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Apples Prevent Mammary Tumors in Rats

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Regular consumption of fruits and vegetables has been consistently shown to be associated with reduced risk of developing chronic diseases such as cancer and cardiovascular disease. Apples are commonly consumed and are the major contributors of phytochemicals in human diets. It was previously reported that apple extracts exhibit strong antioxidant and antiproliferative activities and that the major part of total antioxidant activity is from the combination of phytochemicals. Phytochemicals, including phenolics and flavonoids, are suggested to be the bioactive compounds contributing to the health benefits of apples. Here it is shown that whole apple extracts prevent mammary cancer in a rat model in a dose-dependent manner at doses comparable to human consumption of one, three, and six apples a day. This study demonstrated that whole apple extracts effectively inhibited mammary cancer growth in the rat model; thus, consumption of apples may be an effective strategy for cancer protection.

KEYWORDS: Diet and cancer; phytochmicals; cancer prevention; breast cancer; fruits

Epidemiological studies have consistently shown that regular consumption of fruits and vegetables is associated with reduced risk of chronic diseases such as cancer and cardiovascular disease (*1*−*3*). However, the individual antioxidants of these foods studied in clinical trials, including β-carotene, vitamin C, and vitamin E, do not appear to have consistent preventive effects comparable to the observed health benefits of diets rich in fruits and vegetables (*4*−*7*). Smokers gained no benefit from supplemental β-carotene (*5*) with respect to lung cancer incidence and possibly even suffered a deleterious effect, exhibiting a significant increase in lung cancer and total mortality (*6*, *7*). We previously reported that fresh apples have potent antioxidant activity, and whole apple extracts inhibit the growth of colon and liver cancer cells in vitro in a dose-dependent manner (*8*), suggesting that natural phytochemicals in fresh fruits could be more effective than a dietary supplement. Here we show that 100 g of fresh apples has an antioxidant activity equivalent to 1700 mg of vitamin C and that whole apple extracts prevent breast cancer in a rat model in a dose-dependent manner at doses comparable to human consumption of one, three, and six apples a day. This study demonstrated that whole apple extracts effectively inhibited mammary cancer growth in the rat model; thus consumption of apples may be an effective means of cancer protection. Our results also indicate that antioxidants are best acquired through whole foods consumption, not from expensive dietary supplements.

Extracts of Red Delicious variety apples were prepared using 80% acetone (*8*), and their phenolic content was determined (*9*): the apple extracts contained 272.1 ± 6.2 mg of phenolics per 100 g of apples. Antioxidant activities of the apple extracts were determined using the total oxyradical-scavenging capacity (TOSC) assay (*8*). This activity was 97.6 ± 4.6 μmol of vitamin C equiv per 100 g of fresh apples; in other words, the antioxidant activity of 100 g of apples is equivalent to 1700 mg of vitamin C. The standardized apple extracts were freeze-dried and stored at −40 °C until initiation of the feeding study.

Female Sparague−Dawley rats treated with the carcinogen 7,12-dimethylbenz[*a*]anthracene (DMBA) at 50 days of age developed mammary tumors with 71% tumor incidence during a 24-week study (**Figure 1**). No tumors were detected in the negative control group untreated with DMBA. Rats were administered by gavage control extracts or whole apple extracts 2 weeks prior to the treatment with DMBA and throughout the 24-week study. A dose-dependent inhibition of mammary carcinogenesis by whole apple extracts was observed. Application of low, medium, and high doses of whole apple extracts, comparable to 3.3, 10, and 20 g of apples/kg of body weight, reduced the tumor incidence by 17, 39 ($p < 0.02$), and 44% ($p < 0.01$), respectively (**Figure 1d**); this is comparable to human consumption of one (∼200 g/60 kg), three, and six apples per day. Cumulative tumor numbers in the groups receiving low, medium, or high doses of apple extracts were reduced by 25, 25, and 61% ($p < 0.01$), respectively, after 24 weeks (**Figure 1e**). The time to mammary tumor appearance after DMBA treatment was 11 weeks for rats fed no apple extracts, 12 weeks for rats fed low- and medium-level extract dosages, and 13 weeks for rats fed the high-level dosage. The delay of tumor onset was dose-dependent. The tumor burden was 4.79 ± 5.90

