Antimutagenic Effect of Fruit and Vegetable Ethanolic Extracts against *N*-Nitrosamines Evaluated by the Ames Test

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The inhibitory effect of nine fruit and vegetable ethanolic extracts against the mutagenicity of *N*-nitrosodimethylamine (NDMA), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosodibutylamine (NDBA), and *N*-nitrosopiperidine (NPIP) was evaluated by means of the Ames test. Licorice ethanolic extract was the only one that showed an inhibitory effect (ranging from moderate to strong) against mutagenicity of all *N*-nitrosamines tested. This ethanolic extract showed the greatest inhibition effect against NPIP (72%), NDMA (45%), and NPYR (39%). The greatest inhibition effect (51%) of the mutagenicity of NDBA was shown by kiwi ethanolic extract. Vegetable and fruit ethanolic extracts that exhibited an antimutagenic effect (at the range 50−2000 μg/plate), in decreasing order, against NDMA and NPYR were as follows: licorice > kiwi > carrot and licorice > broccoli > pineapple > kiwi, respectively. Decreasing orders against NDBA and NPIP were, respectively, kiwi > onion > licorice = garlic > green pepper > carrot and licorice > garlic > pineapple > carrot.

Keywords: *Vegetables; fruits; N-nitrosamines; mutagenicity; Ames test*

INTRODUCTION

Diet has been suggested to have a significant impact on the cancer process (Armstrong and Doll, 1975; Doll and Peto, 1981). Some research findings suggest that several dietary constituents may be important factors capable of increasing or decreasing cancer incidence (Wattenberg, 1983). Thus, specific and predetermined manipulations of the diet may turn out to be a promising noninvasive approach to minimize cancer (Liu et al., 1992).

The consumption of fruits and vegetables has been associated with lower incidence and lower mortality rates of cancer in several human cohorts and case-control studies for all common cancer sites (Ames et al., 1993; Dragsted at al., 1993; Willett, 1994). In animal experiments, vegetables common in human diets have been found to have antitumorigenic effects (Maltzman et al., 1989; Stoewsand et al., 1989; Bingham, 1990; Bresnick et al., 1990; Wattenberg and Coccia, 1991). The protection that fruits and vegetables provide against mutagenicity and cytotoxicity has been attributed to the various antioxidants contained in them (Ames, 1983; Steinberg et al., 1989; Gey, 1990; Steinberg, 1991; Cozzi et al., 1997). At present, there is overwhelming evidence indicating that free radicals cause oxidative damage to lipids, proteins, and nucleic acids. Therefore, antioxidants, which can neutralize free radicals, may be of central importance in the prevention of several disease states. Fresh fruits and vegetables contain a great variety of different antioxidant components. Most of the antioxidant capacity of a fruit or vegetable can come from compounds other than vitamin C, vitamin E, or β-carotene (Wang et al., 1996). Many studies have demonstrated that carotenoids from green and yellow vegetables have an anticarcinogenic effect in humans (van Poppel, 1996; PoolZobel et al., 1997). However, in clinical trials, β-carotene has recently been shown to be an ineffective agent and, perhaps, to be harmful (Potter, 1997).

Vegetables such as broccoli, green pepper, apple, and pineapple present activity as antimutagens against the mutagenicity of tryptophan pyrolysis products (Kada et al., 1978). Antimutagenic activities have also been found in greengage, kiwi, mangos, and plums against the mutagenic activity induced by 2-amino-3-methylimidazo-[4,5-*f*]quinoline (Edenharder et al., 1994). Onion, garlic, leek, and other vegetables belonging to the *Allium* genus, used extensively as flavoring agents, have been used also for medicinal purposes for centuries. These vegetables contain a wide variety of specific compounds that act as antimutagens in vitro and also seem to be anticarcinogens in vivo (Ip and Lisk, 1994; Dorant et al., 1995; Malaveille et al., 1996; Tsai et al., 1996; Ernst, 1997). Recent epidemiological studies have revealed that gastric cancer mortality was lower in areas where *Allium* vegetable consumption is high (Mei et al., 1985; You et al., 1989).

Thus, a great variety of naturally occurring compounds or their mixtures have been shown to inhibit the mutagenicity or carcinogenicity of several chemicals. However, information about the effect of fruit and vegetable extracts on the mutagenicity and carcinogenicity of *N*-nitrosamines is scarce. Human exposure to these compounds can be by ingestion or inhalation of preformed *N*-nitroso compounds as well as by endogenous nitrosation of naturally occurring precursors (Ohshima and Bartsch, 1981; Bartsch and Montesano, 1984). It is now well established that a wide variety of foods, particularly cured meat products, smoked fish, dried malt, and beer, contain trace levels of volatile

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10.1021/jf990166n CCC: $18.00
Published on Web 07/15/1999

N-nitrosamines (Scanlan, 1983; Havery and Fazio, 1985; Österdahl, 1991).

