Pyrogallol Induces Selective Cytotoxicity in SH-SY5Y and Cortical Neuron Cells

Betul Cicek[1], Ali Taghizadehghalehjoughi[2], Murat Senturk[3], Esra Senturk[4], Mustafa Gul[5], Fatma Yesilyurt[6], Ahmet Hacimuftuoglu[6,*]

[1]Department of Physiology, Faculty of Medicine, Erzincan Binali Yidirim University, 24100 Erzincan, Türkiye
[2] Department of Medical Pharmacology, Bilecik Şeyh Edebali University Faculty of Medicine, 11230 Bilecik, Türkiye
[3]Department of Biochemistry, Agrı Ibrahim Cecen University Faculty of Pharmacy, 04100 Agrı, Türkiye
[4]Department of Physiology, Faculty of Medicine, Agrı Ibrahim Cecen University, 04100Agrı, Türkiye
[5]Department of Physiology, Atatürk University Faculty of Medicine, 25240 Erzurum, Türkiye,
[6]Department of Pharmacology, Atatürk University Faculty of Medicine, 25240 Erzurum, Türkiye,

Corresponding author: Betul Cicek, Department of Physiology, Faculty of Medicine, Erzincan Binali Yidirim University, 24100 Erzincan, Türkiye. Phone: +904462261824, Fax: +904462261819, e-mail: betulll.cicekk@gmail.com

Received: March 6, 2023 **Accepted:** April 28, 2023 **Published:** April 30, 2023
To cite this article: Cicek B, Taghizadehghalehjoughi A, Senturk M, Senturk E, Gul M, Yesilyurt F, Hacimuftuoglu A. (2023). Pyrogallol Induces Selective Cytotoxicity in SH-SY5Y and Cortical Neuron Cells. Recent Trends in Pharmacology, vol 1, issue 1: 1-10.

Abstract

Objective: We aimed to evaluate whether pyrogallol (PG) has selective cytotoxic effects on neuroblastoma (SH-SY5Y) cells and cortical neuronal cells. **Materials and Methods:** SH-SY5Y cells and cortical neuronal cells were treated with PG at different concentrations for 24 h. Cytotoxicity was assessed by MTT test. Catalase (CAT), glutathione reductase (GR) activities and glutathione (GSH) level were also quantified. **Results:** Treatment of neuroblastoma cells with PG (20–200 μM) significantly decreased cell viability. It had no effect on cell viability of cortical neuronal cells at 20-80 μM. However, it decreased the viability in cortical neuronal cells at 200 μM. CAT, GR, and GSH levels were notably reduced in neuroblastoma cells treated with 200 μM of PG; but, only GSH was reduced in cortical neuronal cells at this concentration. **Conclusion:** PG had a specific cytotoxic effect in neuroblastoma cells but generally spared neuronal cells. PG decreased the antioxidant enzyme activities in neuroblastoma cells suggesting that it kills cancer cells by causing increased oxidative stress. However, having a cytotoxic effect in lower concentrations of PG without decreasing antioxidant enzyme activities suggests that PG may have alternative mechanisms for cytotoxicity in lower concentrations.

Keywords: Polyphenolic compound, pyrogallol, SH-SY5Y, cortical neuron, antioxidant enzymes

Introduction

Neuroblastoma is a malignant tumor that is commonly seen in early childhood and originates from the sympathetic nervous system (Bhoopathi, Mannangatti, Emdad, Das, & Fisher, 2021). It is a dense tumour located in the body except cranium, which is common in pediatric patients under 5 years of age with a bad prognosis (Brodeur, 2018; Qureshi et al., 2018). Neuroblastoma is a disease with very heterogeneous features, from spontaneous regression to recalcitrant tumor development (Brodeur, 2018). Although various treatment modalities, including surgery, radiotherapy, and chemotherapy, have been used; it was realized that they are generally ineffective due to the lack of selectivity, non-specific toxicities, and decreased post-recurrence survival (Zafar et al., 2021). For this reason, it is necessary to explore the effect of novel candidate drugs against neuroblastoma.

Cancer cells are hallmarked by producing excessive free radicals compared with healthy cells (Moloney & Cotter, 2018).

