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The Study of The effect of two Flavone Isomers Derived from Gnaphalium elegans and Achyrocline bogotensis in breast cancer.

Jessica J. Walker
East Tennessee State University

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The Study of The effect of two Flavone Isomers Derived from Gnaphalium elegans and Achyrocline bogotensis in breast cancer

Thesis submitted in partial fulfillment of Honors

By

Jessica Walker

The Honors College

Midway Honors Program

East Tennessee State University

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Dr. Victoria Ramsauer, Faculty Mentor

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Dr. Cerrone Foster, Faculty Reader

_____

Dr. Allan Forsman, Faculty Reader

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Abstract

Flavonoids are ubiquitous to all terrestrial plants and many are known to have anti-tumor activities. In this research project we studied the differential cytotoxic effects of two flavone isomers on human breast cancer cells (BT-474, SKBR-3, and MCF-7) and normal breast cells as a control (MCF-10A). The basis of the relationships between chemical structures, type and position of substituent groups and the effects these compounds exert specifically on cancer cells are not completely elucidated. These flavones are derived from plants native of Colombia, South America. These plants of the family Asteraceae, genera Gnaphalium and Achyrocline are reputed to have anti-cancer properties. The flavones are non-toxic to normal human cells. Our studies indicate that 3,5-dihydroxy-6,7,8-trimethoxy-2-phenyl-4H-chromen-4-one (3,5-dihydroxy-6,7,8-trimethoxy flavone, flavone B) is highly cytotoxic to poorly differentiated carcinomas of the breast such as SK-BR3, with minimal activity against more differentiated carcinomas of the same organs such BT-474. Immunoblot analysis suggests that the anti-tumor effects of flavone B in SKBR-3 may be mediated through the down regulation of the ERK pathway. ERK activation promotes cell proliferation, differentiation and survival. Additionally, flavone B down regulates PS6 in SKBR-3. PS6 controls protein translation by phosphorylating the S6 protein of the 40S ribosomal subunit. On the other hand, neither flavone A or B has a significant inhibitory effect on breast cancer cell line BT-474. The flavones were tested in the human fibrocystic mammary tissue MFC-10A, defined as a normal breast cell line, to demonstrate their lack of toxicity against normal cells.

Introduction

Breast Cancer is the leading cancer death among women of all races. According to the American Cancer Society, approximately 288,130, women were expected to be diagnosed in 2011 and 57,650 women were expected to die of breast cancer. Breast cancer begins in breast tissue, which is made up of glands for milk production, called lobules, and in the ducts that connect the lobules to the nipples. There are two main types of breast cancer, ductal carcinoma and lobular carcinoma. Ductal carcinoma is cancer in the lining of the milk ducts. Some breast cancers are referred to, as in situ, because they are confined. However, most breast cancers are invasive or infiltrating [1]. In addition to ductal and lobular carcinoma, there is also another form of breast cancer known as inflammatory breast cancer. Inflammatory breast cancer is a rare and very aggressive disease in which cancer cells block lymph vessels of the breast [18].

According to the American Cancer Society, cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues [1]. Cancer is a genetic disorder that starts at the DNA level. In a normal cell, when its DNA becomes damaged, the cell either repairs the damaged DNA or the cell dies. This death is a form of apoptosis defined as a genetically determined process of cell self-destruction that is marked by fragmentation of the DNA. In cancer cells, the damaged DNA is not repaired and the cells do not die like they should. Instead these cells undergo mitosis, generating new cells with damaged DNA [2].

There are two important mechanisms involved in cell survival and proliferation. One of these mechanisms is known as Mitogen-activated protein

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kinase/<u>E</u>xtra-cellular signal <u>R</u>elated <u>K</u>inase (MAPK/ERK) and the other known as

AKT. The name AKT originates from early studies done on mice that developed

thymic lymphomas due to the AKT8 retrovirus. The "T" in AKT stands for

"transforming." The letter t was added when a transforming retrovirus AKT8 was

isolated from a spontaneous thymoma, tumor of the thymus, of an AKR mouse strain

[3]. Previous studies suggest that the ERK cascade functions in cellular proliferation

including the regulation of mitosis and meiosis. Inappropriate activation of ERK is a

common occurrence in human cancers. During growth factor stimulation, the ERK

phosphorylation cascade is linked to cell surface receptor tyrosine kinases (RTKs) and

other upstream signaling proteins with known oncogenic potential. Downstream,

activated ERK regulates growth factor-responsive targets in the cytosol. Moreover,

ERK translocates to the nucleus where it phosphorylates a number of transcription

factors regulating gene expression [4]. On the other hand, the intracellular signaling

pathway via AKT is poorly understood due to its complexity. However, it has been

suggested that AKT plays important roles in insulin regulation, activation of

translation of growth factor genes, and inhibition of apoptosis [3].