N-Nitrosamines are formed by *N*-nitrosation reactions between various secondary or tertiary amines and nitrite or other nitrosating agents (Lijinsky, 1992). Reactions between nitrite and secondary amines can produce these *N*-nitroso compounds under gastric conditions (Sander, 1968; Mirvish, 1975). Nitrite may be readily derived by bacterial metabolism (Ayanaba and Alexander, 1973) or salivary reduction (Ishiwata et al., 1975) from the nitrate present in a wide variety of food products. In addition, nitrite is also an important and widely used additive in human food (Gangolli and Phillips, 1985; Hotchkiss, 1987).

Although the evidence that endogenously formed *N*-nitrosamines are involved in human cancers is far from conclusive, it is important to discover naturally occurring or synthetic compounds which can suppress or prevent the toxicity, mutagenicity, and carcinogenicity of *N*-nitrosamines. Several studies have quantified the inhibition of nitrosation by food components including ascorbic acid in vitro (Mirvish et al., 1983; Bartsch et al., 1988) and in vivo (Lin et al., 1986; Liu et al., 1986; Leaf et al., 1987; Mei et al., 1989; Helser et al., 1991, 1992). However, few authors have quantified the inhibition of *N*-nitrosamines effect by natural compounds present in fruits and vegetables.

The present study is part of a line of research aimed at evaluating the antimutagenic and protective effect of fruit and vegetable extracts against the mutagenicity and cytotoxicity of *N*-nitrosamines. In previous works, a protective effect was found in onion, carrot, licorice, and broccoli extracts against cytotoxicity of several *N*-nitrosamines using the MTT assay (Martinez et al., 1998), the BrdU assay, and the determination of total cellular DNA content (Martinez et al., 1999a). On the other hand, aqueous extracts from several fruits and vegetables had an antimutagenic effect against NDMA, NPYR, NDBA, and NPIP (Ikken et al., 1998a). In this paper we report the effect of fruit and vegetable ethanolic extracts against mutagenicity of *N*-nitrosamines using the Ames test.

MATERIALS AND METHODS

Materials. Samples of the six vegetables and three fruits under investigation, onion *(Allium cepa)*, garlic *(Allium sativum)*, green pepper *(Capsicum annuum)*, broccoli *(Brassica oleracea)*, carrot *(Dacus carota)*, licorice *(Glycyrrhiza glabra)*, apple *(Malus domestica* Golden delicious), kiwi *(Ananas sativus)*, and pineapple *(Actinidia diasinensis)*, were purchased from a local food market in Madrid, Spain. These fruits and vegetables were selected on the basis of preliminary in vitro assays described by several investigators (Helser et al., 1992; Bronzetti, 1994; Dorant et al., 1995).

Preparation of Ethanolic Extracts of Fruits and Vegetables. Standard amounts of 100 g of fruits and vegetables were sliced and further homogenized at 4 °C in a homogenizer (Sorvall, Norwalk, CT). Homogenization was performed with 99% ethanol (1/1 w/v), except for licorice *(G. glabra)* extract where the correspondence w/v was 1/6, and before homogenization it was incubated for 72 h. The resulting homogenate was filtered with suction, and the filtrate was centrifuged at 10 000 *g* for 30 min to remove any fruit and vegetable debris. The supernatant was sterilized by filtration through two Millipore filters (0.45 and 0.22 μm). Ethanolic extracts were evaporated to dryness under reduced pressure and stored at −20 °C until use (Martínez et al., 1999a).

Chemicals. NDMA, NPYR, NDBA, and NPIP were purchased from Sigma Chemical Co. (St. Louis, MO). Standard solutions of NDMA and NPYR (5 mg/100 μL) were prepared in PBS (pH 7.4), and NDBA (0.5 mg/100 μL) and NPIP (2 mg/100 μL) were prepared in 12% dimethyl sulfoxide (DMSO; Merck, Darmstadt, Germany). *N*-Nitrosamines are potent carcinogenic agents; safety precautions must be taken for proper handling and disposal of the chemicals. The *N*-nitrosamines tested were chosen because they are the most frequently occurring volatile nitrosamines in foods.