Moderate free radical formation is involved in physiological events such as cellular proliferation and differentiation (Bardaweel et al., 2018). However, elevated rates of free radicals production are considered toxic for cancer cells and this could potentiate the impact of agents, which elicit anti-tumourigenic (Hayes, Dinkova-Kostova, & Tew, 2020). Therefore, manipulation of cellular free radical levels could be a treatment approach to get selective toxicity against cancer cells (Schumacker, 2006).

Many plant-derived compounds have been used in cancer chemoprevention. Recently, many studies have focused on exploring natural compounds with potential anticancer activity (Atanasov, Zotchev, Dirsch, & Supuran, 2021). Natural polyphenolic compounds (NPCs) are found in abundant concentrations in fruits, vegetables, and plant-based beverages (Shahidi & Ambigaipalan, 2015). As anticancer agents, NPCs can block certain stages of carcinogenic processes, prevent cell growth, and/or induce apoptosis in tumour cell (Briguglio et al., 2020). Although anticancer effects of NPCs have been associated with several mechanisms; the most pivotal one is increased production of free radicals (Sun et al., 2019).

Pyrogallol (1,2,3-trihydroxybenzene; PG), a NPC, is known as a superoxide anion (O^{-}_{2}) generator and widely used in evaluating the role of oxidative stress in biological systems(Koo, Lee, Chung, Ko, & Kim, 2004; Yang et al., 2009). PG generates free radicals owing to its auto oxidation property, which lead to the formation of hydrogen peroxide (Siegel & Siegel, 1958). It was demonstrated that PG could stimulate O^{-}_{2} intervened death of various cancer cells; such as lung cancer, human glioma, and hepatocellular carcinoma cells (Ahn et al., 2019; Yang et al., 2009).

It was demonstrated that PG could stimulate O^{-}_{2} intervened death of various cancer cells; such as lung cancer, human glioma, and hepatocellular carcinoma cells. In this context, the precise role of PG in neuroblastoma and cortical neurons and whether it has a selective cytotoxicity in cancer cells are still obscure. Hence, the present research

was planned to evaluate the possible cytotoxicity and the underlying mechanism of action of PG on human neuroblastoma cells.

Materials and methods

Cell culture and drug application

Human neuroblastoma (SH-SY5Y) cell line was purchased from ATCC (Wesel, Germany). Cells were cultured at 37 °C under 5% CO_2 atmosphere in DMEM media containing 10% fetal bovine serum (FBS) and 100 units/mL penicillin and 100 µg/mL streptomycin. Frozen rat cortical neuronal cells were received from the Pharmacology and Toxicology Department of Veterinary Medicine, Faculty of Ataturk University (Erzurum, Türkiye). Neuronal cells were resuspended in neurobasal medium containing B27, 10% FBS and 0.1% antibiotic (penicillin–streptomycin-amphotericin B). Incubation conditions were 37°C in 5% CO_2 humidified air. Human neuroblastoma cells and neuron cultures were exposed to different concentrations (20, 40, 80 and 200 µM) of PG (Sigma, Taufkirchen, Germany) dissolved in medium up-to 24 h. All experiments were conducted with permission from the Ataturk University Ethics Committee of Experimental Animals (Document number E-42190979-000-E.2200123448).

Determination of cytotoxicity

Cytotoxicity was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye reduction assay. Neuronal and neuroblastoma cells were seeded in a flat-bottomed 96-well plate at a density of 2×10^5 and 5×10^3 cells/cm^2 respectively and then applied with PG as at the concentrations mentioned for 24h. Cells treated with MTT reagent were incubated in a humidified chamber for 4 hours. Then the medium was taken out, and the 100 µl DMSO was added to dissolve crystals. Absorbance (A) at 570 nm was assigned using Multiskan™ GO Microplate Spectrophotometer reader (Thermo Scientific, Canada, USA). The cell viability (%) was calculated by the formula below;(Ferah Okkay et al., 2021)

