Alsufyani AM, et al., J Food Sci Nutr 2018, 4: 040
DOI: 10.24966/FSN-1076/100040

HSOA Journal of Food Science and Nutrition

Research Article

Anti-Cancer Effect of Plum Extract on U-87 Glioblastoma Multiforme Brain Cancer Cells

Ashwaq M Alsufyani[1,2], Haiwen Li[1], Jason Younkin[2], Andrea R Beyer[2] and Rafat A Siddiqui[1]*

[1]Nutrition Science and Food Chemistry Laboratory, Agricultural Research Station, College of Agriculture, Virginia State University, Virginia, USA

[2]Department of Biology, College of Natural and Health Sciences, Virginia State University, Virginia, USA

Abstract

Brain cancer is a very devastating disease which is difficult to treat and the survival rate of brain cancer patients is low. Present treatments have limited success; therefore, there is a constant search for new brain cancer treatments and strategies. Oxidative stress plays a role in the initiation and progression of brain cancer. Fruits and vegetables are rich in phenolic compounds that have superior antioxidant properties. Among fruits, plum extract contains high levels of phenolic compounds and has been shown to exhibit anticancer properties. The objective of this study was to determine effects of plum extract on proliferation of brain cancer cells. A standardized preparation of plum extract, PE60, was used to determine its total polyphenols content, total flavonoids content, and anti-oxidation activity. Aqueous, methanolic, and DMSO solutions of PE60 were used to determine their effects on cell viability of U-87 cells by MTT assay. Apoptosis was assayed by analyzing nuclear morphology and caspase activation. PE60 was found to be rich in polyphenols (575-600mg/g of dry weight). The antioxidant activity in PE60 solutions ranged from 3250-3500mM equivalent of Trolox/g dry weight. When cells were treated with aqueous PE60 solution, the cells' viability was significantly by 20% ($p<0.05$) at 25μg/ml, and about 80% ($p<0.05$) of cells died at 250μg/ml PE60 solution. When cells were treated with PE60 dissolved in methanol or DMSO, the cells' viability was decreased significantly by 30-40% at 250μg/ml. Our data show that the effect of plum extract was mediated through an apoptotic pathway involving caspase-3 activation. In conclusion, this study showed that aqueous PE60 solution was better than that of methanolic or DMSO solutions for inhibiting U-87 brain cancer cell growth. Our data suggest that plums have nutritional compounds that can potentially be effective for preventing or inhibiting brain cancer.

Keywords: Antioxidants; Brain cancer; Plum extract; Polyphenols

***Corresponding author:** Rafat A Siddiqui, Nutrition Science and Food Chemistry Laboratory, Agricultural Research Station, College of Agriculture, Virginia State University, Virginia, USA, Tel: +1 8045245957; E-mail: rsiddiqui@vsu.edu

Citation: Alsufyani AM, Li H, Younkin J, Beyer AR, Siddiqui RA (2018) Anti-Cancer Effect of Plum Extract on U-87 Glioblastoma Multiforme Brain Cancer Cells. J Food Sci Nut 4: 040.

Received: October 10, 2018; **Accepted:** November 08, 2018; **Published:** November 22, 2018

Introduction

Cancer is considered to be a major public health problem worldwide and is the second leading cause of death globally, causing an estimated 9.6 million deaths in 2018 [1]. According to the National Cancer Institute (2016), the most common causes of cancer deaths are lung, bronchus, colorectal, and prostate cancers in men and lung, bronchus, breast, and colorectal cancers in women [2]. Brain and other nervous system cancers are relatively rare compared to other cancers [3], and are most frequently diagnosed among people aged 55-64 years though they can develop in any age group [4]. According to the World Health Organization (WHO), there are 120 different types of brain cancers [5]. However, some of the most common brain tumor types include oligodendroglioma, astrocytoma, and meningioma [6]. Gliomas make up about 30% of all brain tumors and about 80% of all malignant brain tumors [7]. The brain tumors arise from glial cells which are normally found in brain tissues [8]. Glial cells support nerve cells with energy and nutrients and maintain the blood-brain barrier [9]. About 5% of brain tumors are caused by hereditary genetic conditions such as neurofibromatosis, tuberous sclerosis, Li-Fraumeni syndrome, and Turcot's syndrome [10].

The hereditary and somatic mutations in oncogenes and tumor suppressor genes play an important role in tumor initiation and progression [11]. Tissues with decreased concentrations of antioxidant enzymes and enhanced concentrations of Reactive Oxygen Species (ROS) initiate mutagenic and carcinogenic conditions that have potential for genomic alteration [12]. The brain, because of its high metabolic activity and relatively decreased capacity for cellular regeneration, is particularly susceptible to the damaging effects of ROS [13]. Reactive species, such as hydroxyl radicals, can react with nucleotide bases. Additionally, they can damage chromatin proteins and cause modifications and genomic instability in chromosomes, therefore, resulting in gene expression alterations [14]. The carcinogenic potential induced by oxidative stress is related to a balance between ROS generation and its neutralization by anti-oxidation processes. An excess ROS accumulation may induce DNA damage and then interfere with crucial cellular processes [14].