Antimutagenicity Assay. The antimutagenic effect of each fruit and vegetable ethanolic extract was assayed according to the Ames method with a 20 min preincubation at 37 °C (Maron and Ames, 1983). The histidine-requiring strain of *Salmonella typhimurium* TA100 was kindly supplied by Dr. B. N. Ames (University of California, Berkeley). The S9 mix (IFFA CREDO; Institut Francais de la Fièvre Aphteuse-Centre de Recherche et d'Elevage du Domaine des Oncins, Lyon, France) was prepared from Sprague−Dawley male rats treated with Aroclor 1254 according to the method of Ames et al. (1975). The indirect mutagens used for *S. typhimurium* TA100 were NDMA, NPYR, NDBA, and NPIP, which required S9 mix for metabolic activation. The mutagen (0.1 mL) was added to the mixture of *S. typhimurium* TA100 strain (0.1 mL) and each concentration of fruit and vegetable ethanolic extracts (0.1 mL) with S9 mix (0.1 mL; 30% S9) (Ikken et al., 1998b). The entire mixture was preincubated while shaking at 37 °C for 20 min before molten top agar (2 mL) was added; the mixture was poured onto a minimal medium agar plate. The his^+ revertant colonies were counted after incubating at 37 °C for 48 h. Each sample was assayed using triplicate plates, and the data presented are mean ± standard error of three independent assays. Plates without *N*-nitrosamines and without fruit and vegetable ethanolic extracts were considered as negative controls and plates with *N*-nitrosamines as positive controls. NDMA and NPYR (5 mg/100 μL) gave 233.0 ± 12.7 and 573.0 ± 41.0 colonies, respectively. NDBA (0.5 mg/ 100 μL) and NPIP (2 mg/100 μL) gave 349.0 ± 8.4 and 531.0 ± 33.9 colonies, respectively. Antimutagenic effect is expressed as percentage of inhibition (% inhibition) = $100 - (R_1/R_0 \times 100)$, where R_1 is the number of his^+ revertants/plate of plates exposed to *N*-nitrosamines and fruit or vegetable extracts and R_0 is the number of his^+ revertants/plate of the positive control. The number of spontaneous revertants was subtracted from the numerator and denominator. The mutagenicity of *N*-nitrosamines (positive control) in the absence of fruit and vegetable ethanolic extracts is defined as 0% of inhibition.

The antimutagenic effect was considered moderate when the inhibitory effect of fruit and vegetable extracts was in the range of 25−40% and strong when the inhibitory effect was >40%. An inhibitory effect <25% was considered weak, and it was not recognized as a positive result.

Data Analysis. Simple regression analyses were used to determine the relationship between the percentage of inhibition versus extract concentration. Analyses were carried out using a StateView program (Feldman, D.; Gagnon, J., 1985) in an Apple Macintosh Performa 5260/120 computer.

RESULTS

The antimutagenic effect of fruit and vegetable ethanolic extracts on four *N*-nitrosamines, including NDMA, NPYR, NDBA, and NPIP, was evaluated by means of the Ames test. No mutagenicity and toxicity has been previously described with the fruit and vegetable extracts used under the conditions tested (Martinez et al., 1999b). All ethanolic extracts studied, with the exception of apple extract, showed a moderate or strong inhibitory effect on the mutagenicity of the *N*-nitrosamines tested. Data points are the mean of values of triplicate plates from three independent assays.

The inhibitory effect of ethanolic extracts of onion (*A. cepa*) and garlic *(A. sativum*) against the four *N*-nitrosamines tested appears in Figures 1 and 2. Onion ethanolic extract showed a strong inhibitory effect (42%)

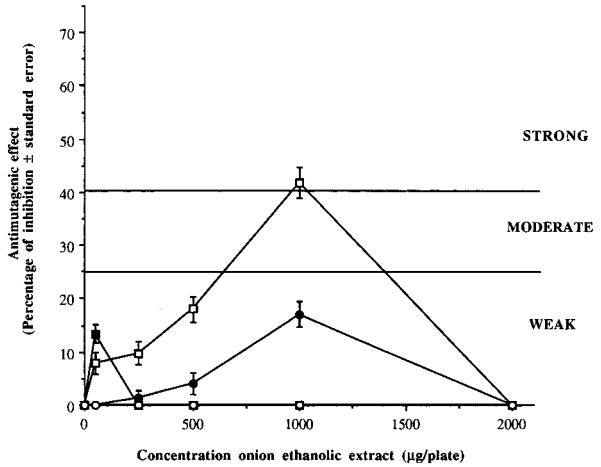

Figure 1. Inhibitory effect of onion *(A. cepa)* ethanolic extract against the mutagenicity of NDMA (○), NPYR (●), NDBA (□), and NPIP (■) to *S. typhimurium* TA100.

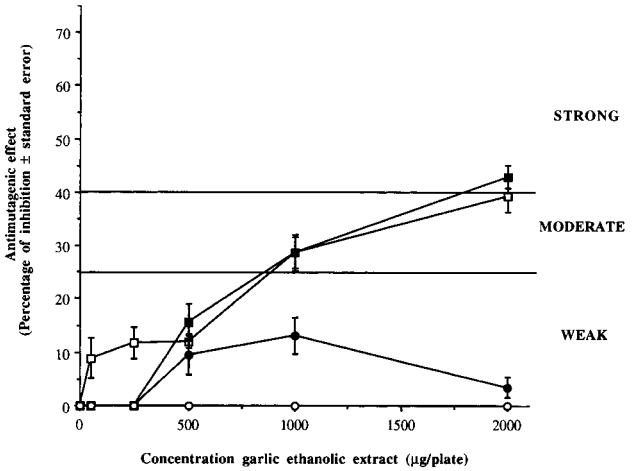

Figure 2. Inhibitory effect of garlic *(A. salivum)* ethanolic extract against the mutagenicity of NDMA (○), NPYR (●), NDBA (□), and NPIP (■) to *S. typhimurium* TA100.

against mutagenicity of only NDBA at a dosage of 1000 μg/plate (Figure 1). The inhibitory effect of garlic ethanolic extract to NPIP ($R^2 = 0.99$; $p \leq 0.01$) and NDBA ($R^2 = 0.99$; $p \leq 0.01$) increased with increasing concentration of this extract (Figure 2). Concentrations ≥1000 μg/plate were required to observe a moderate to strong inhibitory effect against these *N*-nitrosamines (28−43% for NPIP and 28−39% for NDBA). However, this protective effect decreased with increasing concentration of garlic at dosage >2000 μg/plate (data not shown).

