Sorghum phenolic compounds associate with cell apoptosis in human hepatocarcinoma HepG2 and colorectal adenocarcinoma Caco2 cells

by

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Abstract

Sorghum is a rich source of various phytochemical phenolics including phenolic acids, anthocyanins, and flavonoids, etc. These phenolics have been associated with chronic disease prevention, especially cancer. However, the available data for various varieties of sorghum on cancer are limited. In this study, two different sorghum accessions were selected and the extracted phenolics were tested for their impact on cell growth and apoptosis in both hepatocarcinoma HepG2 and colorectal adenocarcinoma Caco-2 cell lines. Total phenolic contents determined by Folin-Ciocalteu were 2.11 mg GAE/g DW in F10000 and 21.89 mg GAE/g DW in PI329694, respectively. Cells treated with various concentrations of the extracted phenolics at 0-200 μM GAE up to 72 hrs resulted a dose dependent reduction of cell number. The apoptosis as measured by the FITC Annexin V protocol was also resulted a significant increase in the extract of PI329694 when comparing with the extract of F10000. Overall, these results showed a positive evidence that a direct impact of the sorghum phenolics on either HepG2 or Caco-2 cellular growth inhibition and apoptosis induction. Both anti-growth and apoptotic induction seemed associated with their phenolic contents. Therefore, this study suggests that sorghum prevent against cancer through phenolic-mediated cancer cell inhibition and apoptosis induction.

Key words: sorghum, phenolic compounds, cell apoptosis, HepG2, Caco-2

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Chapter 1 - Literature review

1.1 Sorghum background

As an important crop worldwide, sorghum was ranked as the fifth most valuable crop after wheat, rice, maize, and barley. The United States is the largest producer and exporter of sorghum, accounting for 17% of the world's production and almost 77% of the world's sorghum exports over the past 5 years. Even sorghum is not considered as a traditional food in United States, it is one of the most important staple foods in the impoverished regions of the world. For millions of poor rural people in the semiarid tropics of Asia and Africa, sorghum remains a principal source of energy, protein, vitamins, and minerals. However, because sorghum has poor sensory quality and low digestibility, it is mainly used as a livestock ingredient and biofuel source in developed countries [1]. Stefoska-Needham et al. (2015)[2] claimed that people underestimate the value of sorghum because it is often used as animal feed. In recent years, sorghum has drawn the attention of scientists because its natural "gluten-free" characteristic proved beneficial to people with celiac disease [3]. Sorghum as a valuable, inexpensive, and nutritional crop that may have higher bioactive phenolic contents than major cereals such as wheat, barley, rice, maize, rye, and oats [2]. Research has shown that sorghum is rich in various nutrients, including proteins, vitamins, fiber, and minerals. In addition, sorghum is high in phytochemicals such as tannins, phenolic acids, anthocyanins, phytosterols, and policosanols [4]. These phytochemicals from the biosynthesis of secondary metabolites that provide potential health benefits such as antioxidant activity, cholesterol-lowering properties, and anticarcinogenic properties, which can lead sorghum to be a functional food or ingredient additives [5]. Interest in the long-term use of food-derived products for the prevention of cancer in different populations has been increasing because they are expected to be safe and are not perceived as "medicine" [6],

[7]. Schwedhelm et al. (2016)[8] reported that healthy and high-quality diets can reduce the damage cause by the free radicals and promote health benefit human such as decrease the cancer mortality.

1.2 Phenolic compounds in sorghum

Phenolic compounds comprise one (phenolic acids) or more (polyphenols) aromatic rings with attached hydroxyl groups in their structures. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants [3], [4], [9]–[12]. There are more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins [11], [13]–[15]. Simple phenols are compounds with at least one hydroxyl group attached to an aromatic ring [11]. For example, catechol, resorcinol, and phloroglucinol are classified as simple phenols [11]. Simple phenols are uncommon plant constituents, but Towo, Svanberg, and Ndossi [13] reported catechol and resorcinol in sorghum grains. Phenolic acids and their derivatives are significant phenolic compounds abundantly occurring in sorghum grains, mainly as benzoic and cinnamic acid derivatives [4], [14]. Phenolic acid is likely to be esterified into cell wall polymers and stay in bound form, whereas ferulic acid is the most abundant bound phenolic acid in sorghum [16]. Phenolic acids show good antioxidant activity that may contribute to the potential health benefit from consuming sorghum. Polyphenols are phytochemicals belonging to a vast group of plant-derived compounds involving many phenol structural units [12]. They can be classified into two main groups, non-flavonoids and flavonoids, according to the functions of the aromatic groups containing phenol and the aliphatic carbon skeleton that binds these rings to one another [12]. The main classes of polyphenol includes phenolic acids, flavonoids, stilbenes, and lignans [17]. Flavonoids are the most abundant polyphenol compounds in plants based on the chemical

structure of a C6-C3-C6 skeleton [12]. More than 4000 flavonoids have been discovered, and these can be divided into six subclasses, namely flavanols, flavones, flavanones, flavanols, anthocyanins, and isoflavones, according to their distinct heterocycle aromatic rings [1]–[5], [10], [11], [14], [15], [17]–[26][22]. The color of sorghum reflects its flavonoid content. Sorghum with a black pericarp contains higher levels of flavan-4-ols and anthocyanins than other varieties [27].

1.3 Extraction solvent and phenolic compounds

Phytochemicals such as phenolic compounds are mainly extracted using organic solvent or aqueous and organic solvents. Liu et al. (2009) [28]reported that acetone has a potent capability to extract polyphenols from lychee flowers compared with methanol, water, and ethanol. IIoki-Assanga et al. (2015) [29]suggested that aqueous methanol can be used as a solvent for extracting polyphenols from *Phoradendron californicum* oak. More recently, Metrouh-Amir et al. (2015) [30]suggested that aqueous and organic solvents are more efficient than absolute organic solvents in extracting polyphenols. No best extraction solvent has been proposed for phenolic compounds, but solvents with higher polarity are likely to be used owing to the solubility of their polyphenols.

