details, *J. Environ. Pathol. Toxicol. Oncol.*, 11: 313-316, 1992.
  17. Block, G., Patterson, B., & Subar, A. (1992). Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutrition and cancer*, 18(1), 1-29.
  18. Steinmetz, K. A., and Potter, J. D. (1991). Salads, crops, and most cancers I. *Epidemiology, cancer reasons maneuver*, 2: 325-357.
  19. Wattenburg, L.W. (1992). Inhibition of carcinogenesis accompanying the aid of minor abstinence from food parts, *Cancer Res.*, having 50 of something two: 2085-209-1.
  20. Guthrie, N., Moffat, M., Chambers, A.F., Spence, J.D., and Carroll, K.O., Inhibition of increase of human breast tumor containers by way of naringenin, a flavonoid in grapefruit, *Natl. fir urn Breast Cancer, I I X (abstract)*, 1993.
  21. Guthrie, N., Gapor, A., Chambers, A.F., and Carroll, OK okay. (1997). Palm oils lubricate tocotrienols, and plant flavonoids act synergistically with each of the various accompanying tamoxifens in preventing conception and the boom of estrogen receptor-distressing MDA-MB-435 and overwhelming MCF-7 human breast malignancy containers in sodality, *Asia Pm. J. Clin. Nutr.*, 6: 4-45.
  22. Hansen, M.B., Nielsen, S.E., and Berg, OK. (1989). Re-test and further happening of a singular and breakneck dye form for weighing container increase/traveling cancel, *J. Immunol. policies*, 1 19: 203-2 10.
  23. So, F.V., Guthrie, N., Chambers, A.F., and Carroll, k.ok. (1997). Inhibition of conception of estrogen receptor-precise MCF-7 human conscience malignancy contains indirect flavonoids inside the vicinity and deficiency of extreme estrogen, most cancers *Lett.*, 1 12: 127-33.
  24. Guthrie, N., and Carroll, okay.okay. (1998). Inhibition of mammary malignancy through citrus flavonoids, in *The Living System*, Manthey, J., and Buslig, B., Eds., Plenum Press, New York.
  25. Guthrie, N., Gapor, A., Chambers, A.F., and Carroll, OK okay., Inhibition of increase of estrogen receptor-negative MDA-MB-435 and -excellent MCF-7 human bosom most cancers containers by way of touch lubricate tocotriols and tamoxifen, by myself and together, *J. Nutr.*, 127: 5448-5488, 1997.
  26. Rosc, D.P., Connolly, J.M., and Liu, X.-H. (1995). results of linoleic acid and  $\gamma$ -linoleic acid on the boom and change of a human conscience tumor traveling line in without clothes, covering rodents, and on allure boom and obtrusive capability artificial, *Nutr: tumor*, 24: 33b45.
  27. Guthrie, N., Chambers, A.F., and Carroll, ok.ok., results of apricot liquid squeezed from the plant, grapefruit liquid squeezed from the plant, and their constituent flavonoids at the increase of a human breast malignancy natural line in without clothes, covering rodent, given at the 16th Int. Cong. Nutr., p.66 (abstract PW10.8), 1997.
  28. Guthrie, N. and Carroll, K.K. (1998). Inhibition of human breast cancer container tumors and often major in nude rodent by citrus juices and their constituent flavonoids, in *Biological Oxidants: Molecular Mechanisms and Health & Factors*, Packer, L., and Ong, A.S.H., Eds., AOCSS Press, Champaign, IL.
  29. Hasegawa, S., Miyake, M., & Ozaki, Y. (1994). Biochemistry of citrus limonoids and their anticarcinogenic activity.
  30. Lam, L.K.T., Zhang, J., Hasegawa, S., and Schut, H.A.J., Inhibition of chemically persuaded carcino creation by citrus limonoids, in *Food Phytochemicals for Cancer Prevention I*, Fruits and Vegetables, Huang, M.T., Osawa, T., Ho, C.T., and Rosen, R.T., Eds., American Chemical Society, Washington, D.C., 1994.



31. Guthrie, N., Chambers, A. F., & Carroll, K. K. (1997). Inhibition of MDA-MB-435 estrogen receptor-negative human breast cancer cells by citrus limonoids. In *Am Assoc Cancer Res* (Vol. 38, p. 113).
32. Connor, S.L. and Connor, W.E., Pathogenic and protective about food determinants in heart failure and courage disease, in *Current Perspectives on Nutrition and Health*, Carmella, K.K., Ed., McGill-Queen's University Press, Montreal, PQ, 1998.
33. Cook, N.C. and Samman, S. (1996). Flavonoids: allure, metabolism, cardioprotective properties, and abstinence from food beginnings, *J. Nut% Riochmn.*, 7: 66-76.