Another important signaling protein we tested is phospho-S6, which

phosphorylates ribosomal protein S6, located in the 40s subunit. It is thought that

ribosomal protein S6 is required for the translation of a subset of mRNAs that contain

a 5'-oligopyrimidine tract at their transcriptional start sites. They encode many of the

components of the translational apparatus, including ribosomal proteins and

elongation factors that are necessary for cell cycle progression [5].

Flavonoids, present in flowering plants, have been found to have a variety of

anti-cancer effects. There are over 4000 chemically unique compounds. Flavonoids are

classified into different subclasses including: flavones, flavanols, flavanones, isoflavones, catechins, arthocyanidins, proanthocyanidins, flavans and aurores[8]. Flavones define one of the largest of the subgroups. In plants, they play a protective role in the absorption of UV radiation in the range that is detrimental to nucleic acids. Flavones also contribute in the attraction of pollinators and protecting the plant against insect and other organisms that could harm the plant [12]. In addition to their contribution to plants, flavones are important compounds to human nutrition and health. They are part of our daily diet and are ubiquitous components of edible fruits, nuts, vegetables, seeds, and tea. The roles of flavonoids include the inhibition of proliferation of cancer cells and selective cell death by apoptosis [6]. The flavones that we studied are: 5, 7-dihydroxy-3, 6, 8-trimethoxy flavone, known was flavone A (figure 1A) and 3, 5-dihydroxy-6, 7, 8-trimethoxy flavone known as flavone B (figure1B). Flavone B has an affinity for less differentiated tumorigenic cancer cells while flavone A seems to affect more differentiated tumorigenic cancer cells. According to the National Cancer Institute, cell differentiation refers to what degree a cell resembles a normal cell histologically. As normal cells develop, they become differentiated, and progressively specialized depending on where in the body they are located. If cancer cells look like normal cells, they are said to be better differentiated. On the other hand, if the cancer cells look unlike a normal cell, they are said to be poorly differentiated.

Increasing evidence suggests that breast cancer subtypes defined by expression of the Estrogen Receptor (ER), Progesterone Receptor (PR), and Human Epidermal growth factor Receptor 2 (HER2) represent distinct biological entities with distinct clinical profiles. Breast cancers that are ER+ and/or PR+ are associated with

the most favorable prognosis, in large part because expression of these markers is predictive of favorable response to hormonal therapy (17). Compared to women with tumors that are ER+ and PR+, women with tumors lacking ER and PR expression have approximately 1.5- to 2-fold higher risk of death. The poorly differentiated SKBR-3 cells studied in this work, lack the ER and PR markers [1,11].

Previous studies have shown the cytotoxic effects of Flavone A and B in pancreatic and color cancer cell lines. In pancreatic cancer, Flavone A seems to have a cytotoxicity effect in the more differentiated cells such as panc28 meanwhile flavone B shows cytotoxicity in the less differentiated cells, MIA PaCa. In colon cancer studies, Flavone A decreases viability of the more differentiated cells, Caco-2 colon cancer cells, while Flavone B has cytotoxicity effect on the poorly differentiated color cancer HCT-116 cells (7). Both flavones show cytotoxicity in highly tumorigenic cells; however, the flavones have differences in their selectivity towards cells lines. Flavone A shows cytotoxicity in the more differentiated cells while flavone B shows cytotoxicity in less differentiated cells. The structural differences in the isomer compounds could be the basis of their selectivity.

In our research we are going to test the effects of these two flavone isomers in breast cancer cell lines, BT-474, (non-tumorigenic better differentiated), SKBR-3, (tumorigenic poorly differentiated), and MCF-7 (non-tumorigenic, better differentiated). MCF-10A normal breast cells are used as a control. Our aim is to further study the biochemical signaling induced by the flavones.

Materials and Methods

Extraction of Compounds

Procedure to obtain 5, 7-dihydroxy-3, 6, 8-trimethoxy flavone (flavone A)

1.5 kg of dried Gnaphalium elegans flowers were extracted with chloroform ($CHCl_3$). The extract was concentrated by dry vacuum, dissolved in methanol and filtered to eliminate fats and hydrocarbons. The extract was concentrated and dissolved in benzene (C_6H_6) followed by silica gel chromatography using benzene: acetone (C_6H_6:Me_2CO) (19:1) as eluent. 50 mg of the flavonoid was purified from fractions 12 through 18 by crystallizations in hexane. The compound was identified by its physical and spectroscopic properties as 5,7 dihydroxy-3,6,8 trimethoxy flavone with the following properties: mp 170–171uC, 1H NMR (300 MHz) 3.86 (3H,s), 3.97 (3H,s), 4.20(3H,s), 7.50–7.6 (3H,m), 8.08–8.16 (2H,m) [13].