1.4 Phenolic compounds in sorghum that are associated with cancer prevention

Phenolic compounds are biosynthesized naturally and derived from the secondary metabolites of plants, which have antioxidant, cytotoxic, and antimicrobial bioactivities, among others[4], [10], [12], [31], [32]. Among cereals, sorghum has the highest content of phenolic compounds. In some specialty sorghum varieties, the sorghum phenolic content can reach up to 6%[2], [4]. High levels of phenolic compounds enhance bioactive properties such as antioxidant activity. Awkia et al. (2009) [8]suggested that the anticancer activity of sorghum may be

attributed to its potent antioxidant activity and phase II enzymes. The phenolic compounds found in tea and wine, which are phenol-rich products, have been reported to have anticancer effects [24], [33]–[35]. Reference data from epidemiological and animal studies have shown that phenolic compounds could have potential health benefits such as cancer prevention that are attributable to their biological properties, including antioxidant activity, cell growth inhibition, and induction of cell apoptosis [8], [36]–[39]. Schwedhelm et al. (2016) reported that healthy and high-quality diets are inversely associated with human cancer mortality. Seong-Ho et al. (2020) [40]suggested that a novel high-phenol sorghum bran exhibited mechanisms of anticancer activity.

1.5 Causes of cancer

Cancer, the second leading cause of death worldwide after cardiovascular diseases, is a disease in which some cells in the body grow uncontrollably and spread into other parts of the body[41], [42]. Tumor development relies on the disruption of the extracellular matrix around a cell and begin when DNA in a cell or population of cells is damaged by exposure to carcinogen[43], [44]. The three major cancer-inducing factors are cigarette smoking, infection or inflammation, and nutrition or diet[45].

Cancer development is a multistage process that involves a series of individual steps, including initiation, promotion, progression, invasion, and metastasis. Genetic mutation occurs when the DNA damage escapes repair, which leads to somatic mutation in the damaged cells. The resulting mutated cells can reproduce and form into cloned mutated cells through mitosis[43]. Tumor promotion is a selective process of proliferation and expansion of mutated cells into a larger premalignant tumor cell population, which can be inhibited if diagnosed and intervened in the precancerous stage. During progression, precancerous cells develop into tumors

through clonal expansion. This can further induce tumor invasion and metastasis, which are corresponding processes that stimulate tumor cells to detach from the primary tumor mass and migrate into surrounding tissues toward blood or lymphatic vessels, thereby forming a secondary lesion. Tumor invasion and metastasis are major causes of cancer mortality. Cancer is characterized as the accumulation and alteration of precancerous cells through initiation, promotion, and progression. Therefore, the approach to cancer prevention is to regulate and alter the mechanism of the cell mutation or tumor in the early stage.

1.7 Phenolic compounds vitro study and cell cycle/apoptosis

In cancer development, disruption of the normal regulation of cell-cycle progression and division is an important event. The cell cycle involves a recurring sequence of events that includes the duplication of cell contents and subsequent cell division. For eukaryotic cells, cell cycle is defined as the interval between the completion of the mitosis of a cell and the completion of the mitosis of one or both of its daughter cells. Traditionally, the cell cycle of eukaryotic cells is divided into four phases as follows: Gap phase 1 (G1), DNA synthesis (S), Gap phase 2 (G2), and mitosis (M). Gap phase 2 is the preparation stage for cell division. The mitotic phase involves the separation of chromosomes after cell division. The progression of the cell cycle from one phase to the next involves a sequence of events that are regulated by the activation and inactivation of checkpoints in the mitotic phase, during which the status of the cell and environmental cues can be monitored and evaluated. The G1 checkpoint regulates the cell size, nutrients, and molecular signals, that is, the growth factors, and DNA damage. The G2 checkpoint regulates DNA damage and replication. Finally, the M checkpoint, or spindle checkpoint, regulates the chromosome attachment to the spindle at the metaphase plate.

Apoptosis, or programmed cell death, is an important mechanism in normal cell development and is regulated by various oncogenes[46]–[48]. The programmed cell death allows the cells to self-destruct when stimulated by the tagger. This occurs mainly via extrinsic (death receptor) and intrinsic (mitochondria) pathways that involve condensation of the nucleus and cytoplasm after cellular partitioning into fragments for disposal. Normally, human cells grow and multiply to form new cells as the body needs them. When cells grow old or become damaged, they die and are replaced by new cells. However, cancer cells escape the checkpoints in the cell cycle and grow permanently.

Studies performed *in vitro* have demonstrated that phenolic components can prevent and/or inhibit the development of different types of cancer by regulating cell signal transduction and gene expression. Seong-Ho et al. (2020) [49]found that a novel high-phenol sorghum bran from Puerto Vallarta, Mexico, has the potential to induce apoptosis and suppress the cell growth of human colon cancer cells. Other recent vitro studies correlated sorghum extract treatment with the development of cancer cells, including breast (MCF-7) [50], colon (HT-15) [49], and liver cancer cells (HepG2) [51]. The results showed that treatment with sorghum phenolic extracts consistently induced cell apoptosis and suppressed cell growth.