Viability (%) = A of experiment well/A of control well × 100

Determination of enzyme activities

In this part, cells were added into cell culture petri dish and incubated for 24h. Then, the cells were applied with different PG concentration and incubated nightlong. Following the incubation period, cells bathed with PBS were applied with 400 mL of 1× lysis buffer that contains 1 mM phenylmethanesulfonyl fluoride (PMSF) and kept on ice for 5 min. After that, cells were thrown out, sonicated shortly, and centrifuged at 14,000 g for 10 min in a cold microfuge. Cell supernatants obtained were kept at -80 C to determine enzyme activities later. CAT activity was measured by following the decomposition rate of the substrate H_2O_2 at 240 nm. CAT enzyme activity was given as µmol of H_2O_2 conversion per minute (Habig, Pabst, & Jakoby, 1974). GR activity was measured by determining NADPH oxidation at 340 nm spectrophotometrically. The activity of the enzyme was assigned by the time-dependent

change in NADPH after the supplement of the sample. One unit of GR activity was stated as µmol of NADPH consumed per minute (Wheeler, Salzman, Elsayed, Omaye, & Korte Jr, 1990). GSH activity was measured the rate of of 1-chloro-2,4-dinitrobenzene (CDNB) conjugation at 340 nm (Wendel, 1981). Quantitative protein determination was performed according to the Bradford method. (Bradford, 1976) Bovine serum albumin (BSA) was used as the standard.

Statistical analysis

The data are given as the mean ± SEM. Statistical differences were evaluated using t-test, one-way ANOVA, and Tukey's HSD using the SPSS 22.0 software. P less than 0.05 was accepted significant.

Results

Viability test results

The survival rates of neuronal cells and SH-SY5Y cells after exposure to 24-h PG were calculated using MTT assay test. The cell number decreased during 24 h incubation and was negatively affected by PG treatment dose-dependently in SH-SY5Y cells. Compared with control cells, treatment with 20, 40, 80, and 200 µM/mL PG for 24 h induced a significant ($P < 0.01$) decrease in cell viability by 51.9%, 48.8%, 47.5%, and 39.2%, respectively (Figure 1).

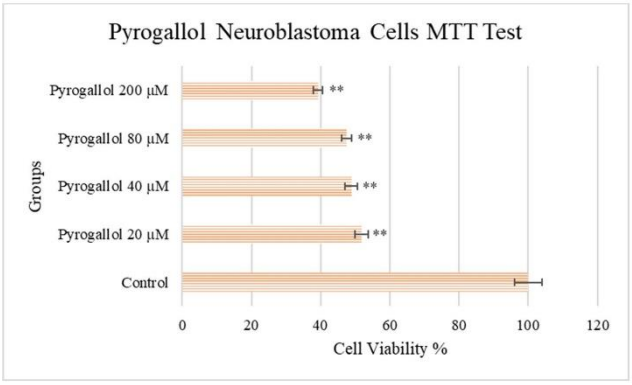

Figure 1. The effect of Pyrogallol on neuroblastoma cell viability after 24 h incubation. (Results are stated as mean ± SEM, *vs. control (untreated) cells, $P < 0.05$; **vs. control cells, $P < 0.001$.

As shown in Figure 2, treatment with 200 µM PG significantly decreased the cellular viability of neuronal cells compared with the control cells. Treatment with 200 µM PG noticeably ($P<0.05$) reduced the cell viability by 84.3%, compared to control. It has to be noted that the decreases in cell viability at concentrationsof 20, 40, and 80 µM PG were not statistically meaningful in comparison to control cells ($P > 0.05$).

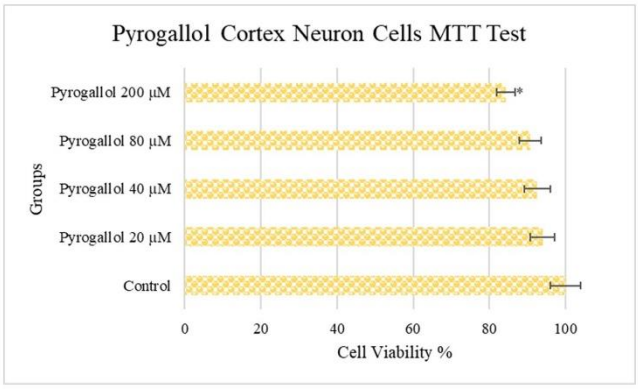

Figure 2. Effect of Pyrogallol on cortical neuronal cell viability after 24 h incubation. (Results are stated as mean ± SEM, *vs. Control cells, $P < 0.05$, **vs. Control cells, $P < 0.001$.