Figure 3 shows the antimutagenic effect of green pepper *(C. annuum)* ethanolic extract to *N*-nitrosamines tested. Only the mutagenicity of NDBA was moderately reduced (37%) by 250 μg/plate of this extract.

Results from Figure 4 show the inhibitory effect of broccoli *(B. oleracea)* ethanolic extract against the mutagenicity of *N*-nitrosamines. This vegetable extract showed a slightly inhibitory effect against mutagenicity of only NPYR (50−250 μg/plate; 30−25%).

Carrot *(D. carota)* ethanolic extract (Figure 5) showed an ability to slightly inhibit the mutagenicity of NDMA (1000 μg/plate; 28%), NDBA (50 μg/plate; 29%), and NPIP (2000 μg/plate; 32%).

Licorice *(G. glabra)* ethanolic extract was the only one that showed an inhibitory effect against mutagenicity of all *N*-nitrosamines tested (Figure 6). This ethanolic

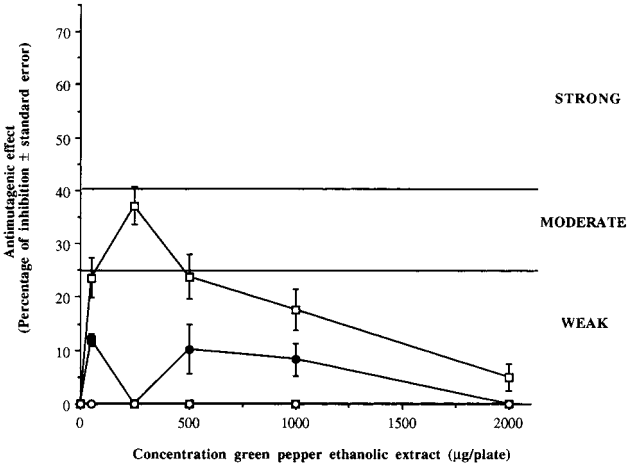

Figure 3. Inhibitory effect of green pepper *(C. annuum)* ethanolic extract against the mutagenicity of NDMA (○), NPYR (●), NDBA (□), and NPIP (■) to *S. typhimurium* TA100.

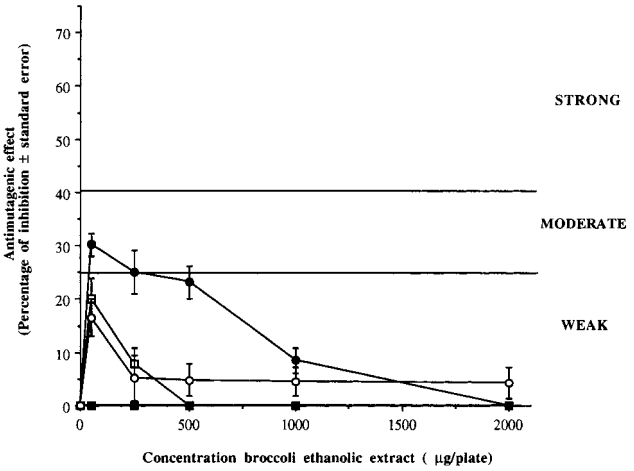

Figure 4. Inhibitory effect of broccoli *(B. oleracea)* ethanolic extract against the mutagenicity of NDMA (○), NPYR (●), NDBA (□), and NPIP (■) to *S. typhimurium* TA100.

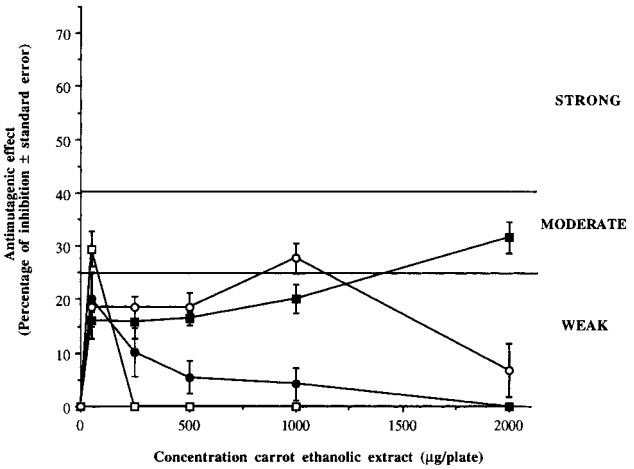

Figure 5. Inhibitory effect of carrot *(D. carota)* ethanolic extract against the mutagenicity of NDMA (○), NPYR (●), NDBA (□), and NPIP (■) to *S. typhimurium* TA100.