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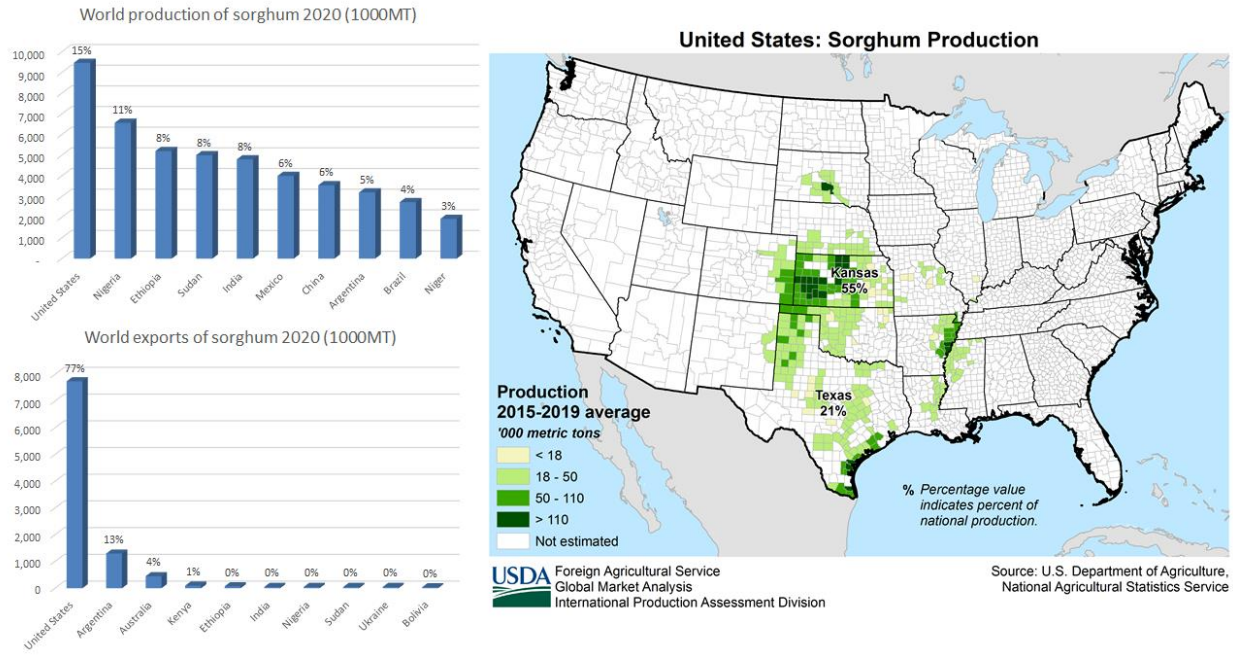


Figure 1.1 United State sorghum production worldwide and in state

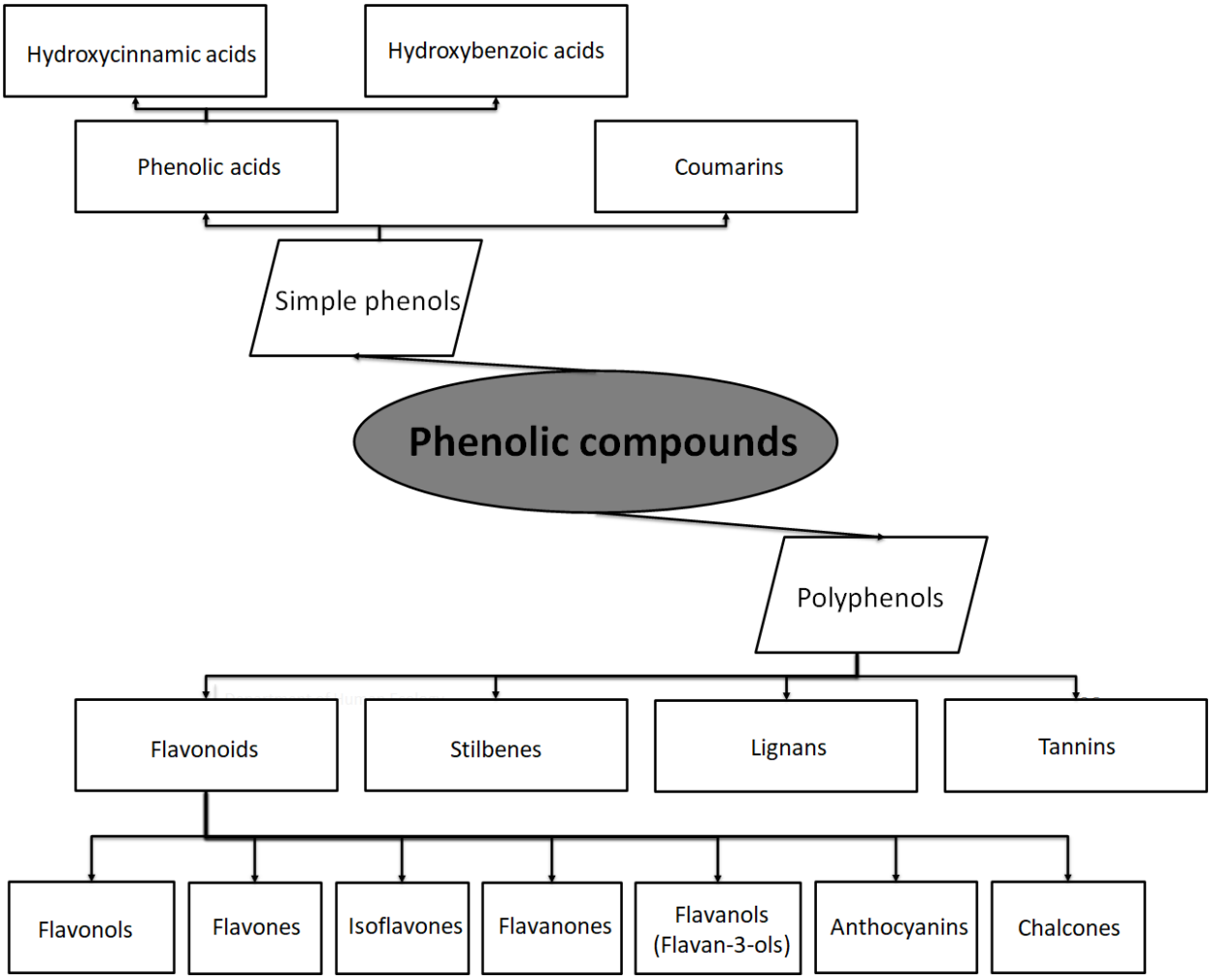
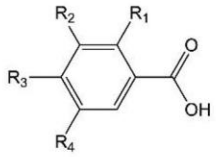