Recent research reports that certain plant chemicals, such as terpenes, polyphenols, and thiols have much more powerful antioxidant

properties than well-known antioxidant vitamins [15]. Plants and vegetables are rich in flavonoids, and their beneficial functions as antioxidant agents and cancer-preventers are well known [16]. In the last decade, bioactive phytochemicals have been regarded as mostly non-toxic compounds for the treatment of many forms of cancers, including brain tumors and gliomas [17]. Several authors reported inhibition of cell proliferation of glioma by compounds such as Curcumin (Cur), isothiocyanate derivatives, resveratrol, and catechins, such as Epigallocatechin-3-Gallate (EGCG) [18-21].

The plum is considered a super food that is rich in phytonutrients [22]. It is a rich source of antioxidant compounds like flavonoids, phenolic acids, and other phenolic compounds, all of which are effective as natural antioxidants and protect the body from unwanted radicals and toxins. Most of its antioxidant power is due to the high levels of neochlorogenic and chlorogenic acids, phenolic acids, and anthocyanins [23,24]. The objective of the present study was to characterize a standard preparation of plum extract for its anti-oxidation activity and to determine its effects on cell proliferation of glioblastoma cells.

Materials and Methods

Cells and reagents

U-87 cells were purchased from ATCC (Manassas, VA). DMEM media, penicillin, and streptomycin were purchased from Gibco-Fisher (Grand Island, NY). Fetal Bovine Serum (FBS) was purchased from RAMBIO (Missoula, MT). The PE60 powder, a standardized preparation of plum extract containing 60% phenolic compounds, (P. L. Thomson, Morristown, MA) was kindly donated by Dr. Suzette Pereira from Abbott Nutrition (Columbus, OH). Folin-Ciocalteu, aluminum chloride, Diphenyl-1-Picrylhydrazyl (DPPH), quercetin, gallic acid, trolox, 3-(4,5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide (MTT), paraformaldehyde, and 2, 2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma Chemical Co. (St Louis, MO). The caspase inhibitors z-VAD-FMK and z-DEVD-FMK were purchased from Biovision (Atlanta, GA).

Preparation of PE60 extracts

PE60 powder was dissolved either in water, 80% methanol, or DMSO (25% or 50%) at 250mg/ml (stock solution). The solutions were centrifuged at 2000g for 10 minutes to remove trace amounts of undissolved material. The clear solution was stored at -20°C until used. The stock solution was diluted with serum-free media to 0-250mg/ml each time when used for cell treatment. The final concentration of methanol and DMSO did not exceed 0.1%. A control containing appropriate amounts of methanol and DMSO was included along with the test samples.

Determination of Total Phenolic Content (TPC)

The total phenolic content of PE60 solutions was determined by using the Folin-Ciocalteu method as previously described [25]. The total phenolic content of the PE60 extract was calculated as gallic acid equivalents (mg GAE/g of dry extract).

Determination of Total Flavonoid Content (TFC)

An aluminum chloride complex-forming assay was used to determine the total flavonoid content of the PE60 solutions [26]. Quercetin was used as a standard and flavonoid content was determined as quercetin equivalents (mg QE/g of dry extract).

Anti-oxidation activity

The anti-oxidation activity of PE60 solutions was determined by using the 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) method [27]. The data is reported as trolox equivalent (mM TE/g of dry extract).

Oxygen scavenging capacity

The oxygen scavenging capacity of PE60 solutions was evaluated according to the previously reported protocol [28,29]. Trolox equivalents were calculated using a standard curve and expressed in mM tolox equivalents per gram of dried plum extract (mM TE/g of dry extract).

Cell culturing and anti-proliferation assay

The U-87 brain tumor cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Invitrogen; Carlsbad, CA) and supplemented with penicillin (100units/ml), streptomycin (100µg/ml) and 10% FBS (complete media). The cells were incubated in a humidified incubator at 37°C with 5% CO_2. Medium was changed every 3 days and cells were subcultured when they became confluent. The effect of PE60 on cell proliferation was determined using a MTT assay as per manufacturer instructions. Briefly, U-87 brain cancer cells (10,000/well) were cultured in a 96 well plate. After 24 hours of incubation in a complete medium, cells were treated with a final concentration of 0 (0.1% methanol or DMSO as control), 10, 25, 50, 100, 150, 200, or 250mg/ml of PE60 plum solution for another 24 hours in serum-free medium. Cells were then incubated with 20ml of MTT solution (5mg/ml in DMSO) for 3 hours and formation of purple crystals were quantified (after dissolving the crystal in 200ml of DMSO) in a 96 well plate reader (Molecular Devices, San Jose, CA) at 570 nm. The results are expressed as % change from control.

Effect of PE60 on nuclear morphology

U-87 brain cancer cells were cultured in a 96 well plate for 24 hours in 100ml of a complete medium. Cells were then incubated with a final concentration of 0, 10, 25, 50, 100, 150, 200, or 250mg/ml of PE60 plum solution for 24 hours. After treatment, the cells were removed and washed once with Phosphate Buffered Saline (PBS) and then fixed with cold 3% paraformaldehyde for 15 minutes on ice. The cells were washed 3 times with PBS and then 50ml of 1:1000 dilution of Hoechst 33342 (10mg/ml) dye was added as per manufacturer's instructions (Thermo Scientific, Rockford, IL). The plate was kept at room temperature for 10 minutes before the cells were washed three times with 100ml PBS. Microscopic images were taken in the dark at different magnifications using a Nikon Fluorescence Microscope.