extract inhibited the mutagenicity of NPIP from 56% to 72%. The mutagenicity of NDBA was markedly reduced (25−46%) by concentrations ≥500 μg/plate of licorice ethanolic extract. The antimutagenicity effect against NPIP ($R^2 = 0.99$; $p < 0.01$) and NDBA ($R^2 = 0.81$; $p \leq 0.05$) increased with increasing concentration

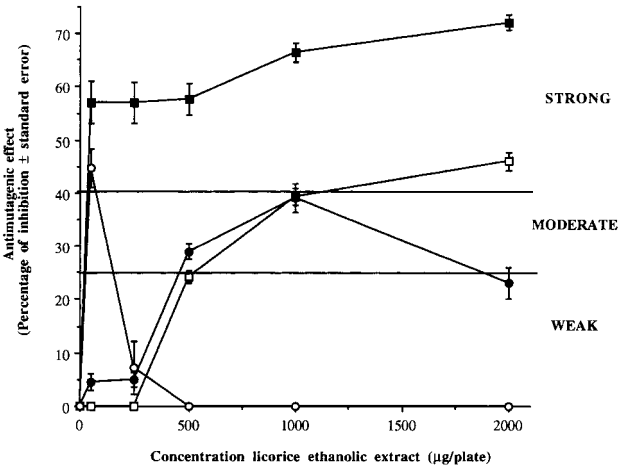

Figure 6. Inhibitory effect of licorice *(G. glabra)* ethanolic extract against the mutagenicity of NDMA (○), NPYR (●), NDBA (□), and NPIP (■) to *S. typhimurium* TA100.

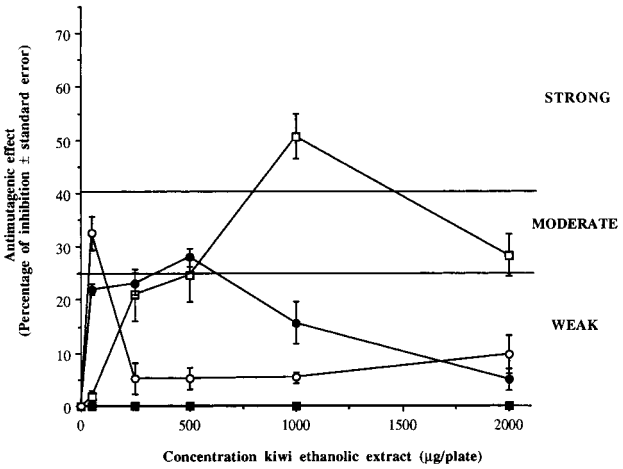

Figure 7. Inhibitory effect of kiwi *(A. sativus)* ethanolic extract against the mutagenicity of NDMA (○), NPYR (●), NDBA (□), and NPIP (■) to *S. typhimurium* TA100.

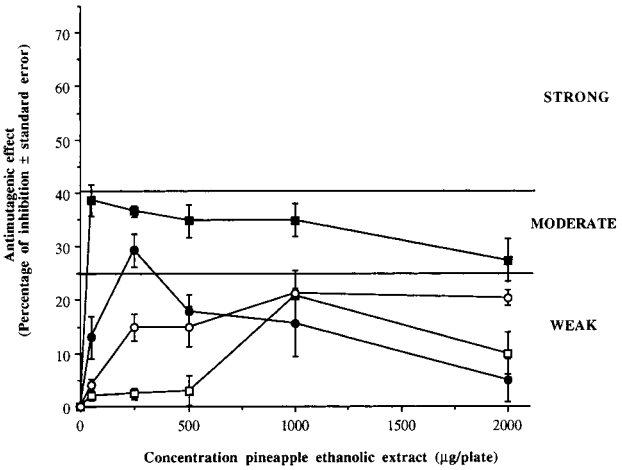

Figure 8. Inhibitory effect of pineapple *(A. diasinensis)* ethanolic extract against the mutagenicity of NDMA (○), NPYR (●), NDBA (□), and NPIP (■) to *S. typhimurium* TA100.

of the extract up to 2000 μg/plate and decreased at concentrations >2000 μg/plate (data not shown). Licorice ethanolic extract showed a strong antimutagenic effect against NDMA (50 μg/plate; 45%) but only a moderate inhibitory effect against NPYR (500−1000 μg/plate; 29−39%).

Kiwi *(A. sativus)* ethanolic extract (Figure 7) showed a slight inhibitory effect against the mutagenicity of NDMA (50 μg/plate; 32.5%) and NPYR (500 μg/plate; 28%). The antimutagenic activity of kiwi against NDBA increased with increasing concentrations of extract from 50 to 1000 μg/plate. A dosage of 1000 μg/plate was required to observe a strong inhibitory effect (51%), whereas concentrations of 2000 μg/plate showed a moderate antimutagenic activity (28%).