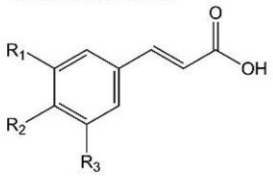
Figure 1.2 Classification of phenolic compounds

Benzoic aicd



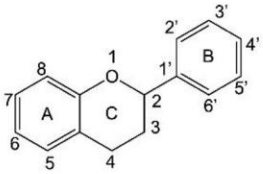
Benzoic acids	R_1	R_2	R_3	R_4
Gallic acid	H	OH	H	OH
Protocatechuic acid	H	OH	OH	H
Vanillic acid	H	OCH_3	OH	H
p-Hydroxybenzoic acid	H	H	OH	H
Syringic acid	H	OCH_3	OH	OCH_3
Gentisic acid	OH	H	H	OH
Salicylic acid	OH	H	H	H

Cinnamic acid

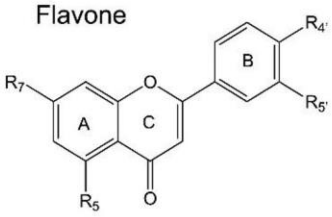


Cinnamic acids	R_1	R_2	R_3
Ferulic acid	OCH_3	OH	H
Caffeic acid	OH	OH	H
Sinapic acid	OCH_3	OH	OCH_3
p-Coumaric acid	H	OH	H
Cinnamic acid	H	H	H

Flavan skeleton

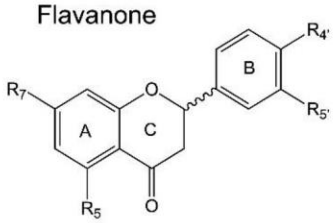


Flavone



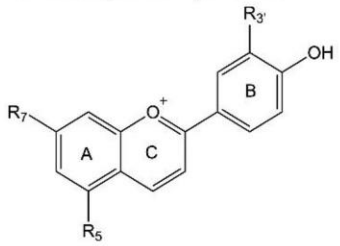
Flavones	R_5	R_7	$R_{4'}$	$R_{5'}$
Apigenin	OH	OH	OH	H
Luteolin	OH	OH	OH	OH
Their derivatives	R_7, R_8 = OH, OCH_3 and /or O-glycosides			

Flavanone



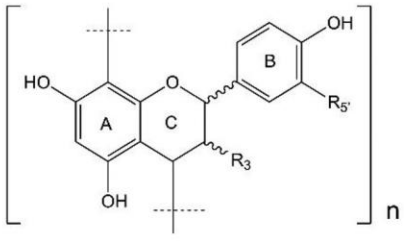
Flavanones	R_5	R_7	$R_{4'}$	$R_{5'}$
Naringenin	OH	OH	OH	H
Eriodictyol	OH	OH	OH	OH
Their derivatives	R_7, R_8 = OH and /or O-glycosides			

3-Deoxyanthocyanidin



3-Deoxyanthocyanidins	R_5	R_7	$R_{3'}$
Apigeninidin	OH	OH	H
Luteolinidin	OH	OH	OH
Apigeninidin 5-glucoside	O-Glu	OH	H
Luteolinidin 5-glucoside	O-Glu	OH	OH
7-Methoxy-apigeninidin	OH	OCH_3	H
7-Methoxy-luteolinidin	OH	OCH_3	OH
5-Methoxy-luteolinidin	OCH_3	OH	OH
7-Methoxy-apigeninidin 5-glucoside	O-Glu	OCH_3	H
7-Methoxy-luteolinidin 5-glucoside	O-Glu	OCH_3	OH
5-Methoxy-luteolinidin 7-glucoside	OCH_3	O-Glu	OH

Proanthocyanidin



Proanthocyanidins	R_3	R_5 & R_7	$R_{5'}$
Procyanidin	OH	OH/OCH_3	OH
Proapigeninidin	H	OH/OCH_3	H
Proluteolinidin	H	OH/OCH_3	OH