Role of caspases in PE60-induced cell death

Cells were cultured in a 96 well plate as described above, and treated with a final concentration of either 0.1% DMSO (control), 10µM of z-VAD-FMK (generic caspase inhibitor) or 10µM of z-DEVD-FMK (specific caspase-3 inhibitor) for 1 hour prior to treatment with PE60. After caspase inhibitor pretreatment, cells were incubated with or without PE60 (100mg/ml) for 24 hours in a CO_2 incubator as described above. The effect of caspase inhibitors on PE60-induced cell death was determined by assaying total viability of U-87 cells using a MTT assay as described above.

Results

Characterization of PE60 preparations for anti-oxidation activity

The different preparations of PE60 were characterized for anti-oxidation potential by assessing their total phenolic content, total flavonoid content, anti-oxidation activity, and total oxygen scavenging activity. The data shown in figure 1 indicate that water- and DMSO-extracted PE60 contained TPC of 575-600mg Gallic Acid Equivalents (GAE)/g of dry weight. However, the methanol extract had a significantly lower TPC concentration of around 450mg/g of dry extract.

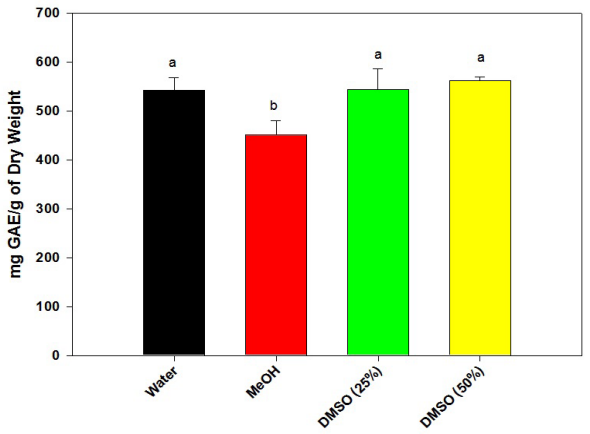

Figure 1: Total phenolic content in PE60 preparations.

Note: The TPC content of PE60 preparations was determined using Folin-Ciocalteu reagent as described in the "Materials and Methods" section. Gallalic acid was used as a standard to determine TPC content. Results are expressed as mean±SD for at least 3 replicates. All comparisons were made by one-way ANOVA with Tukey's-HSD post-hoc test and represented by alphabets. Data between treatment groups indicated by different alphabets represent a significant difference at $p<0.05$ where as data between treatment groups indicated by similar alphabets are non-significant.

The amount of TFC in different PE60 preparations is shown in figure 2. Similarly, the water and DMSO preparations contained TFC of 525-550mg Quercetin Equivalents (QE)/g of dry weight, whereas the methanol extract contained a significantly lower amount of TFC (425mg QE/dry weight) compared to that of other preparations.

The anti-oxidation activity in different preparations of PE60 is shown in figure 3. The data indicate that all preparations have a similar extent of anti-oxidation activity and represented about 3250-3500mM equivalence of Trolox activity/g of dry weight.

The oxygen scavenging activity of different preparations of PE60 extract is shown in figure 4. It is evident from the data that all preparations contained a similar capacity for scavenging free oxygen. The total oxygen scavenging activity in these preparations varied from 4000-5000mM Trolox equivalent/g of dry weight.

Effect of PE60 preparations on U-87 glioblastoma cell viability

Different solvent preparations (water, methanol, and DMSO) were used to determine the effect of PE60 on U-87 glioblastoma brain cancer cells. When cells were treated with water extract of PE60, cell viability was decreased significantly to 80% ($p<0.05$) at 25µg/ml. Cell viability was decreased further in a concentration-dependent manner by increasing the PE60 concentration over 25µg/ml (Figure 5). A decrease in cell viability was observed and only 20% ($p<0.05$) survived at 250µg/ml.

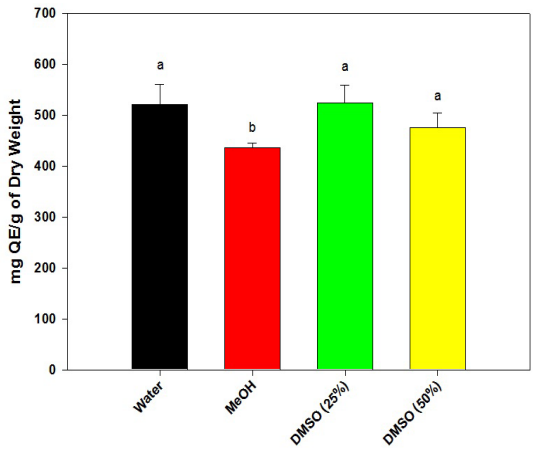

Figure 2: Total flavonoid content in PE60 preparations.

Note: The TFC content of PE60 preparations was determined using AlCl3 reagent as described in the "Materials and Methods" section. Quercetin was used as a standard to determine TFC content. Results are expressed as mean±SD for at least 3 replicates. Data between treatment groups indicated by different alphabets represent a significant difference at $p<0.05$ where as data between treatment groups indicated by similar alphabets are non-significant.

