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UCRL-JRNL-202067

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2/04

Breast Cancer Research and Treatment

Flor-Essence® Herbal Tonic promotes mammary tumor development in Sprague Dawley rats

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Abstract

Background: Women who are diagnosed with breast cancer often self-administer complementary and alternative medicines to augment their conventional treatments, improve health, or prevent recurrence. Flor-Essence® Tonic is a complex mixture of herbal extracts used by cancer patients because of anecdotal evidence that it can treat or prevent disease.

Methods: Female Sprague Dawley rats were given water or exposed to 3% or 6% Flor-Essence® beginning at one day of age. Mammary tumors were induced with a single oral 40 mg/kg/bw dose of dimethylbenz(a)anthracene at 50 days of age and sacrificed at 23 weeks. Rats were maintained on AIN-76A diet.

Results: Control rats had palpable mammary tumor incidence of 51.0% at 19 weeks of age compared to 65.0% and 59.4% for the 3% and 6% Flor-Essence® groups respectively. Overall, no significant difference in time until first palpable tumor was detected among any of the groups. At necropsy, mammary tumor incidence was 82.5% for controls compared to 90.0% and 97.3% for rats consuming 3% and 6% Flor-Essence®, respectively. Mean mammary tumor multiplicity (\pm SES) for the controls was 2.8 (\pm 0.5) and statistically different from the 3% or 6% Flor-Essence® groups with 5.2 (\pm 0.7), and 4.8 (\pm 0.6), respectively ($p \leq 0.01$). As expected, the majority of isolated tumors were diagnosed as adenocarcinomas.

Conclusions: Flor-Essence® can promote mammary tumor development in the Sprague Dawley rat model. This observation is contrary to widely available anecdotal evidence as well as the desire of the consumer that this commercially available herbal tonic will suppress and/or inhibit tumor growth.

Introduction

Complementary and alternative medicines (CAMs) are particularly attractive to women with breast cancer and people with higher education who have chronic diseases (1,2). Cancer patients are motivated to try unconventional therapies in their search for hope (2,3). They do not want to leave unexplored any option that could improve survival or quality of life. However, only 50% of those patients who elect to use CAM discuss it with their physicians. The reasons for this range from doctors not asking about CAM use to the patient thinking it is not necessary to share this information with their doctor. In addition, patients often sense that physicians may be unfamiliar with CAM, might convey their opposition, or may chastise the patient for his/her interest (1,2,4).

Flor-Essence® Herbal Tonic is a widely used and self-administered CAM that cancer patients often use to supplement conventional therapies (2,5-7). Women who have been diagnosed with breast cancer use Flor-Essence® tonics to treat disease as well as to prevent recurrence (2). A recent study reported that 26% of health food stores surveyed recommended one cup of Flor-Essence® daily to treat breast cancer (8). Counseling about the use of Flor-Essence® Herbal Tonic, as for many CAMs, is prevented by a lack of information about its safety, efficacy, or potential drug interactions. Herbal tonics have been used as a breast cancer treatment since the 1920's (5,7,9,10), yet no epidemiologic data are available that support or refute their use as a CAM. Commercially available herbal tonic formulations are expensive products largely used by cancer patients who hope it will improve their health. The available literature focusing on the Flor-Essence® Herbal formulations is widely disseminated in the lay

press (6,9,11) and on numerous web sites where information is often presented as testimonial and is rarely backed by scientific investigation making the patient all that more vulnerable (3,6).

A recent report provides information about the consumers who purchase Flor-Essence® and describes the reasons for its use (2). Of those who use Flor-Essence®, 76% do so because they have been diagnosed with cancer (2,10). The majority (65%) of individuals who use Flor-Essence® report that they heard about it from family or friends with only a small proportion (6%) stating its use was suggested by a traditional health care professional (2). The testimonials circulated about the health benefits of herbal tonics, include their ability to cure cancer, alleviate pain, improve chronic conditions and overall health, as well as other benefits (6,9,10).

