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R. Borucki

A. Blumer

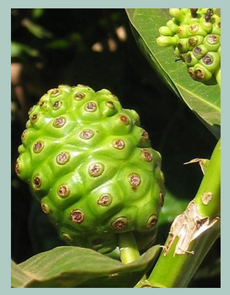
H. Huggins

Y. Wei

A. Tietje

See next page for additional authors

Authors
R. Borucki, A. Blumer, H. Huggins, Y. Wei, A. Tietje, and X. Yang



Compounds from fermented noni exudates (fNE) selectively kill human cancer cells

Alexandra Blumer, Robert Borucki, Shaina Arambula, Haley Huggins, Iris Yang, Ashlee Tietje, Dr. Yanzhang Wei

Clemson University Biological Sciences

Abstract

Our recent studies involving fermented noni exudates (fNE) made from noni (*Morinda citrifolia*) have shown promising anti-cancer activities. Using an *in vitro* cell culture system, this study examines dozens of compounds isolated from fNE in order to identify specific compounds preferentially toxic to tumor cells. The compounds at concentrations of 20, 5, or 2 µg/ml were added to NL-20 non-tumor lung cells or A549 lung carcinoma cells and incubated for 48 hours. The cytotoxicity of the compounds on the cells was measured using the MTS cell proliferation assay. Several compounds were found selectively effective in killing more A549 tumor cells than NL-20 cells. An antioxidant assay was then performed to investigate if a correlation exists between antioxidant activity and selective cancer cell killing in our samples. We found that samples BGS, C2, BGL, and C1 had the highest amount of antioxidant activity. Of these samples, only C2 showed significant selective A549 cell killing (p<.05 at 2µg/ml). Further testing is necessary to determine the mechanism of action and its possible role in immune activation.

Introduction

Noni (*Morinda citrifolia)* is small tree originating in Southern Asia and Polynesia. Parts of this tree, including the fruit, have been used in folk medicine for close to 3000 years (1). It is said to have a broad range of therapeutic effects, including anti-inflammatory, hypotensive, immune enhancing, and anti-tumor effects (2). The Noni fruit has come under study more recently and has been reported to enhance immune activity by activation T and B cells (3). Studies have also shown that intraperitoneal (i.p.) injections of fermented noni exudate (fNE) has caused proliferation of NK and T cells, as well as stimulation of dendritic cells (4, 5).

Recent studies have also shown Noni fruit to have promising anti-tumor properties. C57BL/6J mice with S180 tumor cells received i.p. injections of fNE, which caused both increased granulocyte and NK cell counts, as well as a rejection of the tumors in over 85% of the mice (4). There has been some work that identified the BuOH fraction of fNE as the one with therapeutic properties (6) but little work has been done on specific extracts looking directly at the anti-tumor properties of unique compounds in the fNE. This research looks at several specific compounds in the fNE and their role in preventing tumor growth and cell proliferation in human cell lines.

Materials & Methods

In each trial, the A549 human lung carcinoma and NL-20 non-tumor lung cells were plated on to separate 96 well plates with 5000 cells/well. The noni samples, acquired from collaborators in Hawaii, were diluted to concentrations of 20µg/µl, 5µg/µl, and 2µg/µl, and 20µl of each diluted Noni sample was added in each well. These plates were incubated for 48 hours at 37˚C. The plates were centrifuged at 6000g for one minute to secure cells to the bottom of the well. The media was then removed and 20µl of an MTS/PMS solution was added. In some trials, an additional wash step of 100µl of DPBS solution was utilized. After an additional four hours of incubation at 37˚C, the plates were read at 490nm. The data given from the plate reader was then analyzed using a two-way ANOVA in order to determine the statistical significance of our samples in selectively killing tumor cells.

To further investigate antioxidant activity in our noni samples, we used a working protocol developed from the Sigma-Aldrich Antioxidant Assay Kit. To start, we diluted all of our Noni samples to a concentration of 2µg/µl. As all of our samples had been previously diluted with DMSO at differing concentrations, we created 10%, 20%, and 100% DMSO controls. We also diluted the 10X Assay buffer to a 1X Assay buffer and made a myoglobin working solution and an ABTS working solution according to protocol. Six Trolox standard working solutions were also created according to protocol. In each well, we added 10µl of Noni sample or control, 20µl of myoglobin working solution and 150µl of ABTS working solution. After incubating for five minutes at room temperature, 100µl of stop solution was added. In control cells, 10µl of the sample was added followed by 270µl of ultrapure water. The absorbance was then read at 405nm using a plate reader. To analyze the data, a standard curve was created using the data from the Trolox standards. The equation generated from the line of best fit was then utilized to obtain the antioxidant activity for each sample after adjusting for the dilution factor.

Results

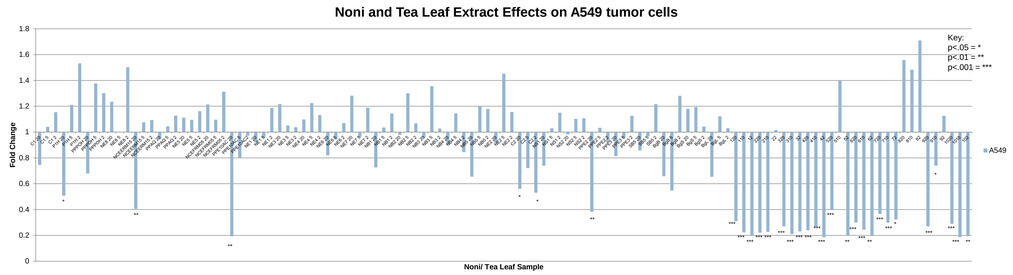


Figure 1: Noni and tea leaf extract effects on A549 tumor cells: After testing each of our various noni and tea leaf samples, we were able to see which samples were effective in killing A549 tumor cells.