Procedure to obtain 3, 5-dihydroxy-6, 7, 8-trimethoxy flavone (flavone B)

200 g of fresh leaves of Achyrocline bogotensis were submerged in chloroform ($CHCl_3$) for 20 minutes. The extract was filtered, concentrated and dissolved in hot methanol. The cold extract was filtered to eliminate fats and was concentrated once again. The obtained solid was dissolved in hot hexane. 100 mg of the purified flavonol was obtained by successive recrystallizations in hexane. The compound was identified by its physical and spectroscopic properties as 3,5- dihydroxy-6,7,8-trimethoxy flavone with the following properties: mp149–150uC , 1H NMR (300 MHz) 3.86 (3H,s), 3.97 (3H,s), 3.99 (3H,s), 4.12 (3H, s), 7.30– 7.45 (3H,m), 8.70–8.82 (3H,m), 11.46 (1H,s) [14].

Cell Culture

Well-differentiated and poorly-differentiated breast cancer cells were obtained from the American Tissue Type Culture Collection (ATCC; Manassas, VA) including: SK-BR3, BT-474, and MCF-7, as well as normal breast MCF-10A. Tumor and normal cells were grown in tissue culture according to ATCC instructions. SK-BR3 cells were grown in McCoy's 5A Medium (GIBCO/ Invitrogen, Carlsbad, CA). BT-474, MCF-7, and MCF-10A were grown in Delbeco's Modified Eagles Medium (DMEM) (GIBCO/ Invitrogen, Carlsbad, CA). All media was supplemented with 10% serum and penicillin/streptomycin. MCF10A cells were additionally supplemented with insulin, EGF, cholera toxin, and hydrocortisone [19]. A technique known as seeding was used to prepare our cells for treatment. A small amount of cells were transferred to a new flask with fresh medium and allowed to grow. After the cells were seeded, they were allowed them to reach approximately 75% confluence before treatment with flavone A, B or vehicle (DMSO at a final maximum concentration of 0.01%). Before cells were dosed with the flavone A or B for 1 hour at 40 μM, they were treated with 0.5% BSA for 6 hours to synchronize them. By synchronizing the cells, it allowed us to bring cell division to a more uniform rate among the cells.

MTT assay

Cells were seeded at a density of 4000 cells/well in 48 well plates, grown overnight and treated with either vehicle, flavone A, or flavone B in concentrations of 5, 10, 20, 40, and 80μM. The dissolution vehicle was dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO) to yield a maximum final concentration of 0.01% in the treated well. After 24 hours of incubation 3-(4, 5-methyl-thiazol-2-yl)-2, 5-diphenyl-

tetrazolium bromide (MTT) (Invitrogen) was added at 100 mg/well for 3 hours. MTT assay is a colorimetric assay that measures the cellular metabolic activity. In living cells MTT is reduced by mitochondrial reductase activity, the reaction is visualized by the changing in color from yellow to a purple formazan product. The intensity of the color is used to measure the viability of the cells. The more cells viable, the higher the intensity of the purple color. The formazan products formed were solubilized with acidified 2-propanol and optical density was measured at 570 nm using a Cary 50 (Varian, Palo Alto, CA). All experiments were done in triplicate. The optical density refers to the intensity of light measured at specific wavelength, in this case at 570nm that passes through the sample.

PAGE and Immunoblot

To determine the presence of proteins of interest and their levels, Western blot was used. Cells were lysed in RIPA buffer (Radio-Immunoprecipitation Assay) containing phosphatase inhibitors that enable efficient cell lysis while avoiding protein degradation and biological activity [22]. Samples were run on SDS-polyacrylamide gel electrophoresis (PAGE) using a polyacrylamide gel of 7 or 10% and then blotted onto nitrocellulose or polyvinylidene difluoride membranes (Schleicher & Schuell Bioscience, Inc., Keene, NH). Membranes were blocked with 5% non-fat dry-milk in tris-buffered saline with 0.1% Tween-20 (TBST). The membranes were incubated with antibodies against the total and phosphorylated forms of ERK (clones 137F5 and 20G11), AKT (clones 73H10 and 193H12), S6 ribosomal protein (clone 61H9), or actin (Sigma) for 1 hour followed by horse-radish-peroxidase conjugated goat anti-rabbit (Promega) or anti-mouse IgG (Pierce) for 1 hour at RT in 3% BSA or dry-milk

with TBST or PBS. The horse-radish-peroxidase allowed us to detect the signal of primary monoclonal antibodies by using secondary affinity and chemiluminescence. The membranes were exposed to X-ray film (Kodak). The intensity of the bands was estimated by digitizing the image (Scion Image) from x-ray film. After subtracting the background, all band intensities were compared against a control.