Flor-Essence® is a complex mixture of eight herbal extracts that includes burdock root (*Arctium lappa*), sheep sorrel (*Rumex acetosella*), slippery elm (*Ulus rubra*), Turkish rhubarb (*Rheum palmatum*), watercress (*Nasturt officinale*), blessed thistle (*Carduus benedictus*), red clover (*Trifolium pratense*), and kelp (*Laminaria digitata*) (10). The lay literature suggests that the components act synergistically to convey beneficial properties (6,9), an assertion not supported or refuted by scientific investigation (10). The herbs in Flor-Essence® are important in the Asian diet and have histories of use in Asian medicine. They are reported to have estrogenic, cytotoxic, anti-estrogenic, anti-mutagenic, and anti-oxidant properties, among others (10). Many of these properties are associated with altering cancer risk in animals and humans.

Estrogen is a known human breast carcinogen and influences mammary cancer risk in rodent models. Red clover, burdock root and sheep sorrel have estrogenic activity whereas watercress and kelp have been characterized as having anti-estrogenic activity(10,12). Consumption of dietary compounds that have estrogenic or anti-estrogenic activity is believed to have an effect on human breast cancer risk. Because Flor-Essence® contains herbs with known

estrogenic and anti-estrogenic activity, we believe that this herbal tonic may have an impact on breast tumorigenesis. However, no clinical trials, animal studies, or basic science reports have been published that evaluate the effect of Flor-Essence® on human health, the breast or in breast tissue, or on other endocrine end organs.

We report here for the first time the effect of Flor-Essence® consumption on mammary tumor development in an animal model. We selected the Sprague Dawley DMBA rat model for this analysis because it is a well-established and highly predictable model system for the evaluating the consequences of environmental exposure on mammary tumor development (13-16). Because the effects of this dietary supplement on tumor development in an animal model had never been reported, this study was designed as a pilot study to determine if further investigations would be warranted.

Flor-Essence® given to rats at physiologically relevant concentrations in the drinking water stimulated the growth of DMBA induced mammary tumors. This observation is contrary to widely available anecdotal evidence as well as the desire of the consumer that the tonic will suppress and/or inhibit tumor growth.

Methods

Animals

Female and male Sprague Dawley rats were purchased (Simonson, CA) and bred to generate the females used in this experiment. Adult rats were housed two or three per cage, depending on their size. The animals were maintained in temperature and humidity controlled room, with a 12-hr dark/light cycle. The rodents were provided the AIN-76A diet (Research

Diets, New Brunswick, NJ) as previously described (17). Rats had access to food and liquid *ad libitum*. Flor-Essence® Herbal Tonic was administered in the drinking water. The care of the animals was in accord with the Lawrence Livermore National Laboratory (LLNL) Institutional Animal Care and Use Committee (IACUC) committee guidelines.

The male progeny were identified at weaning and euthanized in accordance with the SOP for rodent euthanasia as required by the LLNL IACUC and consistent with the recommendation from the Panel on Euthanasia of the American Veterinary Medical Association for animal euthanasia.

Flor-Essence® and Carcinogen administration

Pregnant dams were randomly assigned to three experimental groups on the day they gave birth such that approximately 40 female rat offspring were included in each of three groups. On the day of birth, the dams whose offspring were assigned to the control group received only water while the dams of the offspring assigned to one of the two treatment groups received 3% or 6% Flor-Essence® as their sole drinking source. The offspring were weaned at approximately 24 days and were maintained on the same treatment as their sole drinking source until the termination of the study.

Flor-Essence® Herbal Tonic (Flora Manufacturing & Distributing Ltd., B.C., Canada) was purchased in 500 ml bottles as a pre-prepared and sterile formulation from a local health food store. Flor-Essence® Herbal Tonic was administered to rodents in their drinking water as 3% and 6% solutions and was readily accepted (18,19). The dose of 3% was selected based on the recommendation to cancer patients made by a popular natural health proponent to consume 2 fl oz of Flor-Essence® three times per day (11). The daily consumption of 6 fl oz of Flor-

Essence® by a 130 lb. woman is equivalent to 3 ml/kg/day in rodents. The 6% concentration of Flor-Essence® was also readily accepted by the rodents and was selected to evaluate a dose response. Concentrations administered to rats were calculated based on the average daily consumption of 100 ml/kg of liquid daily.

All experimental rats received a single 40 mg/kg dose of DMBA (Sigma, St. Louis, MO) dissolved in sesame oil (Sigma, St. Louis, MO) by gavage at 7 wk of age to induce mammary tumors (13-16).