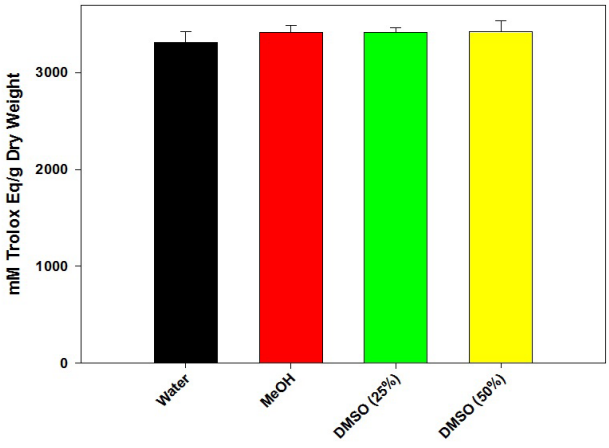

Figure 3: Total anti-oxidation activity in PE60 preparations.

Note: The total anti-oxidation activity in PE60 preparations was determined using a DPPH assay as described. Tolox was used to quantify the total anti-oxidation activity and data is reported as mM Tolox equivalent. Results are expressed as mean±SD for at least 3 replicates. All comparisons were made by one-way ANOVA with Tukey's-HSD post-hoc test. No significant difference was observed between treatment groups.

When cells were treated with methanol extract of PE60, cell viability was decreased significantly to 75% ($p<0.05$) at 25mg/ml. Cell viability was decreased further in a concentration-dependent manner but only to 60% ($p<0.05$) at 250mg/ml. When cells were treated with PE60 extract in 25% DMSO, a non-significant effect on cell viability

was observed up to 200mg/ml. However, when the concentration of PE60 was increased to 250mg/ml, a significant reduction in cell viability of 30% was observed (Figure 5). However, when cells were treated with PE60 extract in 50% DMSO, a non-significant effect on cell viability was observed at all tested concentrations (Figure 5).

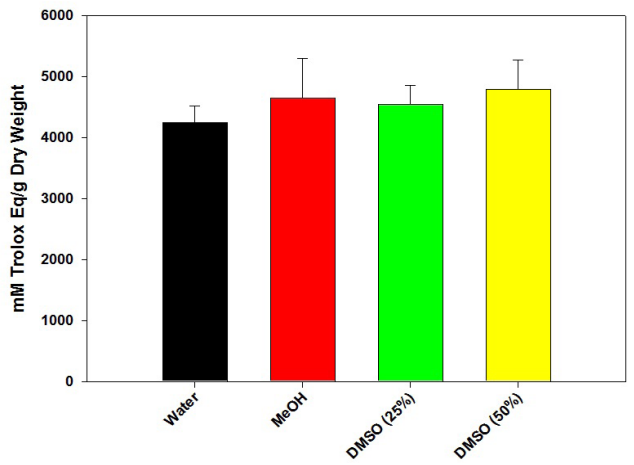

Figure 4: Oxygen scavenging activity in PE60 preparations.

Note: The oxygen scavenging activity in PE60 preparations was determined using an ABTS assay as described in the "Materials and Methods" section. Trolox was used to quantify the oxygen scavenging activity and data are reported as mM Trolox equivalent. Results are expressed as mean±SD for at least 3 replicates. All comparisons were made by one-way ANOVA with Tukey's-HSD post-hoc test. No significant difference was observed between groups.

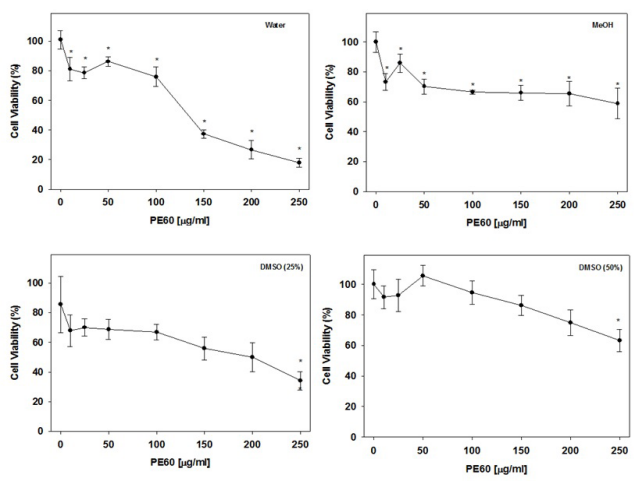

Figure 5: The effect of PE60 preparations on U-87 brain cancer cells.

Note: Cells (10,000/well) were incubated with different concentrations of water, methanol, and DMSO solutions of PE60 in a CO_2 incubator at 37˚C for 24 hours as described. After treatment with PE60 solutions, 20µl of MTT was added to each well and the plate was read at 570nm in a 96 well plate reader. Results are expressed as mean±SD for at least 3 replicates. All comparisons were made by one-way ANOVA with Tukey's-HSD post-hoc test. All significant differences between control and treated cells are reported at p<0.05 and indicated by "*" whereas non significant differences are not marked.