Immunofluorescence

Cells grown to confluence on 12-mm round coverslips (Fisher Scientific) or 6-mm Transwell-Clear TM filters (Corning Costar) were processed for immunofluorescence studies 2, 6, or 12 hours after dosing with vehicle or flavone A or B. The cells were fixed with 3% paraformaldehyde for 20 min at room temperature. After rinsing, the cells were permeabilized with 0.2% Triton-X100 for 5 min or 0.1% saponin throughout the procedure. The permeabilization was followed by rinsing and quenching of the aldehyde groups in 50 mM NH4Cl, after which the cells were incubated with primary antibody for 1 h at room temperature. The primary antibody was diluted in 1% bovine serum albumin. In co-localization experiments, 0.1% immunoglobulin G of the same species as the secondary antibody was used instead of 1% bovine serum albumin for rinsing steps and dilution of the primary antibody. Once this first incubation was completed, the cells were rinsed and then incubated with the secondary antibody conjugated to the fluorescent dye Alexa Fluor® 488 or Texas Red® (Molecular Probes, Eugene, OR) for 1 h at room temperature in the dark. Dapi (4'6-Diamino-2-Phenylindole, Dilactate) a blue fluorescent nucleic acid stain was added and incubated for 5 minutes. The slide was rinse several times to remove all free DAPI. The cells were then mounted in 10% polyvinyl alcohol, 30% glycerol, 1%

n-propyl gallate, and Slow Fade ™ (Molecular Probes) at a dilution of 5:1.

Fluorescent images were obtained using an EVOS fluorescent microscope (AMG,
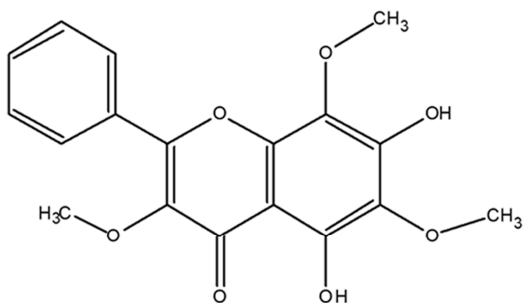
Bothell, WA).

Figures

Figure 1. This picture represents Gnaphalium elegans, flavone A (5, 7-dihydroxy-3, 6, 8-trimethoxy flavone) was extracted from this type of plant.
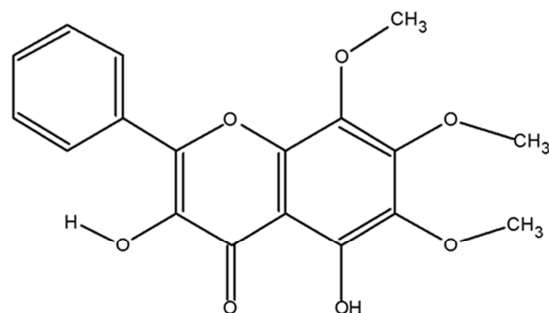

Figure 2. This picture represents Achyrocline bogatensis, flavone B (3, 5-dihydroxy-6, 7, 8-trimethoxy flavone) was extracted for this type of plant.

A.

B.

Figure 3. Molecular structures of flavones A and B. A) Flavone A which was identified by its physical and spectroscopic properties as 5,7 dihydroxy- 3,6,8 trimethoxyflavone. B) Flavone B which was identified by its physical and spectroscopic properties as 3,5-dihydroxy-6,7,8-trimethoxyflavone[7].

Results

Recently, we reported cytotoxic activity of flavone B on SKBR-3 [figure 4] but not of flavone A; on MCF-7 cells neither flavone diminished cell viability [figure 7]. In this study, we studied the mechanism of action of flavone B on SKBR3 and cytotoxic activity of the flavones on BT-474.
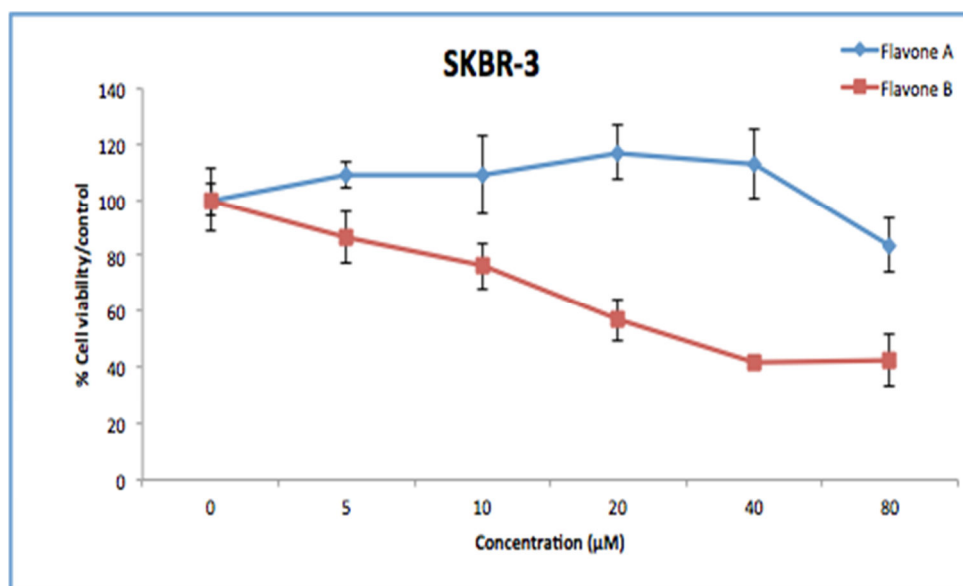