Biochemical measurements

Effects of PG on CAT, GR, and GSH activities in SH-SY5Y cells

In SH-SY5Y cell line, PG (200 μM) decreased CAT, GR, and GSH enzyme activities compared to controls, 20, 40, and 80 μM concentration of PG GR activity was decreased in 80 μM PG group compared to control and group treated with 40 μM PG. However, group treated with 80 μM PG had a significant decrease in GR activity compared to group treated with 200 μM PG (Table 1).

Table 1. Effects of PG treatment on CAT, GR, and GSH enzyme levels in neuroblastoma cells.

Pyrogallol (μM)	CAT (μmol/min/ mg protein)	GR (nmol/min /mg protein)	GSH (nmol/min /mg protein)
0	6.4 ± 0.6	21.4 ± 0.8	17.8 ± 0.9
20	6.1 ± 0.5	21.1 ± 0.7	18.1 ± 1.1
40	5.7 ± 0.4	20.7 ± 0.7	16.7 ± 0.9
80	5.3 ± 0.4	19.6 ± 0.6$^{\sharp}$	16.3 ± 0.9
200	3.8 ± 0.2*	15.1 ± 0.5*	12.8 ± 0.6*

*: 200 μM *vs*. 20, 40, and 80 μM; $^{\sharp}$: 80 μM *vs*. control, 40 and 200 μM (The data are given as the mean ± SEM. Data were analyzed using t-test, one-way ANOVA, and Tukey's HSD. $P<0.05$ was accepted as the level of statistical significance. CAT:catalase; GR:glutathione reductase; GSH:glutathione.

Effects of PG on CAT, GR, and GSH activities in cortical neurons

PG had no effect on CAT and GR activities, however, it significantly reduced GSH activity at a concentration of 200 μM PG compared to 20, 40 and 80 μM in cortical neurons (Table 2).

Table 2. Effects of PG treatment on the levels of CAT, GR, and GSH expressed in cortical neuronal cells.

Pyrogallol (μM)	CAT (μmol/min/ mg protein)	GR (nmol/min/mg protein)	GSH (nmol/min/mg protein)
0	7.3 ± 0.8	23.8 ± 1.2	19.2 ± 1.1
20	7.1 ± 0.6	23.4 ± 0.9	19.1 ± 0.8
40	7.2 ± 0.6	22.9 ± 0.9	19.2 ± 0.7
80	7.0 ± 0.5	22.5 ± 0.8	18.7 ± 0.9
200	6.4 ± 0.4	20.1 ± 0.4	17.8 ± 0.7*

*: 200 μM vs. 20, 40 and 80 μM (The data are given as the mean ± SEM. Data were analyzed using t-test, one-way ANOVA, and Tukey's HSD. $P<0.05$ was accepted as the level of statistical significance. CAT:catalase; GR:glutathione reductase; GSH:glutathione.

Discussion

Numerous anticancer drugs fail to selectively kill cancer cells; they damage normal cells as well cause various side effects (Blagosklonny, 2005). Limited amount of free radicals has some functions in normal cells, i.e. moderate levels of free radicals take part in the activation of transcription factor, gene expression, differentiation and proliferation in cells (Schieber & Chandel, 2014). On the other hand, cancer cells are more susceptible to free radical-modulating drugs that enhancement of free radical generation above the threshold level of redox homeostasis in cancer cells leads to cell death (Briguglio et al., 2020). Throughout the literature, a variety of

compounds were found to be selective against cancer cells without killing normal cells (Briguglio et al., 2020; Tang et al., 2018).