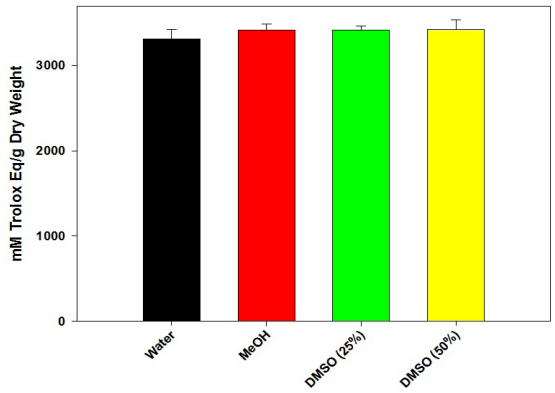

Figure 6: The effect of PE60 on nuclear morphology.

Note: U-87 brain cancer cells were incubated with 0, 10, 25, 50, 100, 150, 200, or 250µg/ml of PE60 water extract for 24 hours as described in the "Materials and Methods" section. After fixing, the cells were stained with Hoechst 33342 (10mg/ml) nuclear dye. The images were taken with 100X magnification using a Nikon Fluorescent Microscope.

Effect of PE60 on cell death through apoptosis

The effect of PE60 on programmed cell death (apoptosis) was determined by using caspase inhibitors (apoptosis inhibitors). One of the hallmarks of apoptosis is the blebbing of cellular nuclei, as examined by microscopy. The data shown in figure 6 indicate that upon increasing the concentration of water soluble PE60, the number of intact nuclei was reduced in a dose-dependent manner. The effect of PE60 on cell viability, as determined by MTT assay, in the presence of caspase inhibitors is shown in figure 7. The PE60 treatment at 100mg/ml caused a significant reduction in cell viability to 40% ($p<0.05$). However, PE60 treatment (100mg/ml) in the presence of a specific caspase-3 inhibitor (10µM) improved cell viability significantly to 55% ($p<0.05$), whereas a generic caspase inhibitor did not cause any significant improvement in PE60-induced reduction in cell viability (Figure 7).

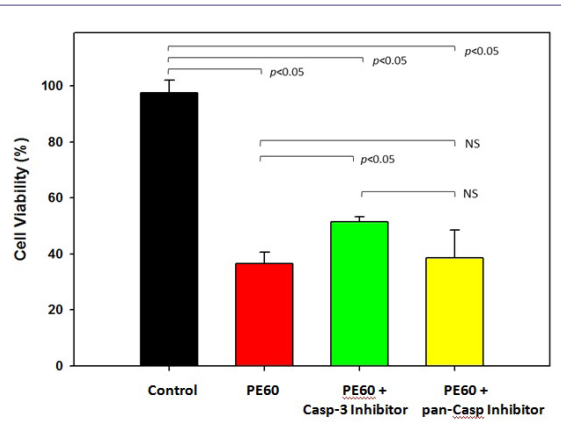

Figure 7: The effect of PE60 on cell viability in the presence of caspase inhibitors.

Note: Effect of a caspase-3 inhibitor (z-DEVD-FMK) and a generic caspase inhibitor (z-VAD-FMK) on water extract of PE60-induced inhibition of U-87 cells viability was determined using the MTT assay. Results are expressed as mean±SD for at least 3 replicates. All comparisons were made by one-way ANOVA with Tukey's HSD post-hoc test using SPSS statistic 20 software. A "P<0.05" represents a significant difference whereas "NS" indicates a non-significant difference between groups.

Discussion

Plant extracts are typically reconstituted in water or DMSO for cell culture studies. During our preliminary studies, we observed variable results when PE60 extract powder was reconstituted in water or DMSO, suggesting that different solvents may have dissolved different compounds from the extract. During this study, we therefore investigated the effect of PE60 extract dissolved in three different solvents. Water, 80% methanol, 25% DMSO and 50% DMSO were used to dissolve components from PE60 for the purpose of testing their effects on U-87 glioblastoma multiforme brain cancer cells.

Our data indicate that water as a solvent was more effective against brain cancer cells than methanol or DMSO. The solubility of polyphenol highly depends on the polarity of the solvent. These solvents were chosen because of their capabilities for dissolving compounds with variable polarity. Methanol is less polar than water because of a nonpolar carbon being attached to a hydroxyl group. The more polar alcohol has a shorter nonpolar carbon chain attached to a polar hydroxyl group. Therefore, dimethyl sulfoxide, DMSO, is less polar than methanol and the data indicate that water soluble polar polyphenols or other water soluble compounds may be more active than that of less-polar polyphenols dissolved in methanol or DMSO. The solvents commonly used in polyphenol extractions are water, ethanol, methanol, ethyl acetate, and acetone [30]. However, the most widely used solvents are ethanol and water because they are only mildly toxic and have relatively high polarities resulting in the highest yields [31]. The use of ethanol is restricted because of local regulatory laws. Other non-polar solvents such as acetone or chloroform were not used during the present investigation because of their toxic solvent residues, and the safety issues regarding worker exposure, disposal of waste, and environmental pollution.

