

Sweet leaf tea extract shows real promise in preventing cancer recurrence

Zhijun Liu, Eugene A. Woltering and Peiying Yang

After initial treatments of cancer through surgery, chemotherapy or radiation therapy, the next task is to prevent the cancers, especially recurrence-prone cancers, from recurring. This is because remnant cancer cells that survived the initial treatment or metastasized tend to grow, first by initiating new blood vessel formation when the environment is favorable.

Because preventing cancer recurrence requires long-term management, a safe regimen with no or minimal adverse effects would provide an optimal profile for managing the disease. Current therapeutics directed toward the prevention or treatment of cancer are often highly toxic at effective doses. Targeted therapies such as agents that inhibit angiogenesis – the process by which new blood vessels initiate and grow – offer great hope in cancer management.

Avastin, a U.S. Food and Drug Administration-approved antibody prescription drug targeted at the angiogenic

growth factor VEGF, is administered by injection, but has limited efficacy and serious adverse effects. This plus its high cost make it an impractical cancer-management agent.

While the pharmaceutical industry searches for single-entity drugs that target angiogenesis, many efforts have also been made to search for anti-angiogenic agents from botanical sources based on their medicinal-use records. LSU AgCenter researchers have identified a number of such botanical agents. One that has shown great promise is Chinese sweet leaf tea. Sweet leaf tea water extract at a dose of 0.45 grams per pound of body weight completely and totally blocked blood-vessel initiation and growth in a human tissue-based angiogenesis test.

The sweet leaf tea plant, native to the Guangxi-Guizhou regions of southwestern China, has been consumed as a beverage tea for years, mainly for its naturally sweet taste (said to be 300 times sweeter than cane

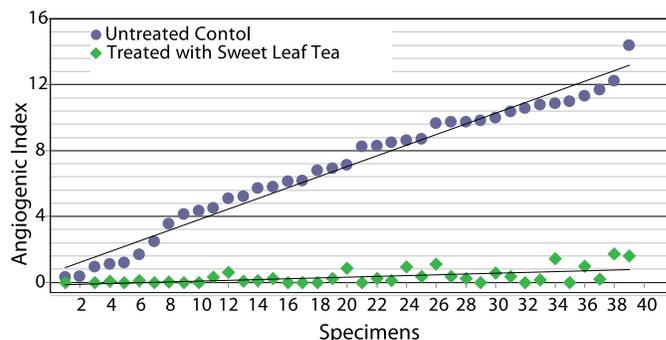
sugar). This human-use history, plus the scientific discovery of its anti-angiogenic property, prompted a multi-institutional investigation led by the LSU AgCenter and supported by the National Center for Complementary and Alternative Medicine of the National Institutes of Health. The ongoing two-year project focuses

on pre-clinical evaluation of the sweet leaf tea extract, with the goal of spinning it off into human clinical investigations as a cancer-preventative agent.

Although the enormous advantages of using safe botanical extracts as cancer-preventative agents are yet to unfold, the greatest challenge is to standardize the extract so that the batch-to-batch variations are assessed and controlled. This is especially challenging given the natural variations of botanical materials and the nature of mixed compounds in a single-leaf extract compared to single-entity drugs, such as aspirin.

The lack of quality control of botanical extracts often results in the lack of reproducible results of clinical efficacy. This investigation placed great emphasis on achieving standardization using multiple chemical markers that have proven combined anti-angiogenic activity. A chemical fingerprinting analysis method has been developed to assess the

Figure 1. Effects of sweet leaf tea extract on the angiogenesis of tumor tissues harvested from patients with mid-gut carcinoids. The standardized sweet leaf tea extract at 0.1% w/v dose consistently suppressed overall angiogenic response (expressed as Angiogenic Index) in comparison to the untreated controls.



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Plants prompt search for cancer prevention

For more than 15 years, Zhijun Liu with the School of Renewable Natural Resources has been investigating plants for medicinal properties. He started by looking at plants that traditionally have been used as folk remedies to treat diseases such as hypertension, diabetes and cancer.

For the past 10 years, his focus has been on angiogenesis inhibitors, hoping to find compounds that will prevent the growth of blood vessels and can be used to treat diseases such as cancer, obesity and psoriasis.

"Inhibiting angiogenesis can prevent cancer – and perhaps even fat tissue – from developing beyond the simple limits of existing blood vessels," he said.

Turning these discoveries into practical therapies presents major obstacles, which are typical in the use of botanical extracts for health care and therapeutics, Liu said.

"To be effective, the active ingredients must be concentrated enough to achieve a therapeutic effect," he said.

Liu first screens extracts by fractionating – dividing the material into smaller segments – and then further subdivides the fraction that holds the most promise for delivering an effective compound. Finally, he purifies the extract fraction to produce an effective concentration of the compound.

"We use bioassay-directed isolation to trace down the molecules responsible, then expand to similar chemical structures," Liu said.