Pineapple (*A. diasinensis*) ethanolic extract (Figure 8) showed a moderate inhibitory effect against NPYR (29%; 250 μg/plate) and NPIP (50 μg/plate; 38%). The inhibitory effect against NPIP decreased as the concentration of this extract increased ($R^2 = 0.99$; $p < 0.01$).

The inhibitory activity against the mutagenicity of *N*-nitrosamines tested was not observed with apple *(M. domestica* Golden Delicious) ethanolic extract under the conditions used.

DISCUSSION

Mutagenic assays such as the *S. typhimurium* test has been widely used to assess the antimutagenic and anticarcinogenic activity of various compounds. Recent studies (Ikken et al., 1998a) from our laboratory have evaluated the antimutagenic effect of fruit and vegetable aqueous extracts against *N*-nitrosamines using *Salmonella*/reversion assay (Ames test). The aim of the present study was to evaluate the antimutagenic effect of fruit and vegetable ethanolic extracts against *N*-nitrosamines by using the Ames test.

Numerous epidemiological studies have consistently reported that a diet rich in vegetables and fruits is associated with a decreased risk of cancer (Potter and Steimmetz, 1996; Riboli et al., 1996; Frohlich et al., 1997). *Allium* vegetables (such as garlic and onion) are consumed all over the world, not only as a spice and food but also as a popular folk remedy for a variety of ailments, such as heart disease, several types of cancer, and blood clotting (Lea, 1996; Ernst, 1997). Wargovich et al. (1988) found that diallyl sulfide, a flavor component of garlic, reduced the incidence of colon cancer in mice treated with dimethylhydrazine and completely inhibited *N*-nitrosomethylbenzylamine-induced esophageal tumor formation (Wargovich et al., 1988; Sumiyoshi and Wargovich, 1990). Sparnins et al. (1986) had also shown that the administration of allyl methyl trisulfide, a constituent of garlic oil, reduced benzopyrene-induced tumors by 70%.

The ability of garlic and onion to inhibit cancer development does not appear to be limited to a specific species, a particular tissue, or a specific carcinogenic activity (Rao et al., 1990; Unnikrishnan and Kuttan, 1990; Liu et al., 1992; Hatono et al., 1996). In this sense, the ability of naturally occurring organosulfur compounds present in these *Allium* vegetables to reduce the formation and bioactivation of carcinogenic nitrosamines has been described (Shenoy and Choughuley, 1992; Dion et al., 1997). Diallyl sulfide has proved to have chemoprotective effects against mutagenesis induced by NDMA (Surh et al., 1995). Organosulfur compounds were found to inhibit the formation of mutagenic heterocyclic aromatic amines (Tsai et al., 1996) and reduce the mutagenicity induced by benzo-[α]pyrene, 4-nitro-1,2-phenylenediamine and aflatoxin (Ishikawa et al., 1996; Soni et al., 1997). In the present study, garlic ethanolic extracts showed a moderate to

strong inhibitory effect against the mutagenicity of NPIP and NDBA evaluated by the Ames test. Onion ethanolic extracts exhibit a strong protective effect against the mutagenicity of NDBA. In previous published results (Ikken et al., 1998a), we reported that the constituents of onion and garlic aqueous extracts exhibit an inhibitory effect against several *N*-nitrosamines. Taken together, the results obtained by Ikken et al. (1998a) and the results of the present study clearly document the ability of onion constituents to inhibit the mutagenicity of NDMA, NDBA, and NPYR. Compounds of onion extracts, however, did not show a protective effect against NPIP, under our experimental conditions. On the other hand, the studies from our laboratory have also shown that garlic constituents are effective in reducing the mutagenic effect of NDMA, NDBA, and NPIP. The mechanism whereby these extracts prevent the mutagenicity of these *N*-nitrosamines remains unclear.

Edenharder et al. (1994) reported that green pepper juice was inactive against the mutagenic activities induced by heterocyclic amines. The results of the present study have shown that green pepper (*C. annuum*) ethanolic extract only presented a moderate inhibitory effect against mutagenicity of NDBA. This *N*-nitrosamine was not inhibited by green pepper aqueous extract constituents (Ikken et al., 1998a), and this antimutagenic effect could, therefore, be attributed to the ethanol-soluble compounds.