When examining Total Polyphenol Content (TPC), we validated that the PE60 has 575-600mg/g and about 60% polyphenols, which is the same as what is reported by the manufacturer [32]. The water and DMSO solutions have a similar amount of TPC while the methanol solution has less than 60% polyphenols. It appears that some polyphenols may be less soluble in methanol than in water. Similar to TPC, we found that the methanol solution has less flavonoid than water or DMSO solutions. There is no difference in anti-oxidation activity between any of our PE60 preparations. In methanol, we found that there is less TPC and TFC but the anti-oxidation activity is similar to water and DMSO. It is possible that some flavonoids in the methanol solution may have been more active than in water or DMSO solutions as different polyphenols contain variable hydroxyl groups. For oxygen scavenging capacity, all preparations were in the same range. Flavonoids and polyphenols exert vital effects on the central nervous system by shielding neurons against stress-induced damage, suppressing neuroinflammation, and improving cerebral functions [33]. They potentially act as neuromodulators by protecting the brain from several pathological conditions that include neuro-degenerative disorders such as Alzheimer's and Parkinson's disease, diseases of the cardiovascular system, infections, brain cancer, and many others [34]. Conclusively, flavonoids and polyphenols provide anti-oxidant and pro-oxidant functions to the human body and have an extensive range of benefits, such as to destroy brain cancer and improve other cerebral disorders [35]. Polyphenols have various beneficial health effects such as antioxidant activities and may neutralize the destructive reactivity of undesired reactive oxygen/nitrogen species produced as a byproduct during metabolic processes in the body. These molecules can be found naturally in fruits, vegetables, cereals and beverages.

The water solution of PE60 was more effective for reducing cell viability of U-87 glioblastoma cells than that of methanol or DMSO solutions. This may be due to the fact that there are some specific components of polyphenols or flavonoids in water preparations that have better anticancer effects against brain cancer cells. It is also possible that water soluble PE60 solution may contain other polar anti-oxidants such as vitamin C, which is also known for its anti-oxidation and anti-cancer effects [36]. During our investigations, we did not identify the individual components of water, methanol, 25% and 50% DMSO preparations as it was beyond the scope of the present study. Future additional studies are required to identify the components in PE60 preparations using analytical approaches such as HPLC and/or HPLC-MS.

Many anticancer drugs cause cancer cell death due to induction of apoptosis which involves a cascade of caspase activation from proteolytic enzymes present in an inactive state as "procaspases" [37]. The initiator caspases (caspase-8 or caspase-9) cause the activation of executory caspase-3. We found that PE60 causes changes to nuclear morphology, and indicator of apoptosis resulting from caspase activity. In order to validate the role of PE60 in inducing apoptosis, we used caspase-3 inhibitor. We found that the PE60 treatment in the presence of a specific caspase-3 inhibitor improved cell viability significantly by 15% while the generic caspase inhibitor did not cause any significant improvement. The inhibition of cell death by caspase-3 inhibitor validated the involvement of caspase 3 activation and supports the theory of induction of apoptosis by PE60 in U-87 cells. Apoptosis is a vital biological process that aids organisms in disposing of unwanted cells from bodily development, homeostasis and disease [38]. Caspase-3 inhibitor is a protein under the Inhibitor of Apoptosis (IAP) family that suppresses apoptosis by providing a mechanism to control the unwanted death of cells and regulate cell division [39]. Conversely, caspase-3 is significant for cell death in crucial tissues and cell types, and is vital for several characteristic changes in the morphology of cells as well as various biochemical processes connected with apoptosis [40]. Caspase-3 is processed by autoproteolytic cleavage that assembles active heterotetrameric enzymes. Consequently, caspase-3 selectively acts on tissues, and this can be explained by two mechanisms: A shortage of the vital caspase-3 protease that supplements for caspase-3 at a crucial stage in the neural process of apoptosis or caspase-3 may position itself at the center of a vital neural death pathway. Therefore, caspase-3 brings about essential effects in organisms that include cell deaths and crucial nuclear and morphological processes that ensure the well-being of the life of organisms [41]. We found that caspase-3 inhibitor improved cell viability while generic caspase inhibitor had no effect, suggesting that PE60 induced its effects through apoptosis. Once caveat to this study is that the inhibitor amounts used were not at a high enough concentration to block the activation of all caspases. Further studies are required to determine the actual chemical nature of the PE60 solutions. The present study has identified that water extract of PE60 containing water soluble compounds including polyphenols may have the potential to inhibit U-87 glioblastoma brain cancer cells.

Authors' Contribution

Concept, design, and manuscript preparation, editing, and review: Rafat A Siddiqui.

Review of results, and preparation, editing, and review of the manuscript: Andrea R. Beyer and Jason Younkin.

Experimental studies, data acquisition, data analysis, statistical analysis: Haiwen Li and Ashwaq Alsufyani

Financial Support and Sponsorship

This work was supported by funds from the Evans-Allen Research program FY2017, the United States Department of Agriculture, and the Agricultural Research Station at, Virginia State University, Petersburg, Virginia. Financial support for Ashwaq Alsufyani's Master's studies was provided by Saudi Arabian Culture Mission, Kingdom of Saudi Arabia.

Conflicts of Interest

The authors have no conflicts of interest to declare.