Figure 1.3 Structure of major sorghum phenolic compounds

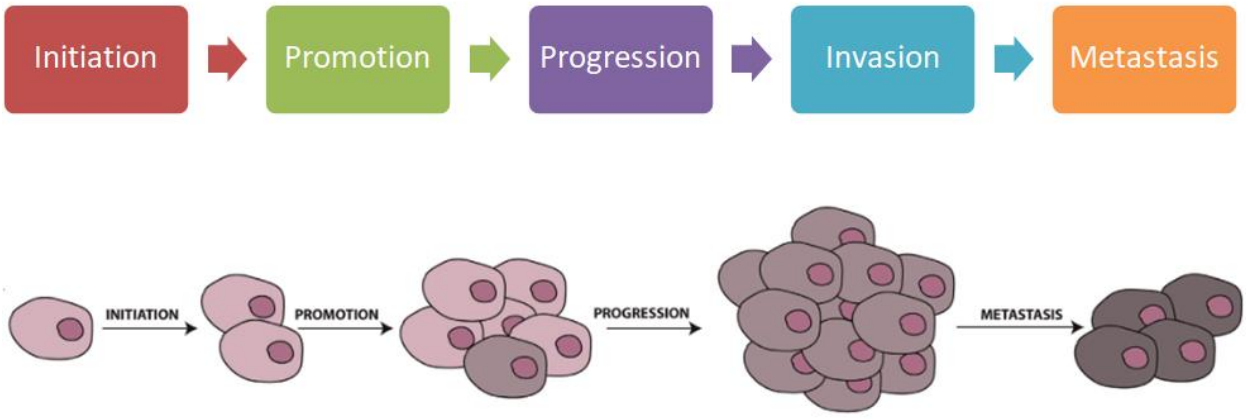


Figure 1.4 Cancer development with each phase

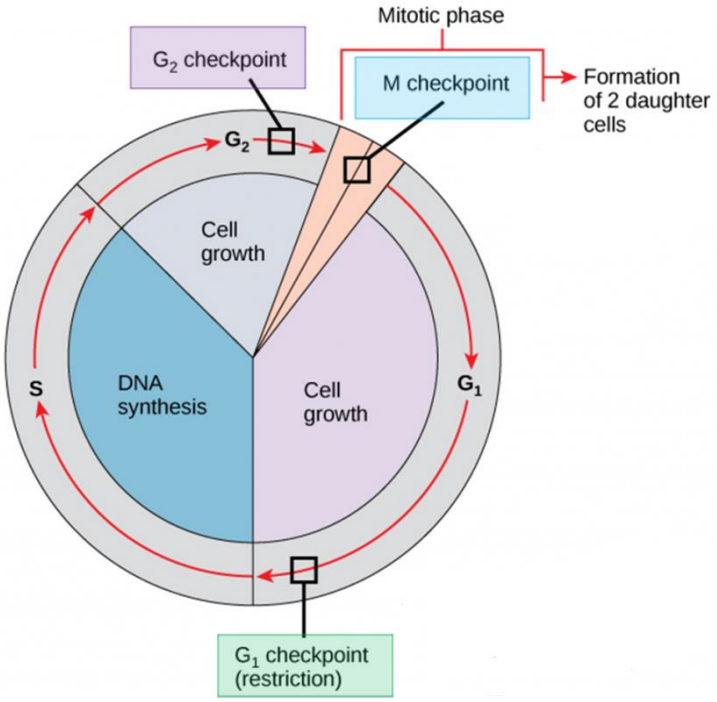


Figure 1.5 Cell cycle with checkpoints

Chapter 2 - Experiments

2.1 Abstract

Sorghum is a rich source of various phytochemical phenolics including phenolic acids, anthocyanins, and flavonoids, etc. These phenolics have been associated with chronic disease prevention, especially cancer. However, the available data for various varieties of sorghum on cancer are limited. In this study, two different sorghum accessions were selected and the extracted phenolics were tested for their impact on cell growth and apoptosis in both hepatocarcinoma HepG2 and colorectal adenocarcinoma Caco-2 cell lines. Total phenolic contents determined by Folin-Ciocalteu were 2.11 mg GAE/g DW in F10000 and 21.89 mg GAE/g DW in PI329694, respectively. Cells treated with various concentrations of the extracted phenolics at 0-200 μM GAE up to 72 hrs resulted a dose dependent reduction of cell number. The apoptosis as measured by the FITC Annexin V protocol was also resulted a significant increase in the extract of PI329694 when comparing with the extract of F10000. Overall, these results showed a positive evidence that a direct impact of the sorghum phenolics on either HepG2 or Caco-2 cellular growth inhibition and apoptosis induction. Both anti-growth and apoptotic induction seemed associated with their phenolic contents. Therefore, this study suggests that sorghum prevent against cancer through phenolic-mediated cancer cell inhibition and apoptosis induction.

Key words: sorghum, phenolic compounds, cell apoptosis, HepG2, Caco-2

2.2 Introduction

Sorghum, one of the cereal crops consumed worldwide, was ranked as the fifth most valuable crop after wheat, rice, maize, and barley [1]. The United States is the largest producer and exporter of sorghum, accounting for 17% of the world's production and almost 77% of the world's sorghum exports over the past 5 years (USDA-ARS). Numerous people in developing countries use sorghum as a food source. However, in developed countries, sorghum is not consider as a traditional food and mostly used as a livestock ingredient and in biofuel production [2]. Stefoska-Needham et al. (2015) [3]claimed that people underestimate the value of sorghum because it is often used as animal feed. In recent years, sorghum has drawn the attention of scientists because its natural "gluten-free" characteristic proved beneficial to people with celiac disease [4]. Sorghum as a valuable, inexpensive, and nutritional crop that may have higher bioactive phenolic contents than major cereals such as wheat, barley, rice, maize, rye, and oats [3]. The present of phenolic compounds in sorghum are naturally synthesis by the secondary metabolites of plants, which arise the interested as a functional food or ingredients, especially for cancer prevention[1], [5]–[8]. Interest in the long-term use of food-derived products for the prevention of cancer in different populations has been increasing because they are expected to be safe and are not perceived as "medicine"[9], [10]. Schwedhelm et al. (2016) [11]reported that healthy and high-quality diets are inversely associated with human cancer mortality.

Reference data from epidemiological and animal studies have shown that phenolic compounds could have potential health benefits such as cancer prevention owing to their biological properties, including antioxidant activity, cell growth inhibition, and induction of cell apoptosis [12]–[16][17], [18] . Studies found that the phenolic compounds in tea and wine, which are phenol-rich products, have anticancer effects [19]–[22]. More recently, Seong-Ho et

al. (2020) [23]found that a novel high-phenol sorghum bran from Puerto Vallarta, Mexico, has the potential to induce apoptosis and suppress the cell growth of human colon cancer cells. Other in vitro studies published recently have correlated sorghum extract administration with the development of cancer cells, including breast (MCF-7) [8], colon (HT-15) [23], and liver cancer cells (HepG2) [23]. The results showed that administration of sorghum phenolic extracts consistently induced cell apoptosis and suppressed cell growth. Many studies have shown that cancer prevention correlates with the consumption of fruits and vegetables containing phenolic compounds, but sorghum phenolic compounds as a new arises topic have lag data compare phenolic rich product such as wine and tea[17], [18], [20], [22], [24]–[28]. Therefore, demonstrated more studies on sorghum phenolic compounds *in vitro and in vivo* are important.