34. Bors, W. (1990). Flavonoids as antioxidants: rate constants for reaction with oxygen radicals. *Methods. Enzymol.*, 186, 343-355.
35. Choi, J.S., Yokogawa, T., and Oura, H. (1991). Antihyperlipidemic effect of flavonoids from *Pru nus clavidiaua*; *J. Nut. Prod.*, 54: 218-224.
36. Rajendran, S., Deepalakshmi, P. D., Parasakthy, K., Devaraj, H., & Devaraj, S. N. (1996). Effect of tincture of *Cra taegus* on the LDL-receptor activity of hepatic plasma membrane of rats fed an atherogenic diet. *Atherosclerosis*, 123(1-2), 235-241.
37. Yotsumoto, H., Yanagita, T., Yamamoto, K., Ogawa, Y., Cha, J. Y., & Mori, Y. (1997). Inhibitory effects of Oren-Gedoku-to and its components on cholesteryl ester synthesis in cultured human hepatocyte HepG2 cells: evidence from the cultured Hep2G cells and in vitro assay of ACAT. *Planta medica*, 63(02), 141-145.
38. Anthony, M. S., Clarkson, T. B., Hughes Jr, C. L., Morgan, T. M., & Burke, G. L. (1996). Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. *The Journal of nutrition*, 126(1), 43-50.
39. Kurowska, E. M., Moffatt, M., & Carroll, K. K. (1994). Dietary soybean isoflavones counteract the elevation of VLDL but not LDL cholesterol produced in rabbits by feeding a cholesterol-free, casein diet. In *Proc. Can. Fed. Biol. Soc* (Vol. 37, p. 126).
40. Tovar-Palacio, C., Potter, S. M., Hafermann, J. C., & Shay, N. F. (1998). Intake of soy protein and soy protein extracts influences lipid metabolism and hepatic gene expression in gerbils. *The Journal of nutrition*, 128(5), 839-842.
41. Hodgson, J. M., Puddey, I. B., Beilin, L. J., Mori, T. A., & Croft, K. D. (1998). Supplementation with isoflavonoid phytoestrogens does not alter serum lipid concentrations: a randomized controlled trial in humans. *The Journal of nutrition*, 128(4), 728-732.
42. Baum, J. A., Teng, H., Erdman Jr, J. W., Weigel, R. M., Klein, B. P., Persky, V. W., ... & Potter, S. M. (1998). Long-term intake of soy protein improves blood lipid profiles and increases mononuclear cell low-density-lipoprotein receptor messenger RNA in hypercholesterolemic, postmenopausal women. *The American journal of clinical nutrition*, 68(3), 545-551.
43. Kurowska, E. M., Hrabek-Smith, J. M., & Carroll, K. K. (1989). Compositional changes in serum lipoproteins during developing hypercholesterolemia induced in rabbits by cholesterol-free, semipurified diets. *Atherosclerosis*, 78(2-3), 159-165.
44. Kurowska, E.M. and Borradaile, N.M., Hypocholesterolemic belongings of dietary citrus juices in animals, *Nut% KPS.*, 20: 121-129, 2000.
45. Carroll, K., & Hamilton, R. M. G. (1975). Effects of dietary protein and carbohydrate on plasma cholesterol levels in relation to atherosclerosis. *Journal of Food Science*, 40(1), 18-23.
46. Cheeke, P.R., *Nutrition and Food ailments*, in *The Biology of the Laboratory Rabbit*, Manning, P.J., Kingler, D.H., and Newcomer, C.E., Eds., Academic Press, San Diego, CA, 1994.
47. Borradaile, N. M., Carroll, K. K., & Kurowska, E. M. (1999). Regulation of HepG2 cell apolipoprotein B metabolism by the citrus flavanones hesperetin and naringenin. *Lipids*, 34(6), 591-598.
48. Thrift, R. N., Forte, T. M., Cahoon, B. E., & Shore, V. G. (1987). Characterization of lipoproteins produced by the human liver cell line, Hep G2, under defined conditions. *Journal of Lipid Research*, 27(3), 236-250.
49. Wilcox, L. J., Borradaile, M., Kurowska, E. M., Telford, D. E., & Huff, M. W. (1998, October). Naringenin, a citrus flavonoid, markedly decreases ApoB secretion in HepG2 cells and inhibits acyl CoA: Cholesterol acyltransferase. In *Circulation* (Vol. 98, No. 17, pp. 537-537). 227 EAST WASHINGTON SQ, PHILADELPHIA, PA 19106 USA: LIP-PINCOTT WILLIAMS & WILKINS.