References

1. World Health Organization (2018) Data visualization tools for exploring the global cancer burden in 2018. World Health Organization, Cancer Today, Geneva, Switzerland.
2. Davidson NE, Armstrong SA, Coussens LM, Cruz-Correa MR, DeBerardinis RJ, et al. (2016) AACR cancer progress report 2016. Clin Cancer Res 19: 1-137.
3. Brem SS, Bierman PJ, Black P, Blumenthal DT, Brem H, et al. (2005) Central nervous system cancers: Clinical practice guidelines in oncology. J Natl Compr Canc Netw 3: 644-690.
4. Etxeberria J, Román ES, Burgui R, Guevara M, Moreno-Iribas C, et al. (2015) Brain and central nervous system cancer incidence in Navarre (Spain), 1973-2008 and projections for 2014. J Cancer 6: 177-183.
5. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, et al. (2016) The 2016 World Health Organization classification of tumors of the central nervous system: A summary. Acta Neuropathol 131: 803-820.
6. Bondy ML, Scheurer ME, Malmer B, Barnholtz-Sloan JS, Davis FG, et al. (2008) Brain tumor epidemiology: Consensus from the Brain Tumor Epidemiology Consortium (BTEC). Cancer 113: 1953-1968.
7. Goodenberger ML, Jenkins RB (2012) Genetics of adult glioma. Cancer Genet 205: 613-621.
8. Swartling FJ, Hede SM, Weiss WA (2013) What underlies the diversity of brain tumors? Cancer Metastasis Rev 32: 5-24.
9. Mamelak AN, Jacoby DB (2007) Targeted delivery of antitumoral therapy to glioma and other malignancies with synthetic chlorotoxin (TM-601). Expert Opin Drug Deliv 4: 175-186.
10. Reilly KM (2009) Brain tumor susceptibility: The role of genetic factors and uses of mouse models to unravel risk. Brain Pathol 19: 121-131.
11. Lee EY, Muller WJ (2010) Oncogenes and tumor suppressor genes. Cold Spring Harb Perspect Biol 2: 3236.
12. Waris G, Ahsan H (2006) Reactive oxygen species: Role in the development of cancer and various chronic conditions. J Carcinog 5: 14.
13. Salim S (2017) Oxidative stress and the central nervous system. J Pharmacol Exp Ther 360: 201-205.
14. Rinaldi M, Caffo M, Minutoli L, Marini H, Abbritti RV, et al. (2016) ROS and brain gliomas: An overview of potential and innovative therapeutic strategies. Int J Mol Sci 17: 984.
15. Pandey KB, Rizvi SI (2009) Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev 2: 270-278.
16. Romagnolo DF, Selmin OI (2012) Flavonoids and cancer prevention: A review of the evidence. J Nutr Gerontol Geriatr 31: 206-238.
17. Wang H, Khor TO, Shu L, Su Z, Fuentes F, Lee JH, et al. (2012) Plants against cancer: A review on natural phytochemicals in preventing and treating cancers and their druggability. Anticancer Agents Med Chem 12: 1281-1305.
18. Alexandru O, Georgescu AM, Ene L, Purcaru SO, Serban F, et al. (2016) The effect of curcumin on low-passage glioblastoma cells *in vitro*. J Cancer Res Ther 12: 1025-1032.
19. Zhu Y, Zhuang JX, Wang Q, Zhang HY, Yang P (2013) Inhibitory effect of benzyl isothiocyanate on proliferation *in vitro* of human glioma cells. Asian Pacific J Cancer Prev 14: 2607-2610.
20. Jiao Y, Li H, Liu Y, Guo A, Xu X, et al. (2015) Resveratrol Inhibits the invasion of glioblastoma-initiating cells via down-regulation of the PI3K/Akt/NF-κB signaling pathway. Nutrients 7: 4383-4402.
21. Le CT, Leenders WPJ, Molenaar RJ, van Noorden CJF (2018) Effects of the green tea polyphenol epigallocatechin-3-gallate on glioma: A critical evaluation of the literature. Nutr Cancer 70: 317-333.
22. Stacewicz-Sapuntzakis M, Bowen PE, Hussain EA, Damayanti-Wood B, Farnsworth NR (2001) Chemical composition and potential health effects of prunes: A functional food? Crit Rev Food Sci Nutr 41: 251-286.
23. Chiu HF, Huang YC, Lu YY, Han YC, Shen YC, et al. (2017) Regulatory/modulatory effect of prune essence concentrate on intestinal function and blood lipids. Pharm Biol 55: 974-979.
24. Kim DO, Jeong SW, Lee CY (2003) Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem 81: 321-326.
25. Yu L, Haley S, Perret J, Harris M (2002) Antioxidant properties of extracts from hard winter wheat. Food Chem 78: 457-461.
26. Parry J, Su L, Moore J, Cheng Z, Luther M, et al. (2006) Chemical compositions, antioxidant capacities, and antiproliferative activities of selected fruit seed flours. J Agric Food Chem 54: 3773-3778.
27. Cheng Z, Moore J, Yu L (2006) High-throughput relative DPPH radical scavenging capacity assay. J Agric Food Chem 54: 7429-7436.
28. Llobera A, Cañellas J (2008) Antioxidant activity and dietary fiber of prensal blanc white grape (*Vitis vinifera*) by-products. International Journal of Food Science and Technology 43: 1953-1959.
29. Miller NJ. Rice-Evans CA (1997) Factors influencing the antioxidant activity determined by the ABTS.+ radical cation assay. Free Radic Res 26: 195-199.
30. Quideau SP, Deffieux D, Douat-Casassus CL Pouységu L (2011) Plant polyphenols: Chemical properties, biological activities, and synthesis. Angew Chem Int Ed Engl 50: 586-621.
31. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, et al. (2014) Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatic*. J Food Drug Anal 22: 296-302.
32. Neal CS, Simpson JM, Wheeler KB, Hertzler SR, Seeds J (2013) Pea protein containing nutritional compositions. International Publication Number (patent) WO 2013/148685 Al.
33. Schaffer S, Halliwell B (2012) Do polyphenols enter the brain and does it matter? Some theoretical and practical considerations. Genes Nutr 7: 99-109.
34. Williamson G, Manach C (2005) Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. Am J Clin Nutr 81: 243-255.
35. Spencer JP (2008) Flavonoids: Modulators of brain function? Br J Nutr 1: 60-77.

