Carcinogenicity of Some Folk Medicinal Herbs in Rats[1,2]

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ABSTRACT—Twelve medicinal herbs were bioassayed to correlate a high incidence of esophageal carcinoma in natives of different places with their habitual consumption of these products. Outbred NIH Black rats were given 72 weekly sc injections of the total aqueous extracts of the plant materials. The tannin-rich plant extracts from *Areca catechu* and *Rhus copallina* produced local tumors in 100 and 33%, respectively, of the experimental animals. Other materials included *Diospyros virginiana* and extracts from plants not rich in tannins. *Diospyros* and extracts of *Sassafras albidum* and *Chenopodium ambrosiodes* were tumorigenic in over 50% of the treated animals.—J Natl Cancer Inst 60: 683–686, 1978.

In earlier communications (*1*, *2*) we reported that the aqueous extracts and fractions of a number of plant materials used as home remedies and beverages from Curacao, South Carolina, and other places, can produce malignant fibrous histiocytomas in rats following repeated sc injections. In continuation of such experiments, several additional plant materials were investigated. Results of studies involving 12 plant materials were presented in (*3*).

MATERIALS AND METHODS

Plant materials.—The 12 plants, their scientific and common names, parts used in this experiment, and their sources are listed in table 1.

The parts from 9 species of plants [exceptions were sugarcane (*Saccharum*), sassafras (*Sassafras*), and sagrado (*Chenopodium*)] were powdered, extracted with hot water, lyophilized, and used as total aqueous extract. Sugarcane stems were pressed to obtain the juice, which was lyophilized. Sassafras root bark was extracted in sequence with petroleum ether (40–60° C), methylene chloride, and ethanol to eliminate safrole (a known hepatocarcinogen) and other propenyl benzenes and fat-soluble compounds, which are extracted in the first two solvents. The ethanol extract was then used for bioassay. Because sagrado is rich in volatile oil, an attempt was made to preserve the oil during lyophilization. Its aqueous extract was, therefore, further extracted with methylene chloride. The aqueous extract was lyophilized and mixed with the residue from the methylene chloride extract; the combined material was then used for bioassay.

Bioassays.—Bioassays were conducted on 1–2-month-old outbred NIH Black rats by the procedures used in our earlier studies (*1*, *2*). A dose (in 0.5 ml normal saline) of a plant material that did not produce any systemic toxicity or local necrosis and sloughing and only caused some swelling, which disappeared within 1–2 weeks, was injected sc once a week alternately into each flank of 15 male and 15 female rats; saline was injected similarly into 30 control rats. The experiment was terminated arbitrarily after 78 weekly injections and a subsequent 12-week period of observation. As soon as a tumor was detected, injection was stopped. When the tumor grew to a sufficient size, the animal was killed and tumor tissue and other organs (i.e., regional lymph nodes, lungs, liver, spleen, and kidneys) were collected. Such tissues with or without tumor were also collected when an animal died or was killed for any pathologic reason as indicated in table 1; animals were also killed and tissues collected at termination of the experiment. All tissues were examined grossly for tumor metastasis; tumors and tissues suspected of being tumors were processed and stained for histologic study, as in our earlier experiment (*1*). Tumor incidence in the respective treated groups was compared to that in the saline-treated control group, and the statistical significance of their differences was estimated by the chi-square test.

RESULTS

The results listed in table 1 indicate that the yields of the water-soluble components from the 12 plant materials extracted varied in amount, the highest (25%) being for *D. virginiana* leaves and the least (4.3%) being for *G. obtusifolium*. The maximum dose of the extract selected for sc injection according to the criteria used (*1*) also varied with each plant material. This dose was highest (45 mg) with *B. halimifolia* and least (8 mg) with *M. cerifera*.