Figure 4. The effects of flavone A and flavone B on breast cancer SK-BR3 cells. The effects of flavone A and flavone B on the poorly differentiated breast cancer Sk-BR3 cells were determined by MTT assay and are represented as a percent of the control absorbance at a wavelength of 570 nm. All data were collected at 24 h after treatment. Data shown are from representative experiments (mean 6 SE, n = 3). * p,0.05, significant difference between control and other concentrations for each flavone.
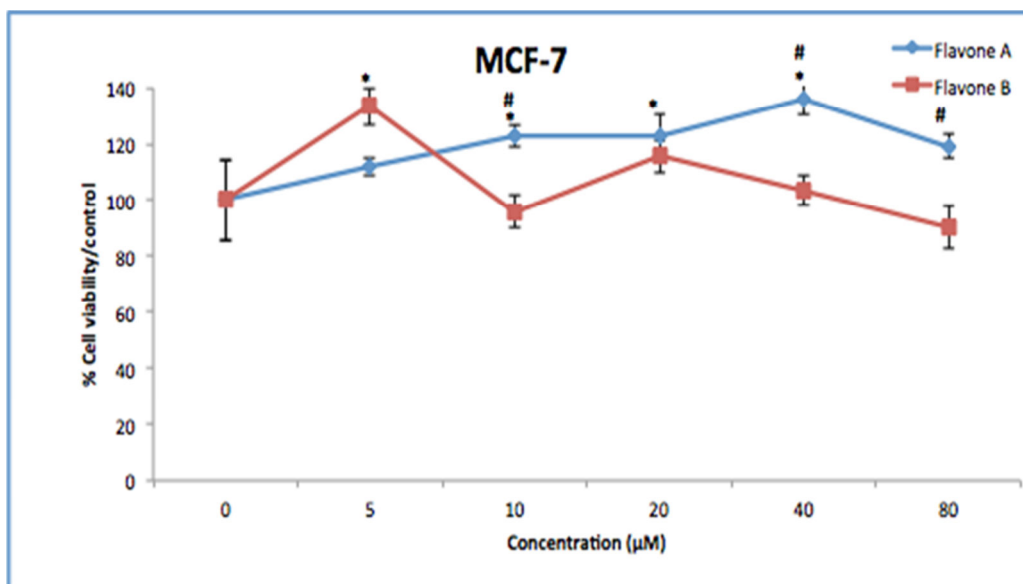
Figure 5. The effects of flavone A and flavone B on breast cancer MCF-7 cells. The effects of flavone A and flavone B on the more differentiated breast cancer MCF-7 cells were determined by MTT assay and are represented as a percent of the control absorbance at a wavelength of 570 nm. All data were collected at 24 h after treatment. Data shown are from representative experiments (mean 6 SE, n = 3). * p,0.05, significant difference between control and other concentrations for each flavone.

Neither flavone A nor flavone B affects cell viability on BT-474 breast cancer cells.

Figure 6 represents the MTT assay for BT474 after 24 hours of treatment with

flavone A and Flavone B. The cells were processed as described above and the data
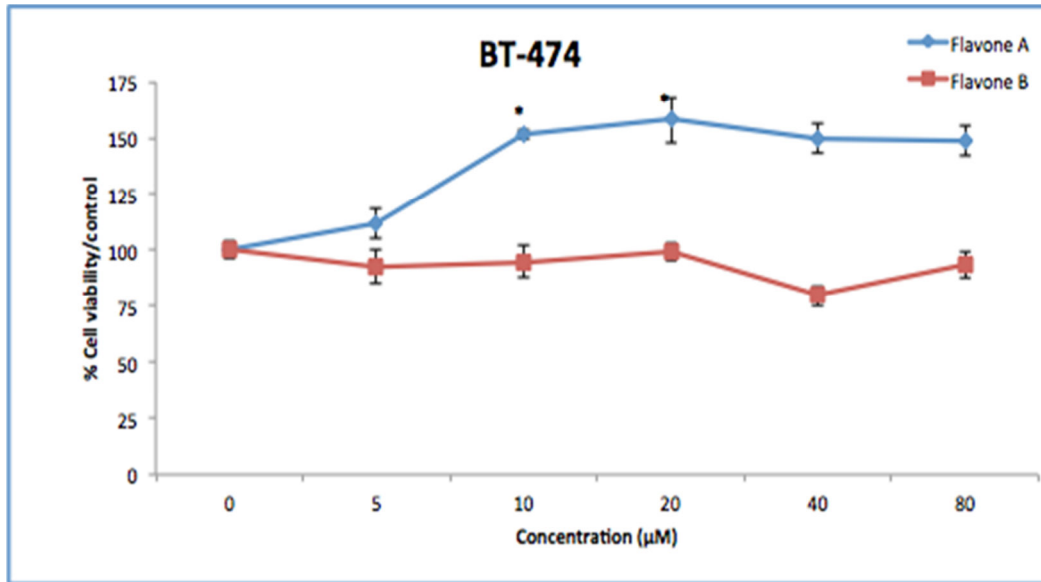
was collected via a spectrophotometer.