36. van der Reest J, Gottlieb E (2016) Anti-cancer effects of vitamin C revisited. Cell Res 26: 269-270.

37. Hassan M, Watari H, AbuAlmaaty A, Ohba Y, Sakuragi N (2014) Apoptosis and molecular targeting therapy in cancer. Biomed Res Int 2014: 150845.

38. Elmore S (2007) Apoptosis: A review of programmed cell death. Toxicol Pathol 35: 495-516.

39. Hashimoto T, Kikkawa U, Kamada S (2011) Contribution of caspase(s) to the cell cycle regulation at mitotic phase. PLoS One 6: 18449.

40. Shiri R, Yari F, Ahmadinejad M, Vaeli S, Tabatabaei MR (2014) The caspase-3 inhibitor (peptide Z-DEVD-FMK) affects the survival and function of platelets in platelet concentrate during storage. Blood Res 49: 49-53.

41. Bell RAV, Megeney LA (2017) Evolution of caspase-mediated cell death and differentiation: Twins separated at birth. Cell Death Differ 24: 1359-1368.

Journal of Anesthesia & Clinical Care

Journal of Addiction & Addictive Disorders

Advances in Microbiology Research

Advances in Industrial Biotechnology

Journal of Agronomy & Agricultural Science

Journal of AIDS Clinical Research & STDs

Journal of Alcoholism, Drug Abuse & Substance Dependence

Journal of Allergy Disorders & Therapy

Journal of Alternative, Complementary & Integrative Medicine

Journal of Alzheimer's & Neurodegenerative Diseases

Journal of Angiology & Vascular Surgery

Journal of Animal Research & Veterinary Science

Archives of Zoological Studies

Archives of Urology

Journal of Atmospheric & Earth-Sciences

Journal of Aquaculture & Fisheries

Journal of Biotech Research & Biochemistry

Journal of Brain & Neuroscience Research

Journal of Cancer Biology & Treatment

Journal of Cardiology & Neurocardiovascular Diseases

Journal of Cell Biology & Cell Metabolism

Journal of Clinical Dermatology & Therapy

Journal of Clinical Immunology & Immunotherapy

Journal of Clinical Studies & Medical Case Reports

Journal of Community Medicine & Public Health Care

Current Trends: Medical & Biological Engineering

Journal of Cytology & Tissue Biology

Journal of Dentistry: Oral Health & Cosmesis

Journal of Diabetes & Metabolic Disorders

Journal of Dairy Research & Technology

Journal of Emergency Medicine Trauma & Surgical Care

Journal of Environmental Science: Current Research

Journal of Food Science & Nutrition

Journal of Forensic, Legal & Investigative Sciences

Journal of Gastroenterology & Hepatology Research

Journal of Gerontology & Geriatric Medicine

Journal of Genetics & Genomic Sciences

Journal of Hematology, Blood Transfusion & Disorders

Journal of Human Endocrinology

Journal of Hospice & Palliative Medical Care

Journal of Internal Medicine & Primary Healthcare

Journal of Infectious & Non Infectious Diseases

Journal of Light & Laser: Current Trends

Journal of Modern Chemical Sciences

Journal of Medicine: Study & Research

Journal of Nanotechnology: Nanomedicine & Nanobiotechnology

Journal of Neonatology & Clinical Pediatrics

Journal of Nephrology & Renal Therapy

Journal of Non Invasive Vascular Investigation

Journal of Nuclear Medicine, Radiology & Radiation Therapy

Journal of Obesity & Weight Loss

Journal of Orthopedic Research & Physiotherapy

Journal of Otolaryngology, Head & Neck Surgery

Journal of Protein Research & Bioinformatics

Journal of Pathology Clinical & Medical Research

Journal of Pharmacology, Pharmaceutics & Pharmacovigilance

Journal of Physical Medicine, Rehabilitation & Disabilities

Journal of Plant Science: Current Research

Journal of Psychiatry, Depression & Anxiety

Journal of Pulmonary Medicine & Respiratory Research

Journal of Practical & Professional Nursing

Journal of Reproductive Medicine, Gynaecology & Obstetrics

Journal of Stem Cells Research, Development & Therapy

Journal of Surgery: Current Trends & Innovations

Journal of Toxicology: Current Research

Journal of Translational Science and Research

Trends in Anatomy & Physiology

Journal of Vaccines Research & Vaccination

Journal of Virology & Antivirals

Herald Scholarly Open Access, 2561 Cornelia Rd, #205, Herndon, VA 20171, USA.
Tel: +1-646-661-6626; E-mail: info@heraldsopenaccess.us
http://www.heraldopenaccess.us/