In the present study, human hepatocellular carcinoma HepG2 and colon adenocarcinoma Caco-2 cells were selected because the major site for metabolism and absorption including phenolic compounds. Two sorghum accessions with different phenolic contents were selected to examine cell growth and apoptosis throughout the course of the cell treatments. The aim of this study was to investigate the cell inhibition and apoptosis induction effects of sorghum phenolic extracts on cancer cells and correct the apoptosis result presented in previous study.

2.3 Experimental Section

2.3.1. Sorghum accessions

Sorghum accession P1329694 was selected because of its high phenolic contents. Sorghum accession F10000 was selected as a control because of it lower phenolic content. Sorghum samples were collected by the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) Grain Quality and Structure Research Unit (1515 College Avenue, Manhattan, KS, USA).

2.3.2. Standards and reagents

Acetone, ethanol, Folin-Ciocalteu reagent, gallic acid, sodium carbonate, and Dulbecco's phosphate-buffered saline (DPBS) were purchased from Sigma-Aldrich (St. Louis, MO). Cell treatment reagents such as Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), and trypsin-EDTA were purchased from Fisher Scientific Co. L.L.C (Pittsburgh, PA, USA). Reagents for cell apoptosis such as the FITC Annexin V apoptosis detection kit, propidium iodide (PI), annexin-binding buffer, and cell-staining buffer were purchased from BioLegend (San Diego, CA, USA).

2.3.3 Extraction

Sorghum flour (5 g) was mixed with 50 mL of 70% aqueous acetone (*v*/*v*) and shaken using a 211DS shaking incubator (Labnet International Inc., NJ, USA) for 2 h. The samples were then stored at −20°C in the dark overnight to allow the phenolic compounds to completely diffuse into the solvent. On the next day, the extracts were centrifuged at 2970×*g* for 10 min. The residue was rinsed with an additional 50 mL of solvent with 5 min of shaking and centrifuged at 2970×*g* for 10 min. A combination of two aliquots was used for determination of the total phenolic content (TPC) and cell treatment. The extracts for cell treatment were dried under a stream of nitrogen followed by evaporation. After all the water had evaporated, the extracts were freeze-dried to obtain a completely dry powder. The dry extracts were then dissolved in dimethylsulfoxide (DMSO) to make stock solutions and stored at −20°C for further analysis. The stock solution was diluted with fresh medium to achieve the desired concentrations (0–200 μM gallic acid equivalents [GAE]) before each treatment. The final DMSO concentration in each treatment was kept within ≤0.1% to prevent an altered cell growth compared with the vehicle-free medium. All extractions and treatments were performed in triplicate.

2.3.4 Total phenolic content

The TPC of the sorghum extract was evaluated using the Folin-Ciocalteu method (Thamnarathip et al., 2016). Briefly, 0.1 mL of extract sample was diluted in 7.9 mL of water and then mixed with 0.5 Folin-Ciocalteu reagent. Then, 1.5 mL of 20% sodium carbonate solution was added, and the mixture was then reacted for 2 h. The samples were read absorbance at 760 nm by a microplate reader (SynergyHT, Biotek) with the Gen5 TM2.0 data analysis software (Winnoski, USA). Gallic acid (0–0.06 mg/mL) was used as the standard, and the TPC was calculated as mg GAE/g of dry weight (DW).

2.3.5 Cell culture

Human hepatocellular carcinoma HepG2 cells were provided by the Grain Science Department, Kansas State University (Manhattan, KS 66506). Human colorectal adenocarcinoma Caco-2 cells were provided by the USDA-ARS Grain Quality and Structure Research Unit (1515 College Avenue, Manhattan, KS 66502, USA). The complete medium was made by mixing DMEM with 10% FBS and 1% penicillin/streptomycin. Then, the cells were cultured in the supplemented medium at 37°C in a 5% CO_2 humidified atmosphere. The exponential growth phase of the cells was used for all the experiments.

2.3.6 Cell growth

The initial 2-mL cell suspension (1×10^5 cells/mL) were seeded into 6-well plates and cultured in a humidified incubator overnight to allow adhesion. The sorghum extracts at different concentrations (0–200 M) in DMEM were prepared ahead and then used to exchange the original DMEM in the 6-well plates to begin the treatment. Cells were incubated at 37°C for 72 h. All the cells were grown three times separately to minimize the deviation.

2.3.7 Cell concentration and viability

The medium in the 6-well plates was first removed, and DPBS was used next for cell rinsing. The trypsin-EDTA solution (0.05%) at 37°C was used to detach the cells. Then, all the mixtures were collected into a 15-mL tube and centrifuged at 200×*g* for 5 min. The supernatant was removed, and 0.5 mL of medium was added to resuspend the cells. Twenty microliters of resuspended cells was collected in a small vial and mixed with 20 µL of acridine orange and propidium iodide. Twenty microliters of the mixture sample was added into a counting chamber and then inserted in a Nexcelom Auto 2000 cellometer (Nexcelom Bioscience LLC., Manchester, UK). Cell concentration and viability were tested using the cellometer through the system software.