Figure 6. The effects of flavone A and flavone B on breast cancer BT-474 cells. The effects of flavone A and flavone B on the more differentiated breast cancer BT-474 cells were determined by MTT assay and are represented as a percent of the control absorbance at a wavelength of 570 nm. All data were collected at 24 h after treatment. Data shown are from representative experiments (mean 6 SE, n = 3). * p,0.05, significant difference between control and other concentrations for each flavone.

Flavone B downregulates pERK in SKBR3 cells. Based on the analysis of the

Immunoblot of pERK (figure 7), Flavone B down regulates pERK in SKBR3 cells. For

this experiment the cells were synchronized using 0.5% BSA for 6 hours. Then, the

cells were treated for 1 hour with flavone B at 40 µM. The cells were lysed and the

SDS samples of treated cells were separated by PAGE with an SDS extract of cells

exposed to vehicle alone and untreated cells as a control. The immunoblots were

probed with antibodies against the phosphorylated form of pERK . Actin was used as a

loading control. Figure 8, confirms the down regulation of pERK by flavone B in

SKBR-3 Cells by immunofluorescence. DAPI, the blue channel, is used to locate the

nucleus of the cell. The middle column shows the untreated cells (c) and the treated

cells with flavone B (B+) at 40 μM. Lastly, the third column, merges the blue color in

DAPI and the green color in pERK from the respective row. The intensity of the

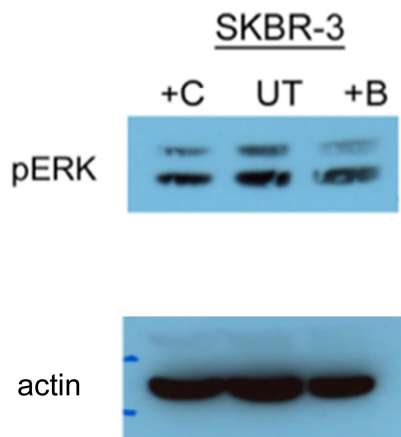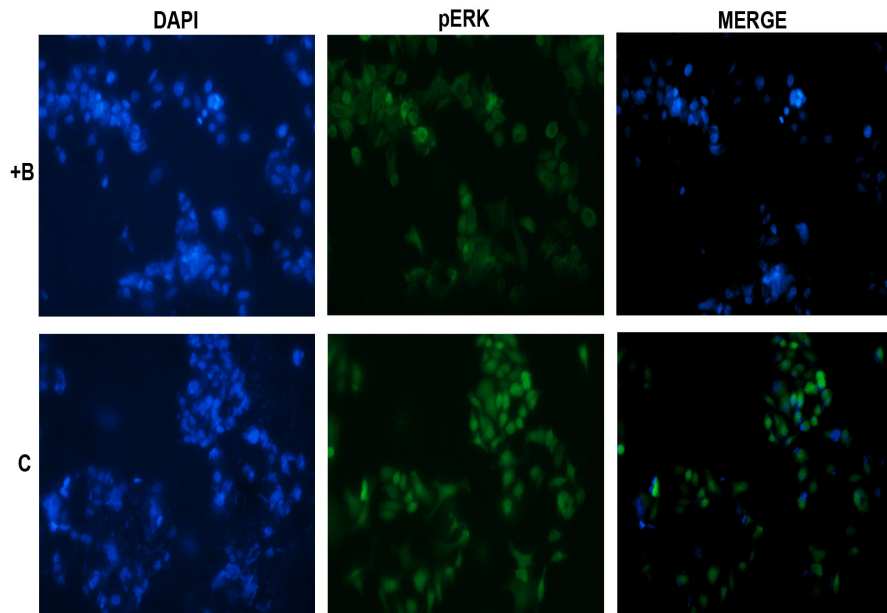fluorescent microscope remained the same throughout the experiment.

Figure 7. Analysis of proliferative signaling pathways in breast cancer cells SKBR-3 treated with compound B and with vehicle as a control (UT), DMSO at 0.01%. Total SDS extracts of PC3 cells were used as positive control for the antibody (+C).

