Dietary Administration of *Asimina triloba* (Paw Paw) Extract Increases Tumor Latency in *N*-Methyl-*N*-nitrosourea–Treated Rats*

Muriel Cuendet,1 Carol P. Oteham,1 Richard C. Moon,1 William J. Keller,2 Paul A. Peaden,2 and John M. Pezzuto1,3

1Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmaceutical Sciences, College of Pharmacy, Nursing, and Health Sciences, Purdue University, West Lafayette, Indiana, USA; 2Nature’s Sunshine Products, Inc., Spanish Fork, Utah, USA; 3University of Hawaii at Hilo, College of Pharmacy, Hilo, Hawaii, USA

**Abstract**

The paw paw tree, *Asimina triloba* (L.) Dunal (Annonaceae), contains more than 50 bioactive components, primarily annonaceous acetogenins. Some therapeutic activities have been associated with this material, but the potential to mediate a cancer chemopreventive effect has not been reported. In this study, a standardized extract from the twigs, in which bullatacin, asimicin, and trilobacin represent the most potent and major bioactive acetogenins, was tested in the *N*-methyl-*N*-nitrosourea–induced mammary carcinogenesis model. With Sprague-Dawley rats given a diet containing paw paw extract (1250 and 2500 mg/kg diet; based on maximum tolerated dose studies), mammary tumor latency was increased from 55 to 66 days. However, mammary tumor incidence and multiplicity were not affected by extract consumption.

**Keywords:** Acetogenins, annonaceous, *Asimina triloba*, bullatacin, carcinogenesis, paw paw tree, prevention of mammary.

**Introduction**

Studies on annonaceous species have intensified in the past 20 years, largely due to the discovery of the annonaceous acetogenins. This class of natural compounds has been reported to mediate anthelmintic, antimalarial, antimicrobial, antiprotozoal, pesticidal, and antitumor activities. The annonaceous acetogenins are powerful inhibitors of complex I (NADH: ubiquinone oxidoreductase) in mammalian and insect mitochondrial electron transport systems (Ahammadsahib et al., 1993; Hollingworth et al., 1994). In addition, they are potent inhibitors of NADH oxidase of the plasma membranes of cancer cells (Morre et al., 1995). These actions decrease oxidative as well as cytosolic ATP production. The consequence of such ATP deprivation is apoptosis (Wolvetang et al., 1994). Also, the acetogenins are especially effective against multidrug-resistant (MDR) tumors in which the resistance is due to ATP-dependent efflux pumps (Oberlies et al., 1997a, b). The acetogenins circumvent MDR by decreasing efflux pump function and increasing cellular drug accumulation (Fu et al., 1999).

The paw paw tree, *Asimina triloba* (L.) Dunal (Annonaceae), is native to the eastern United States; the fruit is edible. Bioactivity-directed fractionation has resulted in the isolation of more than 50 bioactive components, mostly acetogenins (Alali et al., 1999). Nature’s Sunshine Products (Spanish Fork, UT, USA) developed a standardized acetogenin product that uses an extract of the twigs from the paw paw tree (Table 1). Bullatacin (Fig. 1), asimicin, and trilobacin represent the most potent and major bioactive acetogenins that are found in this paw paw extract (Hui et al., 1989; Zhao et al., 1992).

Limited individual studies have been conducted with cancer patients taking one capsule containing 12.5 mg of the standardized extract 4 times a day. Results have suggested decreases in breast tumor antigens, stability of the prostate specific antigen, and reduction of tumor...
Table 1. Composition of the paw paw twigs concentrated powder.\(^a\)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Extraction ratio:</td>
<td>100 kg twigs to 1 kg extract</td>
</tr>
<tr>
<td>Percentage extract in the powder:</td>
<td>20%</td>
</tr>
<tr>
<td>Total active acetogenins:</td>
<td>73 mg/g of powder</td>
</tr>
<tr>
<td>Major acetogenins:</td>
<td>Asimicin (0.01%), bullatacin (0.003%), trilobacin (0.001%), bullatalicin, bullatetrocin, 10-hydroxyasimicin, 10-hydroxytrilobacin</td>
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\(^a\) Product of Nature’s Sunshine Product (Spanish Fork, UT, USA).

Materials and Methods

Animals and reagents

Female Sprague-Dawley rats were obtained from Harlan Sprague Dawley (Indianapolis, IN, USA) at 5 weeks of age. The paw paw standardized extract was from Nature’s Sunshine Products. N-Methyl-N-nitrosourea (MNU) was from Sigma-Aldrich (St. Louis, MO, USA).

Determination of the maximum tolerated dose

Virgin female Sprague-Dawley rats were received from Harlan at 35 days of age and placed on a diet of Teklad 4% rat/mouse chow. After 1 week, at 42 days of age, animals were randomized by weight into six groups and placed on experimental diets containing 1250, 2500, 5000, 10,000, or 20,000 mg extract/kg diet. All animals were weighed weekly. Animals were observed twice daily to assess their general health.

Inhibition of MNU-induced mammary carcinogenesis in rats

Virgin female Sprague-Dawley rats were received from Harlan at 35 days of age and placed on a diet of Teklad 4% rat/mouse chow (Harlan). After 1 week, at 42 days of age, animals were randomized by weight into four groups and placed on experimental diets containing 1250 and 2500 mg extract/kg diet. At 50 days of age, the animals received a single intravenous injection of MNU (50 mg/kg body weight) in acidified saline (pH 5.0) (or vehicle only) following an overnight fast. The rats were maintained on the extract diets until the end of the study (120 days post-MNU). During the experimental period, all animals were weighed weekly. Palpation for mammary tumors began 3 weeks after animals received MNU and continued until termination of the study. The date of appearance and location of all tumors were recorded. Animals were observed twice daily to assess their general health. Moribund animals were sacrificed by CO\(_2\) asphyxiation. Moribund animals or animals found dead were necropsied immediately. At the end of the study, blood samples were collected, and the concentration of acetogenins was determined by liquid chromatography–tandem mass spectrometry (LC-MS/MS).