2.3.8 Cell apoptosis analysis

The cells were detached and washed with a cold cell-staining buffer solution after the treatment. Then, the cells were resuspended in 100-µL Annexin V-binding buffer and then transferred to EDTA tube. FITC Annexin V (5 µL) and 10 µL of PI was added to the cell suspension, which was gently vortexed and incubated for 15 min at room temperature (25°C) in the dark. Annexin V-binding buffer (400 µL) was added to the EDTA tube, and the samples were analyzed using flow cytometry with the appropriate machine settings.

2.3.9 Statistical analyses

All the tests were performed in three repetitions. Statistical analyses were performed using the SAS Studio version 3.8 (2008) software (SAS University Edition) at 5% probability.

2.4 Results

2.4.1 Total phenolic content

The TPCs of the two sorghum accessions were determined using the Folin-Ciocalteu method. The results were expressed as milligram gallic acid equivalent per gram of dry weight (mgGAE/g DW) and shown in Table 1. F10000 with a white pericarp has a low TPC of 2.11 mg GAE/g DW, while PI329694 with a black pericarp has a high TPC of 21.89 mg GAE/g DW.

2.4.2 Cell inhibition

The cell inhibition of the HepG2 and Caco-2 cells treated with the two sorghum phenolic extracts at 0–200 μM GAE for up to 72 h are shown in Figures 1 and 2, respectively. The sorghum accessions at various concentrations in both cell lines showed a dose dependent manner, with significant differences between concentrations. The higher the sorghum phenolic content, the stronger the cell inhibition.

2.4.3 Cell apoptosis analysis

With regard to apoptosis, the results of the cell treatments with sorghum accessions F10000 and PI1329694 at 0–200 μM GAE for up to 72 h are shown in Figures 3 and 4. The results showed that both sorghum accessions significantly increased the percentage of apoptotic cells as the sorghum phenolic extract dose was increased. The flow cytometry results for both cell treatments with the representative sorghum phenolic extracts at 200 μM GAE are shown in Figure 5. The results for the HepG2 cells showed that the number of apoptotic cells was increased by 9% by F10000 with a white pericarp and by 23.1% with PI1329694 with a black pericarp compared with the control. A similar trend but with a mild increase was observed in Caco-2.

2.5 Discussion

The aim of this study was to examine the usefulness of the sorghum accessions of the white (F10000) and black pericarps (PI1329694) in the HepG2 and Caco-2 cell lines for cancer prevention. These two colors of sorghum accessions were selected on the basis of their phenolic contents. Accession F10000 with a white pericarp contained low levels of phenolic compounds, whereas accession PI1329694 with a black pericarp contained high levels of phenolic compounds. The study used two cell lines, one from liver cancer and the other from colorectal cancer. These cell lines were selected because the major site of metabolites and absorption of various substances including phenolic compounds.

In this study, the TPCs of two sorghum accessions were determined using the Folin-Ciocalteu method. The white-pericarp sorghum accession (F10000) had a low TPC of 2.11 mg GAE/g DW, whereas the black-pericarp sorghum accession (PI329694) has a high TPC of 21.89 mg GAE/g DW.

After incubation with each treatment, the two cell lines treated with F10000 and PI329694 extracts showed the phenolic extracts effectively inhibited HepG2 or Caco-2 cancer cell growth in a dose-dependent manner. These results also indicated that sorghum accessions rich in phenolic compounds significantly suppressed the cell growth in both HepG2 and Caco-2 cell. The result demonstrated phenolic extracts from sorghum accessions (F10000 and PI329694) can inhibit the cell growth of HepG2 and Caco-2 cells *in vitro* (Figures 2.1 and 2.2).

After incubation with each treatment, cells were detached by 0.05% trypsin at 37 °C and then suspended in DPBS for further apoptosis analysis. The apoptosis analysis was conducted in accordance with the BioLegend FITC Annexin V staining procedure and detected by flow cytometer and result in a cell distribution graph. Among the data, the phenolic extracts at 200

μM GAE in two sorghum accessions significantly induce the apoptotic cells in HepG2 and Caco-2 cells. Figure 3 is the overall apoptosis result of sorghum extracts from 0 to 200 μMGAE in HepG2 and Caco-2. It shows the phenolic extracts in two sorghum accessions effectively induce HepG2 or Caco-2 apoptotic cell in a dose-dependent manner. These results also indicated that sorghum accessions rich in phenolic compounds significantly induce apoptosis in both HepG2 and Caco-2 cell. The result demonstrated phenolic extracts from sorghum accessions (F10000 and PI329694) can induce cell apoptosis of HepG2 and Caco-2 cells *in vitro*.

In conclusion, the study demonstrated that cell inhibition by the sorghum phenolic extracts was significantly associated with their phenolic content. The Two sorghum accessions selected in this study effectively induced the apoptotic cells in HepG2 and Caco-2 cancer cells and was significantly associated with the concentration of the sorghum phenolic extracts. Overall, this study suggests that sorghum prevent against cancer through phenolic-mediated cancer cell inhibition and apoptosis induction.