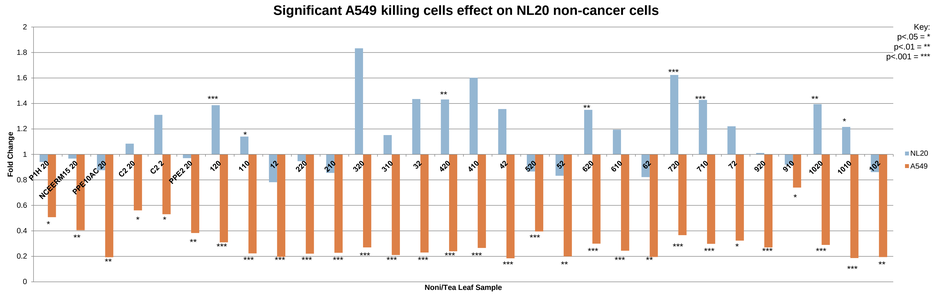


Figure 3: Significant A549 killing cells effect on NL20 non-cancer cells: After looking at which samples were statistically significant in their killing of A549 tumor cells, we examined which samples also left the NL20 non-cancer cells unharmed. We found that many exhibited this trend, while tea leaf samples 1 20, 7 20, and 7 10 were most successful in promoting NL20 cell growth while having a significant decrease in A549 cells

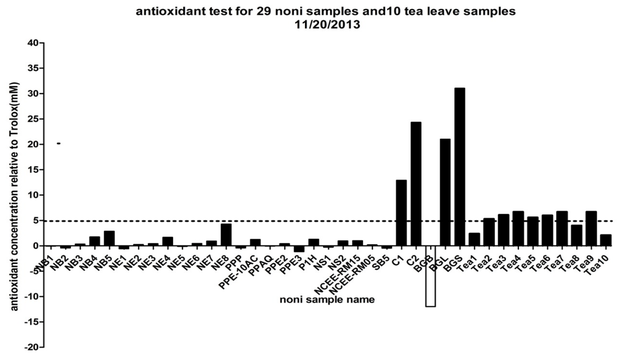


Figure 3: Antixoidant Assay: This figure illustrates the amount of antioxidants present in each noni and tea leaf sample relative to the Trolox standards. As seen here, BGS, C2, BGL, and C1 have the highest amount of antioxidant concentration.

Results & Discussion

Once exposed to noni samples, the cells' viability was determined in both normal (293 and NL-20) and in cancerous (DU-145 and A549) cell lines. A favorable trend was indicated by a decrease in cancer cell survival and an increase or state of stability in the survival of normal cells. Twenty-five samples were tested, but only six samples significantly demonstrated the desired trend after 48 hours of incubation. Certain samples (PPE10AC at 20µg/µl) were able to selectively kill DU-145 cells (p<.001) while not affecting the stability of the 293 cells (Figure 1). Other samples (C2 at 20µg/µl and 5µg/µl) showed the ability to significantly increase the growth of 293 normal cells (p<.001) while also decreasing (20µg/µl, p<.05) or keeping stable (5µg/µl) the DU-145 cancerous cells. We saw similar trends with the A549/NL-20 cell lines (Figure 2). Again, certain samples (NCEERM15, PPE10AC, and PPE2 all at 20µg/µl) displayed the ability to selectively kill A549 cancerous cells (p<.01) while not significantly changing the stability of the NL-20 normal cells. To further investigate how time of noni sample exposure would influence cell proliferation, these samples were analyzed after 24-hour, 48-hour, and 72-hour incubations.

After 24 hours of incubation, NL-20 cells exposed to C2 noni extract at 20µg/µl yielded significantly increased cellular growth (p<.001) and slightly decreased A549 growth (Figure 3).

After 48 hours, these trends continued, and there was a significant decrease in A549 cellular growth when exposed to PPE10AC (p<.001), PPE2 (p<.001), NCEERM15 (p<.05), and P1H (p<.001), each at 20µg/µl dilutions (Figure 4). PPE10AC yielded the greatest decrease in A549 cells of all the extracts.

After a 72 hour incubation, some samples (NCEERM15 at 20µg/µl) increased the significance of selectively killing A549 cells (p<.05 at 48 hours to p<.001 at 72 hours). This suggests that while some samples, such as PPE2, were able to selectively kill A549 cells (p<.001) after only 24 hours, other samples took longer to reach their peak effectiveness (NCEERM15 at 20µg/µl).

Conclusions & Future Directions

This research shows that there is significant evidence that select extracts from Noni juice have the ability to selectively kill cancerous cells while increasing or maintaining the stability of non cancerous cell lines. Certain extracts have proven effective in both DU-145/293 and A549/NL-20 human cell lines. We found that the most potent samples can exhibit significant results in as little as 24 hours, while other compounds take between 48 and 72 hours to display the desired trend. These early results are promising in the hopes of fighting cancer.

Using components of noni fruit to fight cancer is the goal of this research; however, there is a great deal of research to be done before this goal can be met. The next step in this project is to expand upon the cell lines being tested. We have already tested prostate and lung carcinoma cells. Next we will test melanoma and breast cancer cells to determine if the effects of fNE differs between cell lines.

Successful therapeutics are often cocktails of complementing drugs. For this reason, combining extracts to see if the effectiveness increases will also be assessed. For example, C2 enhanced the growth of normal cells and PPE10AC was successful at targeting tumor cells. Therefore, they may work together for a more successful treatment option. Once the basic effects of fNE are determined, the mechanism through which the tumor cells are being killed will be assessed. The methods to determine if fNE is inducing apoptosis in cancerous cells, Caspase and Annexin-V assays, will be utilized.

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