Figure 8. Detection of downregulation of pERK by flavone B in SKBR-3 cells.
Compound B(+B) was dosed at a concentration of 40μM on the less differentiated
SKBR-3 cells. DAPI (blue channel) is used to locate the nuclei of the cells. As the
control(C), SKBR-3 treated with vehicle only, DMSO at a final concentration of
0.01%.

Flavone B down regulates pS6 in SkBR3 but has no effect on pAKT, while flavone A

has no effect in BT-474 cells. We tested the effects of flavone A and B in SKBR-3 and

BT-474 (figure 9). The cells were treated with flavone A and B at 40μM for 1.5 hours.

Then, the cells were lysed; PAGE separated the samples of treated cells with a sample

exposed to vehicle alone being run as a control (first and third lane). The

immunoblots were probed with antibodies against the total and the phosphorylated

forms of pAKT and with pS6 antibodies. The membranes were reprobed for actin as a

loading control (bottom row). The Immunoblot shows the cytotoxic effect of flavone B

in the SKBR-3 cells. Flavone B demonstrates a down regulation of pS6 in while no

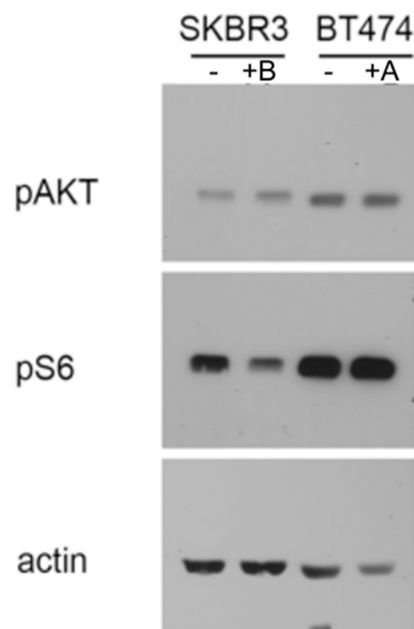change occurs in pAKT. On the other hand, flavone A shows no effect in BT-474.

Figure 9. Analysis of survival and apoptotic signaling pathways in breast cancer cells SKBR-3 and BT-474 treated with compound A and B and the vehicle as a control, DMSO at 0.01%. Analysis of pAKT and ps6 after 1.5 hours treatment of flavone A and B at 40uM. The cells were lysed and the samples of treated cells were separated by PAGE with a sample exposed to vehicle alone being run as a control (first and third lane).The immunoblots were probed with antibodies against the total and the phosphorylated forms of pAKT and with pS6 antibodies. The membranes were reprobed for actin as a loading control (bottom row).

Neither flavone is cytotoxic to normal breast cells . MCF10-A cells were treated with either flavone A, B, or vehicle and processed for MTT as described above. The assay was analyzed spectrophotometrically (figure 10).
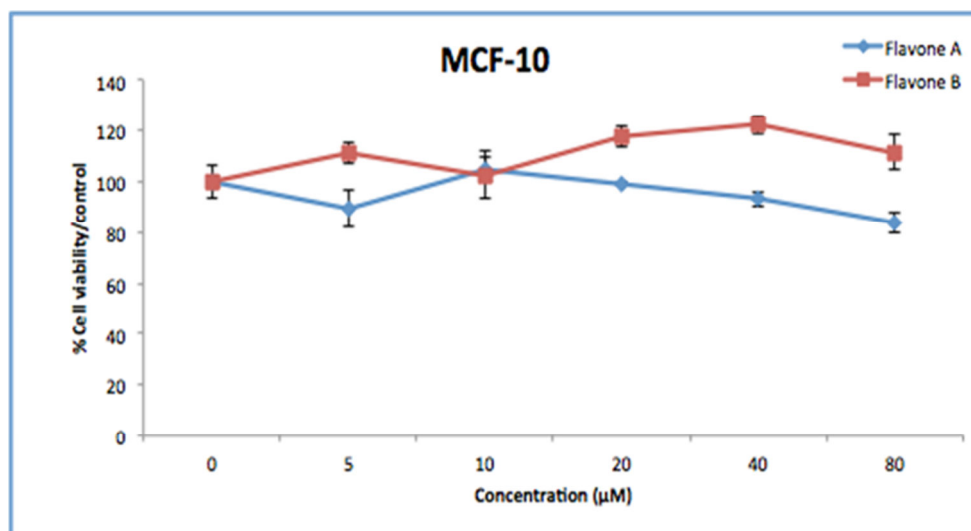
Figure 10. The effects of flavone A and flavone B on breast cancer MCF-10 cells. The effects of flavone A and flavone B on the poorly differentiated breast cancer MFC-10 cells were determined by MTT assay and are represented as a percent of the control absorbance at a wavelength of 570 nm. All data were collected at 24 h after treatment. Data shown are from representative experiments (mean 6 SE, n = 3). * p,0.05, significant difference between control and other concentrations for each flavone.