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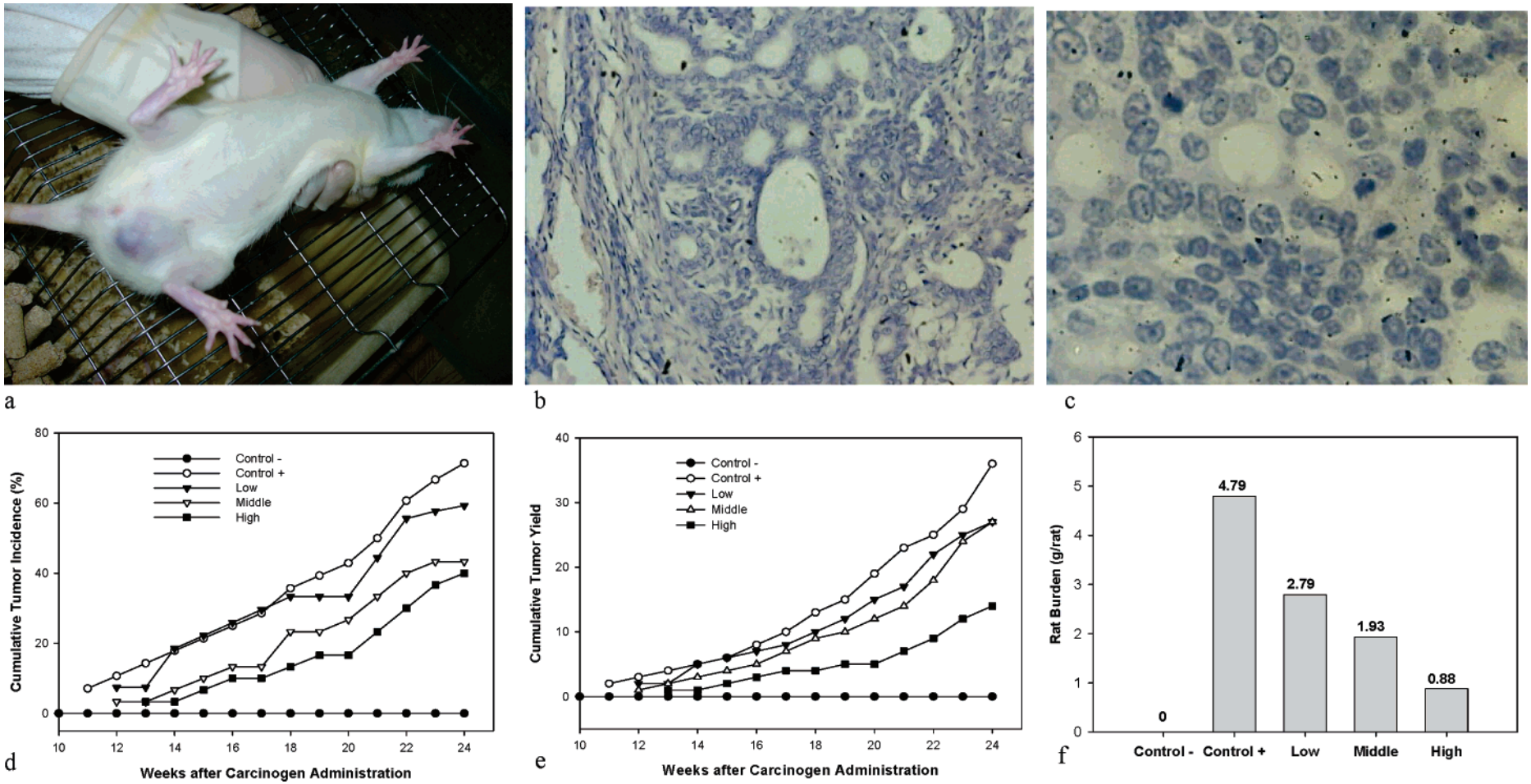