Several lines of investigations support the ability of *Brassica* vegetables, including all types of cabbage, broccoli, cauliflower, and Brussels sprouts to prevent the cancer process (Verhoeven et al.., 1996). The protective effect of *Brassica* vegetables against cancer may be due to their relatively high content of isothiocyanates, mostly in the form of their glucosinolate precursors (Verhoeven et al., 1997). Furthermore, cruciferous vegetables were found to have a variable antimutagenic effect against the mutagenic activities induced by heterocyclic amines (Edenharder et al., 1994) and a protective effect against *N*-nitrosodimethylamine genotoxicity (Knasmuller et al., 1996). Our results indicate that broccoli ethanolic extract only showed a slightly antimutagenic effect against NPYR. Because the results obtained by Ikken et al. (1998a) showed the ability of broccoli aqueous extracts to exert an inhibitory effect (moderate to strong) against the mutagenicity of NDBA, NPIP, NPYR, and NDMA, the antimutagenic effect of broccoli on these *N*-nitrosamines can be attributed to water-soluble compounds.

Epidemiological studies support the ability of carotenoid-rich fruits and vegetables to inhibit the mutagenicity or carcinogenicity of several chemicals (van Poppel, 1996; PoolZobel et al., 1997; Tang and Edenharder, 1997). Ikken et al. (1998a) observed that carrot (*D. carota*) aqueous extract exhibited a strong antimutagenic effect against NDBA and moderate effect against NDMA and NPIP. The present study reflects the ability of ethanol-soluble carrot constituents to inhibit slightly the mutagenicity of these *N*-nitrosamines. These data may suggest the presence of several antimutagenic factors (water and/or ethanol soluble) in this vegetable.

Licorice (*G. glabra*) is an economically important plant that has been used for centuries as a medicine because of its wide range of therapeutic properties. The antitumorigenic activity of licorice has been evaluated in several investigations (Agarwal et al., 1991; Kobayashi, 1995). In this regard, it has been established that glycyrrhizic acid is a potent inhibitor of some enzymes involved in neoplasia (Duax and Ghosh, 1997). Furthermore, glycyrrhizic acid was found to inhibit the mutagenicity induced by benzo[α]pyrene, 2-aminofluorene, and aflatoxin B_1 in *S. typhimurium* TA100 (Wang et al., 1991). The results obtained in the present study demonstrate that licorice root ethanolic extract exhibited the strongest inhibitory effect against the mutagenicity of NPIP (72%), NDMA (45%), and NPYR (39%). The mutagenicity of NDBA was also inhibited by this extract (46%). Previous studies (Ikken et al., 1998a) have shown that licorice aqueous extracts are not effective in inhibiting NPYR, NPIP, and NDBA mutagenicity. Thus, the antimutagenic effect of licorice against these *N*-nitrosamines can be attributed to ethanol-soluble constituents.

Fruits are a good source of antimutagenic factors. Kiwi and pineapple juices have shown antimutagenic activity against heterocyclic amines (Edenharder et al., 1994). Recent investigations reported that the juices from these fruits exerted weak to moderate co-mutagenic activity with respect to mutagenicity induced by nitroarenes in *S. typhimurium* TA98 (Tang and Edenharder, 1997). In previous studies (Ikken et al., 1998a), kiwi *(A. sativus*) aqueous extract was found to have a strong antimutagenic effect against NPIP and slight inhibitory effect against NDMA, NDBA, and NPYR whereas pineapple *(A. diasinensis)* had a moderate effect against NPIP, NPYR, and NDMA. The present study reveals that kiwi ethanolic extract is the most effective in inhibiting NDBA mutagenicity (51%). These data suggest that ethanol-soluble kiwi constituents may be more effective in inhibiting NDBA activity than the water-soluble constituents of this fruit. The kiwi ethanolic extract also showed a slight inhibitory effect against the mutagenicity of NDMA and NPYR. In agreement with the results obtained with aqueous extracts in previous studies (Ikken et al., 1998a), the ethanolic extracts of pineapple had a moderate effect against NPIP and NPYR. These results support the conclusion that the antimutagenic effect against *N*-nitrosamines involves both water- and ethanol-soluble compounds.

We have previously reported (Ikken et al., 1998a) that apple *(M. domestica* Golden Delicious) aqueous extract showed a strong inhibitory effect against the mutagenicity of NPYR and a moderate effect against NDMA. However, in our experimental conditions, apple ethanolic extract was inactive against the mutagenic activities induced by *N*-nitrosamines. From these results, we concluded that only water-soluble compounds are implicated on the apple antimutagenic activity against NPYR and NDMA.

As it might have been expected, most of the results obtained in this work have shown nonlinear dose responses. A similar trend has been observed in our studies with aqueous extracts (Ikken et al., 1998a), and this is a common situation when a complex mixture or combined treatments are tested. The effect of the modifying chemical can be either enhancing or inhibiting depending on its mechanism of action (Wallum et al., 1990). The modifier agent(s) may act outside the bacteria by interfering with the bacterial metabolism. Other modifying effects inside as well as outside the bacteria may act by chemical or enzymatic modifications of the mutagen (Ikken et al., 1998a). Our results clearly

indicate that fruit and vegetable ethanolic extracts have shown antimutagenicity against indirect mutagens (*N*-nitrosamines), requiring S9 activation. One feasible mechanism of antimutagenic action is that fruit and vegetable components may interact with the enzyme systems catalyzing the metabolic activation of the various promutagens, impeding the production of genotoxic intermediates (Ikken et al., 1998a).