Tumor development and body weight

All rats were uniquely identified, were examined weekly for palpable mammary tumors, and were weighed weekly beginning at weaning. Palpable mammary tumor incidence and average body weight was determined weekly for each experimental group. The rats were sacrificed at 23 weeks of age, when tumors have grown larger than 3 cm, or when moribund. At the time of terminal sacrifice, the animals were weighed and the mammary tumors enumerated, their locations on the pelt recorded, dissected from the pelt, and measured. Any tumor measuring greater than 1.5 cm in diameter was weighed after dissection. To confirm tumor type, routine histopathology was performed on tumors from control and treated groups and diagnosed by a veterinary pathologist.

Statistical Analysis

Palpable mammary tumor development was compared among groups using a life-table method (20). Mammary tumor incidence and multiplicity were determined from the data collected at sacrifice. Mammary tumor incidence was compared between the control group and

each of the Flor-Essence® treated groups using a two-tailed Fisher's exact test. The Cochran-Armitage trend test was used to determine the significance of the trend across the three groups (21). Comparisons of mammary tumor multiplicity between the Flor-Essence® treated and untreated groups was performed using the Wilcoxon rank sum test. The Jonckheere-Terpstra trend test was used to evaluate the trend in the number of tumors per animal from control to 3% and 6% Flor-Essence® (22). Comparisons of weight between the control and treated groups were performed using a two-tailed Wilcoxon rank sum test. The Jonckheere-Terpstra trend test was used to evaluate the weights across all three experimental groups. All p-values are two-tailed.

Results

Palpable mammary tumor development

The first palpable mammary tumors were detected in the control and 6% Flor-Essence® treated group at 13 wk and in the 3% group at 15 wk of age (**Figure 1**). Palpable mammary tumor incidence was determined weekly for each group until the end of the study. By 19 weeks of age 51.3% of the control animals had palpable mammary tumors compared to 65% and 59% of the 3% and 6% Flor-Essence® treated groups, respectively. At 23 weeks, and end of the study, the proportion of animals with palpable tumors was 73%, 80%, and 86% for the control, 3% and 6% groups, respectively. Overall, there was no significant difference in the incidence of palpable mammary tumors among groups ($P>0.50$)

Mammary tumor incidence and multiplicity

The terminal necropsy was performed when the animals were 23 weeks of age or when the tumor burden became too great. The mammary tumor incidence values observed at the final necropsy in the control, 3% and 6% Flor-Essence® groups were 82.5%, 90.0%, and 97.3%, respectively and differed from palpable mammary tumor incidence because not all tumors visible at necropsy could be palpated (**Table 1**). The association between increasing incidence of tumors at sacrifice and increasing strength of the Flor-Essence® Herbal tonic exhibited a trend of borderline statistical significance ($p = 0.039$).

At the end of the study, mammary tumor multiplicity in the experimental groups consuming 3% and 6% Flor-Essence® was 1.9- and 1.7-fold greater than for the controls (**Table 1**). Whereas the control group had a mean (\pm SEM) of 2.8 (± 0.5) tumors per animal, the 3% and 6% treated groups had means of 5.2 (± 0.7) and 4.8 (± 0.6), respectively. Mammary tumor multiplicity in both the 3% and 6% Flor-Essence® treated groups was statistically significantly greater relative to controls ($p=0.01$; $p=0.0026$ respectively), and the trend was highly significant ($p=0.0066$).

The tumors in the control, 3% and 6% Flor-Essence® groups were examined as a function of tumor size (**Table 2**). For those animals that had tumors at the termination of the experiment the average number of tumors that measured < 1 cm, 1-1.9 cm, 2-2.9 cm and ≥ 3 cm was determined. The average multiplicity for tumors less than 1 cm in diameter was 1.5, 2.7, and 2.0 in the the control, 3% Flor-Essence and 6% Flor-Essence groups, respectively. The average tumor multiplicity was slightly greater in the 3% and 6% Flor-Essence® treated groups than in the control group for each of the other three class sizes of tumors. Thus, in each case, the numbers of tumors according to size tended to be consistent with overall multiplicity results. As

expected, the majority of mammary tumors isolated from the rats in this study were diagnosed as adenocarcinomas and similar between control and treated rats.