Discussion

Besides the important function in the biochemistry, physiology and ecology in plants, flavones are important compounds to human nutrition and health. Flavones have chemical and pharmacological activities, which are beneficial for human health, including antioxidant, anti-cancerogenic, anti-inflammatory and anti-proliferative. [12]. Flavone A, extracted from Gnaphalium elegans (figure 1), and flavone B extracted from Achyrocline bogatensis (figure 2) are isomers (figure3). Both compounds have the same molecular formula but they differ in the positions of their hydroxyl (-OH) and methoxy groups(-OCH$_3$). Flavone A has hydroxyl substitutions at positions 5, 7 and methoxy substitutions at position 3,6 and 8 while flavone B has hydroxyl substitutions at 2,5 and methoxy substitutions at position 6,7 and 8. These differences in hydroxyl and methoxy substitutions alter their conformation allowing for the difference in their resonance structures, which may be relevant to cellular uptake. In flavone A, both 5- and 7-OH can resonate with the carbonyl, but the 5-OH next to the carbonyl has one more resonance structure. In flavone B, the 5-OH can resonate with the carbonyl but not the 3-OH [7]. This difference in the delocalization and resonance of charge in the two flavones might be part of their properties that gives them the selectivity for the more or the less differentiated cells.

Our findings demonstrate flavone B as a potential anti-tumor agent, particularly against the poorly differentiated cells, SKBR-3. After performing the MTT assay using both Flavone A and B at concentrations 0, 5, 10, 20, 40 and 80 μM as represented in figure 4, flavone B shows a cytotoxic effect to the cells as the concentration increases. At 10 - 20μM, flavone B decreases cell viability in breast cancer cell line SkBR-3, from 80% down to 60%. This suggests only 60% of the cells

survived at that particular concentration. At 80 µM flavone B, only 41% of the cells tested survived the treatment. On the other hand, flavone A does not have a cytotoxic effect to SKBR-3 except at high concentrations. At 80µM, flavone A shows a sharp decrease in cell viability, decreasing from 100% down to 80%. Both of the flavones show a cytotoxic effect on the SKBR-3 cells, however flavone B showed a more marked decrease in cell viability against SKBR-3 cells.

To further study the mechanism of action, figure 7 shows the immunoblot bands of the effects of Flavone B in SKBR-3. The treated cells show a decrease in pERK against the untreated cells and controls cells. In figure 8 we confirm the cytotoxic effects of the activation of apoptosis by compound B (+B) at 40µM on the less differentiated SKBR-3 by immunofluorescence. DAPI (blue channel) is used to locate the nuclei of the cells. As the control(C), SKBR-3 cells were treated with the vehicle only, which is DMSO at a final concentration of 0.01%. On the middle column, we can see a decrease of the pERK when the cells were dosed with Flavone B. In addition to the down regulation of pERK, figure 9 demonstrates that flavone B also down regulates PS6, which controls translation by phosphorylating the S6 protein of the 40S ribosomal subunit [5]. In addition, flavone B does not change the levels of pAKT in SKBR-3 cells. Therefore, flavone B induces apoptosis in SKBR-3 by down regulating the cell's proliferation pathway pERK and the survival pathway PS6.

We also studied the effects of the isomer flavones in a normal mammalian epithelial cell line. To study the effects of Flavone A and B in normal mammalian cells, we used MFC-10A which is normal mammalian cell line derived from a 36 year old female with fibrocystic disease of the breast (ATCC). MCF10A cells have a gene

expression signature that correlates to the normal-like phenotype (16). In figure 10, we show the lack of cytotoxic effect of flavones A and B in normal tissue line, MCF-10A. However, at the high concentration of 80um, flavone A has cell viability of 85%. On the other hand, Flavone A and B does not have a cytotoxic effect to the less tumorigenic, more differentiated cells MCF-7 and BT-474 (figure 6 and 9). Our hypothesis to explain the lack of cytotoxicity against these two cell lines is that neither cell line is highly tumorigenic. According to previous studies, the isomer compounds not only are selective to the differentiation status in the cell but also both flavones are selective to highly tumorigenic cells (7). In breast cancer, high ALDH aldehyde dehydrogenase activity is a marker for highly tumorigenic cells; SKBR-3 has been found to have high ALDH activity while MCF-7 has a low ALDH activity hence a less aggressive cell line (9). This hypothesis allows us to explain the selectivity of flavone B to the highly tumorigenic SKBR-3 cells.

The findings in this work helps to further understand the anti-cancercogenic effects associated with the flavones found in Gnaphalium elegans and Achyrocline bogotensis. The cytotoxic effect of flavone B in the highly tumorigenic and of intermediate differentiation SKBR-3 breast cancer cells is relevant to future studies done in the design of therapeutic and pharmaceutical compounds that can induce apoptosis by interfering in the survival and proliferative pathways in highly tumorigenic cancer cell without affecting the normal cells.

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