Through laborious isolation he has identified gallic acid as a contributor to angiogenesis inhibition. Then he began looking for derivatives – analogs of gallic acid from other sources.

In Chinese sweet tea, for example, the extract did a better job of inhibiting angiogenesis than gallic acid alone.

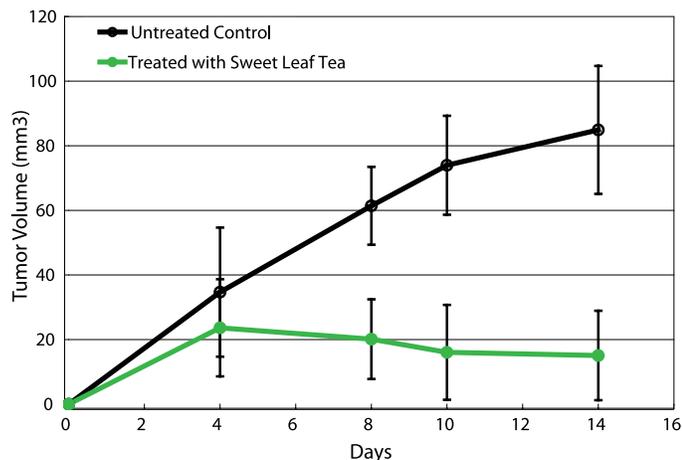
"Based on bioactivity, we determined that the effect was not because of a single molecule," Liu says. "Three different chemicals in an appropriate proportion behave synergistically."

Liu believes he and his colleagues are on the threshold of a breakthrough.

"I used to start with the plant and not stop until I found the molecule," Liu said. "Now, I realize it may not be one compound but several compounds. My interest now is to see if they're synergistic. That's the beauty of natural plants – they produce synergistic compounds."

■ Rick Bogren

Figure 2. Effect of the sweet leaf tea extract on the tumor growth of xenografted SCID mice bearing human breast cancer cells (MDA231). The untreated control group continued to grow, whereas the treated group with 1g/kg body weight dose via oral gavage stopped tumor growth starting on Day 4.



similarity of overall chemical constituents among different batches against a "standard extract" with a defined efficacy. Any batch that falls outside 90 percent similarity to this standard extract is disqualified.

The standardized sweet leaf tea extract was tested against numerous and diverse tumor specimens harvested from patients, and it was found that the extract repeatedly and consistently inhibits the initiation and growth of new blood vessels regardless of each tumor's angiogenic potential (Figure 1). This is very encouraging because it shows efficacy against solid tumors of various types in which growth or recurrence depends on new blood vessel formation.

A positive anti-tumor efficacy result using appropriate animal models is a "gold standard" for beginning human clinical trials. In a preliminary study with mice that are highly susceptible to infection, two groups 25-39 days old were injected with human breast cancer cells to develop a tumor. Immediately after the injection, one group of mice received the standardized sweet leaf tea extract via tubes to the stomach (a process called oral gavage) on days zero, 4, 8, 10 and 14. The control group received only water, which is the vehicle used to prepare the sweet leaf tea extract.

The result of this study shows that tumor volume continued to increase in the nontreated control group, whereas tumor volume in the treated group remained constant starting at day 4 after the initial onset of the tumor growth (Figure 2). This encouraging finding is a proof of concept that the sweet leaf tea

extract is orally active in stopping tumor growth, possibly by inhibiting angiogenesis that deprives the implanted cancer cells from accessing blood vessel networks to feed their growth.

Ongoing animal studies are determining the minimal effective dose in a multiple-dose study and variations in a different cancer type (pancreatic). A toxicity and safety study of the standardized

sweet leaf tea also is being planned to define safe dose range. However, all indications are that it is safe for short-term use and predicted safe in longer-term use as a cancer-preventative agent.

To gain insight on the mechanisms of action, a number of studies were conducted using endothelial cells – the cells that become angiogenic to produce new blood vessels – from human umbilical veins. It was found that the sweet leaf tea extract markedly inhibited the expression of a basic growth factor that stimulates and promotes angiogenesis, and the suppression depended on the concentration of the extract. In contrast, it did not affect other vascular growth factors, including VEGF – the one targeted by the drug Avastin.

The extract did suppress the VEGF receptor on the cells regardless of the presence or absence of VEGF. These results suggest that the observed anti-angiogenic property of sweet leaf tea works by inhibiting receptors on the cell rather than the growth factors themselves. Moreover, the sweet leaf tea extract has the ability to slow the growth of endothelial cells and disrupt blood vessel formation. Additional investigations are ongoing to determine if and how the multiple constituents of the sweet leaf tea extract combine and work together to produce the anti-angiogenic effect.

LSU AgCenter researchers hope to develop the sweet leaf tea extract as an effective and safe cancer preventative agent that will allow cancer patients to manage cancer and keep any residual cancer cells in remission indefinitely. ■