Results and Discussion

One of the primary requirements for chemoprevention studies is that the test agent must be nontoxic, as the overall objective is to delay cancer in healthy populations or in individuals with an increased risk of developing cancer who are otherwise healthy (Kelloff et al., 1994). Thus, before conducting a chemoprevention study, a dose tolerance experiment is usually performed. In the current investigation, a maximum tolerated dose (MTD) study was performed using 1250, 2500, 5000, 10,000, and 20,000 mg extract/kg diet. At doses of 5000, 10,000, and 20,000 mg extract/kg diet, reduced food consumption led to a slight decrease in body weight (Fig. 2). Based on these results, doses of 1250 and 2500 mg extract/kg diet were chosen to conduct a MNU-induced mammary carcinogenesis study.

The MNU model provides an experimental approach for investigating mammary tumorigenesis induced by a
direct-acting carcinogen. Hyperplastic and carcinoma in situ lesions observed in the rat are generally comparable with the lesion types observed in the human breast (Russo et al., 1990; Crist et al., 1992). Premalignant lesions induced by MNU can be dependent (65–80% based on criteria used) or independent of ovarian steroids (Thompson et al., 1998). As MNU causes point mutations, a true anti-initiator often cannot prevent the tumorigenic response.

In the current study, palpable tumors was first observed in the control rats at 53 days of age (Table 2). In contrast, rats provided with diets containing either dose of the paw paw standardized extract did not develop mammary tumors until day 66. The increase in tumor latency was statistically significant compared with the control group (1250 mg extract/kg diet, \( p = 0.025 \); 2500 mg extract/kg diet, \( p = 0.018 \)). However, at the termination of the study, there were no differences in incidence and multiplicity of mammary tumors among the dietary groups (Fig. 3). In general, rats that received the paw paw extract had larger tumors than those of the control group. Some of these tumors became ulcerated and the rats were sacrificed. Rats fed the experimental diets gained weight at similar rates and there were no significant differences in final body weight among the treatment groups that received MNU (Fig. 3).

Table 2. Effect of the paw paw standardized extract on tumor latency.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Time to first tumor (days)</th>
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<tbody>
<tr>
<td>1</td>
<td>MNU</td>
<td>52.8 ± 15.6</td>
</tr>
<tr>
<td>2</td>
<td>MNU and 1250 mg extract/kg diet</td>
<td>67.3 ± 19.0*</td>
</tr>
<tr>
<td>3</td>
<td>MNU and 2500 mg extract/kg diet</td>
<td>65.5 ± 19.1*</td>
</tr>
</tbody>
</table>

*Significantly different from group 1 (\( p < 0.05 \)).
the extract did not prevent tumor formation, nor did it affect the rate of tumor growth. A similar delay in tumorigenesis without prevention of tumor development was observed in c-neu mice treated with a retinoid analogue (Rao et al., 1998) or soy isoflavones (Jin et al., 2002). These results suggest that tumor initiation was delayed by the dietary compound, or that tumor growth was inhibited for a short period. The growth of the tumors was apparently not affected after this period as incidence and multiplicity at termination were not different among the dietary treatment groups compared with controls.

Annonaceous acetogenins may be among the most potent cytotoxic agents with \( ED_{50} \) values of \(<10^{-9}\) ng/mL reported for trilobacin (Zhao et al., 1994) and asiminocin (Zhao et al., 1992) in several human tumor cell lines. However, different tumor cell lines show notable differences in their susceptibility to the same acetogenin (Bermejo et al., 2005). There are multiple factors involved in cell death caused by enzymatic inhibitors of mitochondrial complex I, including acetogenins, such as access of the toxic compound to the target enzyme, and defensive cellular mechanisms against this poisoning. Many studies have reported \textit{in vitro} activity of acetogenins, but only few have shown \textit{in vivo} activity. Uvaricin, rollinones, and asimicin demonstrated activity against P388, a murine lymphocytic leukemia (Jolad et al., 1982; Rupprecht et al., 1990). Also, bullatacin was effective at 50 \( \mu \)g/kg, intraperitoneal, against L1210 in normal mice and in tumor xenografts of a human ovarian carcinoma in athymic mice (Ahammadsahib et al., 1993; Alali et al., 1999).

In the current study, the paw paw extract was administered through the diet. Oral administration is most appropriate for cancer chemoprevention. However, at the end of the carcinogenesis study, LC/MS analyses of blood samples did not show measurable levels of acetogenins, irrespective of sample hydrolysis. The bioavailability of acetogenins, which are fat-soluble compounds, has not been reported. Studies with carotenoids, also liposoluble compounds, showed that the food matrix, the formulation, dietary proteins, and fats were important factors influencing bioavailability (Hosotani et al., 2005; Reboul et al., 2006; Yonekura et al., 2007). In the past, if a compound proved active in a given experimental model, it was believed to serve as a possible chemopreventive agent, possibly for most sites. However, with the accumulation of new data in the literature, it has become apparent that such a universal relationship does not exist. For example, certain retinoids are effective against experimental skin and urinary bladder carcinogenesis but not effective against chemically induced mammary carcinogenesis (Mehta, 2000). This raises an important concern about the appropriate model systems for predicting the response to a given chemopreventive agent. Further studies are warranted to fully evaluate the potential of the paw paw standardized extract as a cancer chemopreventive agent.

### Acknowledgment

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### References