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TABLE 1.—*Development of tumor following sc injections of plant materials in NIH Black rats*

Plant material		Yield[a] %	Dose mg	No. of rats with tumor; mean No. of weekly injections (range)[b]		No. dead (wk)	
Scientific name, common name, and plant part	Source			Males	Females	Males	Females
Areca catechu Betel nut (cured); seed	South India	11.5	12	15; 42 (29-56)[c]	15; 51 (32-54)[c]	0	0
Hamamelis virginiana Witch hazel; leaf	Woods near Huger, S.C.	6	10	3; 73 (72-73)	0	1 (24)[d] 1 (57)[d]	1 (59)[d]
Rhus copallina Shining sumac; root	Brookgreen Gardens, Murrels Inlet, S.C.	5.9	15	7; 65 (50-75)[e]	3; 62 (56-70)	2 (69, 72)[d] 1 (67)[f]	1 (59)[d]
Baccharis halimifolia Sea myrtle; leafy twig	Miami, Fla.	22	45	0	0	1 (58)[f] 2 (67, 71)[g]	1 (76)[f]
Chenopodium ambrosioides Sagrado, wormseed plant, Jerusalem oak; whole plant without root	Johns Island, S.C.	19	10	11; 58 (37-75)[c]	5; 61 (48-76)[h]	1 (64)[d]	1 (76)[f]
Diospyros virginiana Persimmon; leaf	Brookgreen Gardens, Murrels Inlet, S.C.	25	15	14; 69 (56-75)[c]	3; 68 (52-78)	0	2 (52, 55)[d] 1 (78)[f]
Gnaphalium obtusifolium Life everlasting; whole plant without root	City market, Charleston, S.C.	4.3	30	0	0	2 (45, 54)[d] 1 (55)[f] 1 (50)[g]	3
Myrica cerifera Southern bayberry; leaf	Georgetown, S.C.	6.5	8	0	0	1 (32)[d] 2 (64, 76)[f]	1 (76)[i] 1 (33)[f]
Paederia foetida Prasarni; vine	Miami, Fla.	16	40	0	0	1 (39)[f]	1 (78)[i]
Psidium guajava Guava; unripe fruit	Homestead, Fla.	18	35	2; 75 (72, 77)	0	1 (72)[g]	1 (78)[f]
Saccharum officinale Blue Ribbon sugarcane; stem	City market, Charleston, S.C.	8.1	35	0	0	1 (72)[g]	1 (20)[d]
Sassafras albidum Sassafras; root bark	Local market	6.9	15	11; 59 (38-74)[c]	9; 65 (50-78)[c]	0	0
Saline		—	—	0	0	1 (60)[g]	—

[a] Weight of fraction/100 g plant material. Yield indicates total aqueous extract for all plant materials except for the ethanol extract of *Sassafras albidum*.
[b] Each dose was given weekly to 15 male and 15 female rats (by sc injection in alternate flanks) until tumor development was detected.
[c] $P<0.005$.
[d] Died or killed because of lung infection.
[e] $P<0.01$.
[f] Died or killed because of unknown cause.
[g] Died or killed because of ear infection.
[h] $P<0.05$.
[i] Died or killed because of abdominal abscess.

Extracts of 5 of the 12 plant materials were definitely tumorigenic. Among these the uncured seed extract of *A. catechu* caused tumors in all 30 NIH Black rats ($P<0.005$). Furthermore, tumors appeared in these rats much earlier than in the other groups. Tumor incidences were also high in groups treated with *D. virginiana, C. ambrosioides, Sassafras albidum,* and *R. copallina,* particularly in the males ($P<0.005$-<0.01). The extracts of *Psidium guajava* and *H. virginiana* produced tumors in only 2 and 3 rats, respectively, as late as 72 weeks or more; such occurrences were statistically insignificant compared to the controls. No tumors were seen after treatment with the other plant materials.

In most instances, the tumor incidence was greater among the males than among the females. When all the tumor-producing drug groups were combined, the incidence of tumor was 1.8 times higher among the male rats than among the females, and the difference was statistically significant ($P<0.005$).