Table 2-1 Total Phenolic Contents in Sorghum Accessions

Sorghum Accession No.	Phenolic contents (mg GAE/g DW)
F10000	2.11±0.08
PI1329694	21.89±2.54

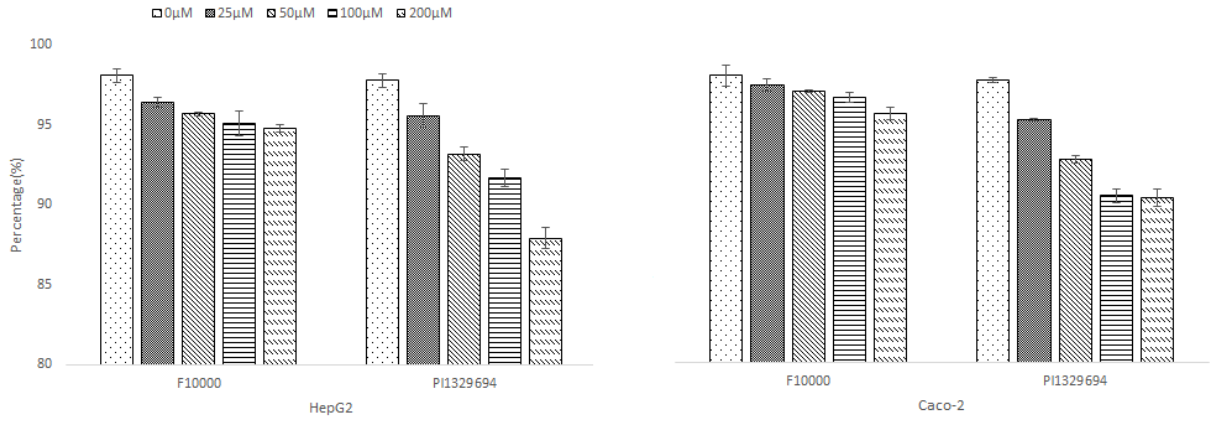


Figure 2.1 Cell inhibition 0-200 μM GAE

The inhibition effect of phenolic extracts from two sorghum ascensions at 0-200 μM GAE in HepG2 cells after 72 hrs. Values are expressed as Mean ± SD (n = 3), Means with different alphabetical letters differ significantly, $p \leqslant 0.05$

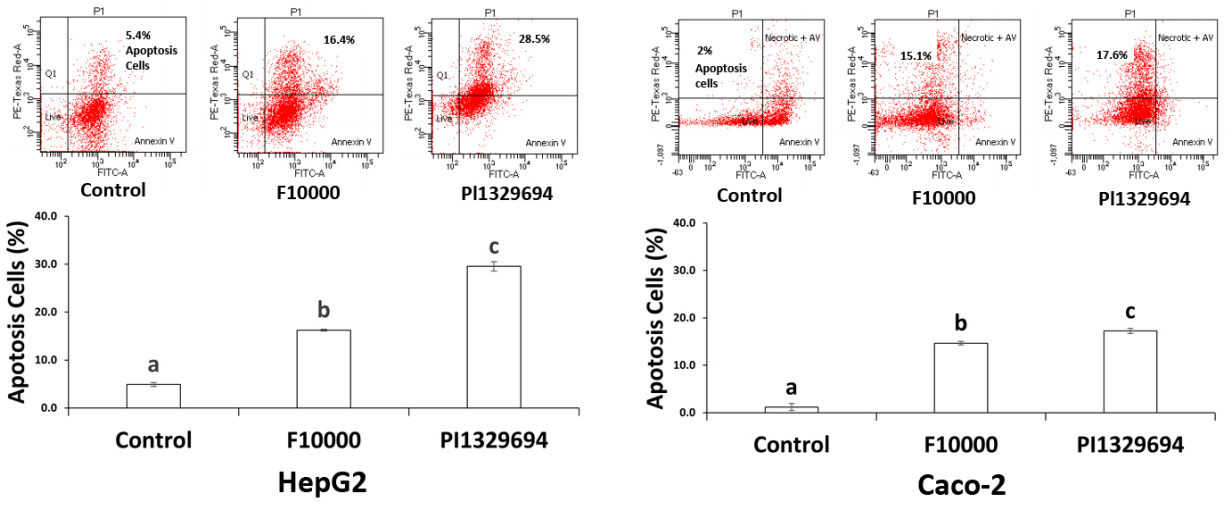


Figure 2.2 Apoptosis induction at 200 µM GAE with flow cytometer graph

Apoptosis induced by representative sorghum phenolic extracts (200 µM GAE) in HepG2 and Caco-2 cell lines. Values are expressed as Mean ± SD (n = 3), Means with different alphabetical letters differ significantly, $p \leqslant 0.05$

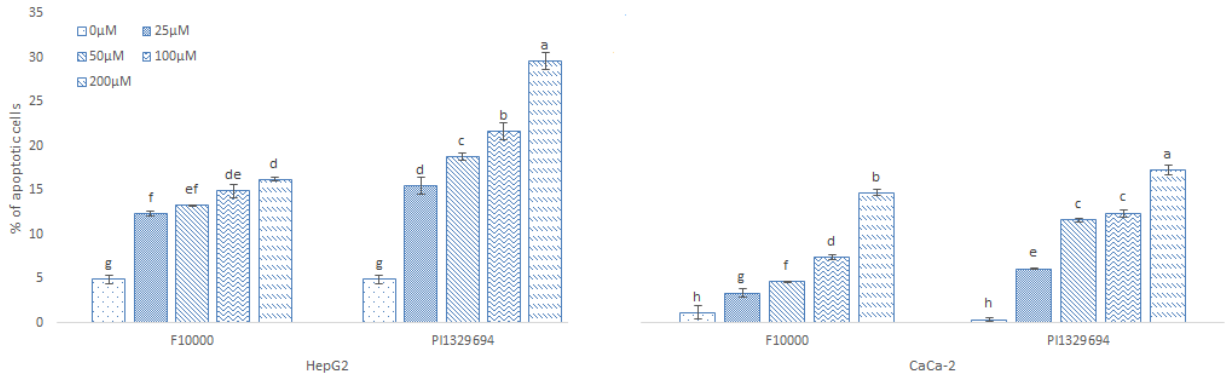


Figure 2.3 Overall apoptosis induction at 0-200 µM GAE

Apoptosis induced by sorghum phenolic extracts from 0-200 µM GAE in HepG2 and Caco-2. Values are expressed as Mean ± SD (n = 3), Means with different alphabetical letters differ significantly, $p \leqslant 0.05$

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