The aqueous and ethanolic extracts from the analyzed fruits and vegetables have shown different antimutagenic activities against structurally nonrelated *N*-nitrosamines. These results suggest that either a great variety of antimutagenic compounds of different structure exist in fruits and vegetables or that groups of similar compounds, with virtually universal distribution in the plant kingdom, may be responsible for the direct interaction between the genotoxic reactive intermediates derived from *N*-nitrosamines and the antimutagenic compounds.

The observed differences in the antimutagenic activity of aqueous and ethanolic extracts from tested fruits and vegetables could be attributed to their antioxidant capacity as reported by some researchers (Gey, 1990; Steinberg, 1991; Ferguson, 1994). Dietary antioxidants present in fruits and vegetables are thought to decrease free-radical attack on DNA and, hence, to protect against mutation that causes cancer (Duthie et al., 1996). Therefore, it would be of interest to measure the total antioxidant capacity of fruits and vegetables. The results obtained by our laboratory indicated that the greatest inhibition effect of the mutagenicity of *N*-nitrosamines tested may be attributed to the kiwi (against NDBA) and licorice (against NPIP, NDMA, and NPYR) ethanolic extracts. Fruits and vegetables contain many different antioxidant components (vitamin C, vitamin E, β-carotene). In this way, kiwi fruits are an excellent source of vitamin C, containing around 5.0 μmol/g (wet wt) (USDA, 1986). However, Wang et al. (1996) suggested that the major source of antioxidant capacity of most fruits may not be from vitamin C and that other antioxidants in fruits should be considered. The results obtained with aqueous (Ikken et al., 1998a) and ethanolic kiwi extracts suggest that the antimutagenic activity against *N*-nitrosamines involved several kiwi compounds (water and/or ethanol solubles).

Some natural plant products, such as phenolics, indoles, and flavonoids (including flavones, isoflavone, flavonones, anthocyanines, catechin, and isocatechin), have been shown to have a strong antioxidant activity and antimutagenic and anticarcinogenic effects against some direct- and indirect-acting mutagens (Wattenberg, 1983; Newmark, 1984; Bors et al., 1990; Teel and Castonguay, 1992; Hanasaki et al., 1994; Wanasundara and Shahidi, 1994; Yen and Duh, 1996). Among all the ethanolic extracts analyzed, only licorice ethanolic extract was effective in reducing the mutagenicity of all *N*-nitrosamines tested. Besides glycyrrhizin, licorice also contains flavonoids and isoflavonoids, chalcones, coumarins, and 45 phenolic constituents (Demizu et al., 1988; Okuda et al., 1989; Fenwick et al., 1990). Gordon and An (1995) suggested that a synergic effect of the flavonoid mixture may be responsible for the high antioxidant activity attributed to licorice (Gordon and An, 1995). In this regard, the ability of licorice ethanolic extract to inhibit the mutagenicity of *N*-nitrosamines could be associated with the high antioxidant activity of the components.

Taken together, the results reported in this work and in previous studies (Ikken et al., 1998a) indicate that fruit and vegetable extracts exert a wide antimutagenic effect against *N*-nitrosamines evaluated by means of the Ames test. Further investigation is needed on the isolation of the antimutagenic compounds from fruits and vegetables and to understand the antimutagenic mechanism by which they may provide protection against mutagenic activities of *N*-nitrosamines.

CONCLUSION

This paper has discussed the inhibitory effect of fruit and vegetable ethanolic extracts against the mutagenicity of NDMA, NPYR, NDBA, and NPIP using the Ames test. Licorice ethanolic extract showed the greatest inhibition effect against NPIP (72%), NDMA (45%), and NPYR (39%). The greatest inhibition effect (51%) of the mutagenicity of NDBA was shown by kiwi ethanolic extract. Vegetable and fruit ethanolic extracts that exhibited an antimutagenic effect in decreasing order against NDMA and NPYR were licorice > kiwi > carrot and licorice > broccoli > pineapple > kiwi. Decreasing orders against NDBA and NPIP were, respectively, kiwi > licorice > onion > garlic > green pepper > carrot and licorice > garlic > pineapple > carrot. The results indicate that vegetable and fruit ethanolic extracts could be useful to humans for the purpose of mutation chemoprevention against *N*-nitrosamines.

ABBREVIATIONS USED

DMSO, dimethyl sulfoxide; NDMA, *N*-nitrosodimethylamine; NPYR, *N*-nitrosopyrrolidine; NDBA, *N*-nitrosodibutylamine; NPIP, *N*-nitrosopiperidine

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Received for review February 17, 1999. Revised manuscript received May 19, 1999. Accepted May 22, 1999. This work has been supported by Grant ALI-94-0993 from the Comisión Interministerial de Ciencia y Tecnología (CICYT). A.M. is recipient of a Fellowship from the Ministerio de Educación y Ciencia, Spain.

JF990166N