Body weight

The body weight of all rats was documented over time. The mean body weight for the control animals was greater at every time point examined compared to the 3% and 6% Flor-Essence® treated groups (**Figure 2**). The animals treated with 3% Flor-Essence® weighed less than the control and the effect was even more pronounced with 6% Flor-Essence®. Overall, this trend was statistically significant at every time point from 14 to 23 weeks. ($p \leq 0.001$).

Conclusions

This is the first report demonstrating that Flor-Essence® Herbal tonic can promote the development of mammary tumors in an animal model. The female rats consuming Flor-Essence® had a significantly greater mammary tumor multiplicity than the controls and there was a trend of borderline statistical significance toward increasing tumor incidence as the strength of the tea increased. However, the herbal tonic did not influence the time at which the first tumor developed. Together, these factors suggest that Flor-Essence® promotes mammary tumor development and is unlikely to contribute to their initiation by DMBA.

Sprague Dawley rats do not develop spontaneous mammary adenocarcinomas; all mammary tumors that develop after DMBA exposure are chemically induced. By one year of age Sprague Dawley rats do begin developing spontaneous fibroadenomas, which can be influenced by environmental exposure but are not believed to progress to adenocarcinomas. DMBA-induced rat mammary tumors are estrogen receptor positive and hormonally responsive (23,24).

Flor-Essence® herbal tonic contains several components with known estrogenic activity including red clover, burdock root, and sheep sorrel. Red clover contains several phytoestrogens including the isoflavones genistein, daidzein, formononetin, and biochanin-A. Administration of red clover to ovariectomized rats results in a dose-dependent increase in uterine wet weight, a biologic measure of estrogenic activity (12). Genistein levels in red clover are ten-fold greater than in soy (10). The long-term consequences of red clover exposure in women has not been reported (25). Burdock root contains the phytoestrogen genistein and sheep sorrel is reported to have high phytoestrogen content (10).

In vitro studies have shown that Flor-Essence® can promote human breast tumor cell growth through an estrogen-dependent pathway (26,27) and at higher concentrations can inhibit cell growth (28, Kulp and Bennett, unpublished observations). It is possible that the estrogen-like components of the Flor-Essence® herbal tonic could play a role in the promotion of mammary tumors in the Sprague Dawley rat. For example, genistein has been shown to modulate mammary tumor development in several studies (14,15,17,29-33).

Flor-Essence® consumption resulted in a significant decrease in average body weight with increasing dose of tonic relative to controls. There were no observable differences in overall health or behavior of the treated rats compared to controls. The mechanistic basis for this observation has not been established.

There are many examples of complex mixtures to which individuals choose to expose themselves. In these instances the perceived risk is acceptable to the individual in spite of abundant data demonstrating adverse human health consequences. The choices to smoke cigarettes, use smokeless tobacco, consume charred meats, or drink alcoholic beverages are just some examples (34). Choices made to self administer commercially available vitamin,

nutritional or dietary supplements, herbal medicines, and plant extracts are usually made because they are perceived as being beneficial to human health and without risk. Promotion by the manufacturer, lay press, and from anecdotal evidence reduces or eliminates perceived health risk by the individual in spite of little or no evidence to support or refute the health claims.

The preliminary study presented here provides a foundation for the continued evaluation of the dietary supplement Flor-Essence® to understand better the consequences of its administration on human breast health. Natural products have that can modify normal mammary gland development as well as carcinogenesis in animal models (13,15,32,33,35). To establish the relevance of these results to humans, it will be critical to design studies to expand the knowledge gained in this investigation by considering exposure in combination with the critical windows of mammary gland development and evaluate the consequences of the herbal tonic on normal mammary gland ductal morphology as well as tumorigenesis. We will determine if the acceleration of tumor growth in the Sprague Dawley rat is associated with exposure during a particular period of mammary gland development or has the same or opposite effect when administered when tumors have developed and the mammary gland is mature. This aspect is especially important since many women choose to self-administer Flor-Essence® after a breast cancer diagnosis and the breast is fully developed, to supplement conventional therapy.

Suggested daily dosage on the Flor-Essence® label include directions for use in children and infants and lack any directions or cautions about use during pregnancy, a time when there are developmental changes occurring in the breast and the effects of *in utero* exposure are unknown. It is critical to understand how Flor-Essence® contributes to tumor growth in the rat, whether timing plays a role, and whether there is an effect on normal mammary ductal morphogenesis as a consequence of treatment during critical windows of development. Future studies will be

designed to answer these questions. Information about how herbal tonics affect normal and neoplastic mammary gland growth and development can be used to make informed decisions about their use and will set the stage for further analysis of this as well as other currently used CAMs.