Figure 1. Mammary cancer prevention of whole apples. Female Sparague–Dawley rats were randomized into five diet groups ($n = 30$/group) and fed with AIN-76 formulation diet. Four of five groups of 30 rats (50 days old) were given by gavage 10 mg of DMBA dissolved in 1 mL of corn oil; the fifth group received no DMBA. Rats were administered control extracts or whole apple extracts starting 2 weeks prior to the treatment with DMBA and throughout the 24-week study. Three levels of low, medium, and high doses of whole apple extracts with 3.3, 10, and 20 g of fresh apples/kg of body weight, which are comparable to human consumptions of one, three, and six apples per day, were given to rats by gavage. Animals were weighed and observed for tumor development weekly: (**a**) gross view of a rat with mammary tumor swelling in the lower left quadrant of the abdomen; (**b**) H&E-stained section of a moderately differentiated mammary adenocarcinoma (10×); (**c**) H&E-stained section of a moderately differentiated mammary adenocarcinoma (40×) (neoplastic cells show great diversity in size and arrangement around lumens at sites, becoming multilayered, palisaded, and showing papillary or even disorganized or solid growth; nuclei are enlarged, euchromatic with multiple nucleoli; cytoplasm is scant, and mitotic figures are common); **(d)** percent incidence of observable mammary tumors; (**e**) total cumulative number of observable mammary tumors per group; (**f**) tumor burden (grams) per rat in each group.

g per rat for rats fed no apple extracts (**Figure 1f**). In the groups receiving low, medium, or high doses of apple extracts, tumor burden was reduced to 2.79 ± 5.00, 1.93 ± 4.58 ($p < 0.02$), and 0.88 ± 1.80 ($p < 0.001$) g per rat, respectively (**Figure 1f**). Body weight and food intake were not affected by any apple extract dosage throughout the 24-week duration when compared to the control group ($p > 0.05$), suggesting there was no toxicity due to apple extracts at any of the doses tested. Thus, this study convincingly demonstrated that consumption of whole apples is an effective strategy to achieve cancer protection in the rat model.

Studies to date have demonstrated that the mechanisms of action of phytochemicals in the prevention of cancer go beyond the antioxidant activity scavenging of free radicals. Phytochemicals in fruits, vegetables, whole grains, and other plant foods can have complementary and overlapping mechanisms of action (*10*), including antioxidant activity and scavenging of free radicals; regulation of gene expression in cell proliferation, cell differentiation, oncogenes, and tumor suppressor genes; induction of cell cycle arrest and apoptosis; modulation of enzyme activities in detoxification, oxidation, and reduction; stimulation of the immune system; regulation of hormone metabolism; and antibacterial and antiviral effects. We have proposed that the additive and synergistic effects of phytochemicals in fruits and vegetables are responsible for their potent antioxidant and anticancer activities and that the benefit of a diet rich in fruits and vegetables is attributed to the complex mixture of phytochemicals present in whole foods (*8*, *10*−*12*). This hypothesis partially explains why no single antioxidant can replace the combination of natural phytochemicals in fruits and vegetables in achieving ultimate health benefits. The pure compounds either lose their bioactivities in isolation or may not behave the same way as the compound in complex whole foods. Our findings suggest that consumers may gain more significant health benefits from including whole foods in their balanced diet than from more expensive dietary supplements, which do not contain the same array of balanced, complex components. Approximately 8000 phytochemicals are present in whole foods. These compounds differ in molecular size, polarity, and solubility, which may affect their bioavailability and distribution in different subcellular organelles, cells, and tissues and organs. This balanced natural combination of phytochemicals present in fruits and vegetables cannot simply be mimicked by dietary supplements. More importantly, dietary intake of antioxidants through consumption of a wide variety of foods is unlikely to result in ingestion of toxic quantities because foods originating from plants contain many diverse types of phytochemicals in varying low quantities in relation to the total fresh weight ingested. Furthermore, the health benefits of the consumption of fruits and vegetables extend beyond lowering the risk of developing cancers and cardiovascular diseases to include preventive effects on other chronic diseases such as cataracts, age-related macular degeneration, central neurodegenerative diseases, and diabetes (*2*).

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