Histologic examination of all induced tumors showed characteristics of malignant mesenchymal tumors. These were similar to those of human malignant fibrous histiocytoma. No significant abnormality was observed in the other organs of treated animals or in controls.

DISCUSSION

Among the 12 extracts bioassayed for carcinogenicity, 3 plants—*A. catechu, R. copallina,* and *H. virginiana*—are known to be rich in tannins. As expected from earlier studies on such plant materials (*1-7*), extracts from the 3 plants produced tumors in varying numbers of test animals. Significant numbers of tumors were produced by the extract of cured *A. catechu* and by *R. copallina* (100 and 33%, respectively). However, *H. virginiana* caused tumors in only 10% of the experimental animals. In the present study, only local tumors were observed, as reported earlier by us and others (*1-5, 7*).

The carcinogenicity of uncured raw betel nuts combined with active shell lime and chewing tobacco (1:1:1), as used by humans, has been studied in mice by Reddy

and Anguli (*8*). Vaginal epithelial papillomatous growths and vaginal mucosal thickening with changes of epithelia and submucosa were observed. Metastases to lungs, kidneys, and intraperitoneal regions were also found. Dunham et al. (*9*) studied the carcinogenicity of arecoline, an alkaloid from *A. catechu*. Proliferative lesions developed in the esophagi of 2 hamsters in a group of 9 that received calcium hydroxide applied to the cheek pouch followed by painting with arecoline.

In the present bioassays, extracts of 4 plant materials not rich in tannins also produced tumors. Among these were the safrole-free ethanol extract of *Sassafras albidum* root bark and the aqueous extract of *D. virginiana*, which produced local tumors in 66 and 56% of the treated animals, respectively. The former plant is known to contain safrole, isosafrole, anethole, and eugenol. The propenyl benzene derivatives eugenol and anethole are known to be weak hepatotoxins (*10, 11*). Safrole has been reported to be a hepatocarcinogen (*12–14*). However, in the extract we used, these volatile compounds and a number of other constituents (*15, 16*) were eliminated. The extracts of *C. ambrosioides* and *Psidium guajava* also produced local tumors in 53 and 6% of the treated rats, respectively.

Ingestion of a number of plant materials, including several that we studied, has been implicated by Morton (*17–19*) as among possible factors involved in development of esophageal cancer in humans. To establish a definitive role of these materials in causing esophageal cancer, ingestion experiments in suitable test animals must be performed. However, such ingestion studies are difficult and require much longer time, particularly when predisposing conditions in the host are unknown and plant materials are too many. Furthermore, their definitive correlation of carcinogenicity in humans is still unproved. Before proceeding with ingestion studies, we were interested in selecting the prime plant materials responsible for causing carcinogenesis by the sc route. Having identified several potent materials as reported herein and earlier (*1, 2*), we are now subjecting some of these "candidates" to ingestion experiments in rats. In all our studies several plant materials as well as saline (as control) injected sc failed to produce any tumor; such evidence indicates that carcinogenicity is related to the material injected and not to the route of administration.

The British Committee on Medical and Nutritional Aspects of Food Policy concluded that sarcomas induced in the subcutaneous tissues of the rats following local administration of a substance is not itself decisive evidence that the substance represents a carcinogenic hazard for other species (*20*). However, recently Tomatis (*21, 22*) has collated and analyzed the data on the 102 chemicals that were tested by sc route and compared the results with those obtained by the use of other routes. He concluded that the sc administration of a chemical produced "false negative" results in 6 (5.6%) of the 102 chemicals tested and, if all the criticisms of this route of administration are considered, false positive results in 9 (8.7%) of the 102 chemicals tested. He pointed out that the sc route of administration is not much worse than any other route, and a similar proportion of false positive and false negative results are obtained in other tests used for the prediction of a possible carcinogenic activity.

Thus, although questions exist as to the validity of results obtained by the sc method, indications suggest that some of the plant materials tested represent a hazard if used for a long time.

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