Acknowledgements

We greatly appreciate the expert assistance with veterinary pathology provided by Dr. Barbara J. Davis and Dr. Sharron Kirchain. This study was funded by the California Breast Cancer Research Program, grant #7IB-0003. This work was performed, in part, under the auspices of the U.S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48.

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Figure 1:

Palpable mammary tumor incidence in Flor-Essence® treated rats compared to controls. All control (triangle), 3% Flor Essence (diamond), or 6% Flor-Essence® (square) rats received a single 40 mg/kg dose of DMBA (Sigma, St. Louis, MO) dissolved in sesame oil (Sigma, St. Louis, MO) by gavage at 7 wk age to induce mammary tumors. All rats were uniquely identified, examined weekly for palpable mammary tumors.

Figure 2:

Comparison of body weight among Flor-Essence® treated and control rats. Average body weight was determined weekly for animals drinking 3% Flor-Essence (diamond), 6% Flor-Essence® (square), or water (triangle).

Table 1. Effect of Flor-Essence® administration on DMBA-induced mammary tumor development

Treatment Group	Number	Mammary tumor		
		First Tumor	Incidence	Multiplicity ^a
Control	35	13 wk	82.5	2.7 (0.5)
3% Flor-Essence®	40	15 wk	90.0	5.2 (0.7) *
6% Flor-Essence®	37	13 wk	97.3	4.8 (0.6) *

^a Values are mean (SEM). *p ≤ 0.01 compared to controls

Table 2. Average tumor multiplicity as a function of size at sacrifice

Group	Multiplicity				
	overall	< 1 cm	1-1.9 cm	2-2.9 cm	≥ 3 cm
Control	2.7	1.5	1.3	0.39	0.18
3%	5.2	2.7	1.8	0.57	0.46
6%	4.8	2.0	2.0	0.4	0.3

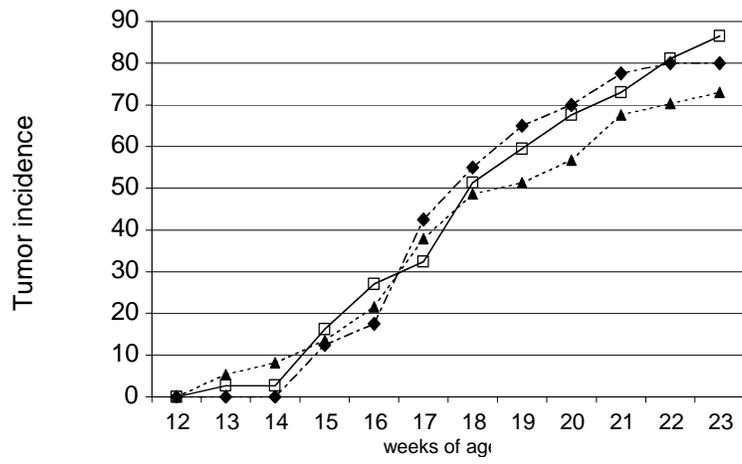


Figure 1

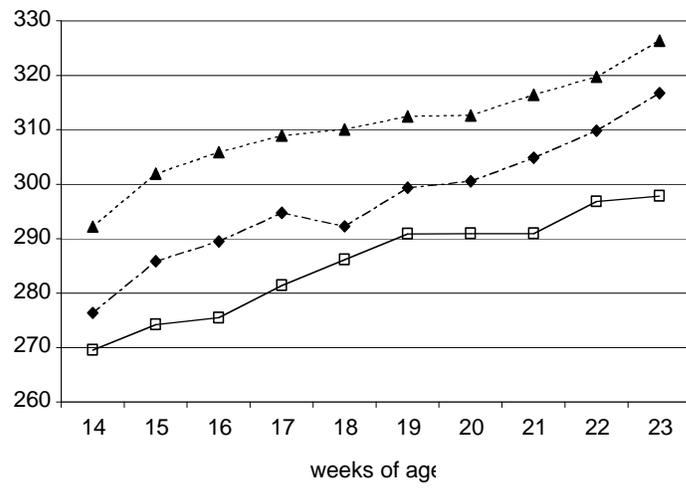


Figure 2