The effects of PG on neuroblastoma and rat cortical neuronal cells were evaluated in this study. The data indicated that application of SH-SY5Y cells with PG resulted in a considerable decrease in cell viability, whereas treatment of cortical neuronal cells with PG did not affect the cell viability significantly at 20, 40, and 80 μM. Treatment with PG (20-200 μM) significantly reduced cell viability in neuroblastoma cells (Table 1). While lower doses of PG (20, 40, and 80 μM) slightly decreased the cell viability, high dose (200 μM) significantly reduced cell viability in cortical neurons. It has been reported that PG decreased cell viability in several types of cells, such as human lymphoma cells (Saeki, Hayakawa, Isemura, & Miyase, 2000), human glioma cells (Sawada et al., 2001), and Calu-6 lung cancer cells (Han, Kim, Kim, & Park, 2008). In the meantime cytotoxic effects of PG on cancer cell line while not affecting normal cells, is in line with the findings of Yang et al (Yang et al., 2009), who showed that PG had cytotoxic effects on human lung cancer cell lines and a little impact on normal human bronchial epithelium cell line (Yang et al., 2009). These results demonstrate that the specificity of cytotoxicity not only associated with different cell types but also PG dose.

Free radicals and oxidative stress have long been related to cancer development. Increasing free radical generation is a common mechanism of most anticancer treatment strategies, such as chemotherapy and radiotherapy (Moloney & Cotter, 2018; Schumacker, 2006). Cancer cells are probably more susceptible to damage by free radical-modulating drugs that augment free radical levels over the threshold of redox homeostasis (Briguglio et al., 2020). Additionally, boosting free radical production and inhibiting cellular antioxidant enzymes would further make the cells more susceptible to cell death in tumors. GSH dependent antioxidant defense system and superoxide dismutase are the important targets of free radicals elevating anticancer agents (Schumacker, 2006; Trachootham et al., 2006). Findings from different studies have demonstrated that inhibition of cellular antioxidant defense system stimulate free radical -mediated cytotoxicity in various types of tumors (Glasauer & Chandel, 2014; Sun et al., 2019). Several chemotherapeutic agents including taxanes and alkaloids interrupt the electron transport chain in mitochondria leading to elevated O_2^-(Glasauer & Chandel, 2014). It has been known that PG produces O_2^- and stimulates death in various cancer cells (Kim et al., 2008; W. H. Park, Y. H. Han, S. H. Kim, & S. Z. Kim, 2007a; W. H. Park, Y. W. Han, S. H. Kim, & S. Z. Kim, 2007b). In this study, 200 μM PG significantly decreased the CAT, GR, and GSH enzyme activities in neuroblastoma cells (Table 1). However, PG in all concentrations did not alter the antioxidant enzyme activitiesin neuronal cells. These data suggest that the effects of PG on the activities of antioxidant enzymes can change according to the

cell types and concentrations. In line with these results, Park et al.(Park et al., 2007a) have shown that PG significantly increased O_2^- level and decreased the intracellular GSH level in As4.1 cells dose-dependently; the apoptotic effects of PG correspond well to intracellular O_2^- level. Additionally, induction of apoptosis and lowering the level of glutathione by an anticancer agent can lead to death of cancer cells (Park et al., 2007a). The study of Han et al.(Han et al., 2008) reported that exposure of Calu-6 cells to PG results in increasing O_2^- level and induces apoptosis. Adding catalase recovered Calu-6 cells from apoptosis due to PG exposure and also avoid the growth arrest by PG that was associated with downregulation of O_2- level (Han et al., 2008).

The findings of this study have some limitations. Our perception is based on the premise that PG can exert a cytotoxic effect on cancer cells while protecting normal neuronal cells. Decrease in the antioxidant enzyme activities in neuroblastoma cells with high concentration of PG suggests that it kills cancer cells by causing increased oxidative stress. However, having cytotoxic effect in lower concentrations without decreasing antioxidant enzyme activities implies that PG might have alternative mechanisms for cytotoxicity. Further studies are needed to clarify other mechanisms or signaling pathways that are activated by PG in neuroblastoma cells.

In conclusion, we have demonstrated that PG has selective cytotoxicity on neuroblastoma cells without affecting cortical neuronal cells much. As a result, PG has a specific cytotoxic effect in tumour cells but spared cortical neurons. The activities of CAT, GR, and GSH enzymes did not change after PG treatment in cortical neuronal cells, however, high concentration of PG (200 μM) significantly decreased the antioxidant enzyme levels in neuroblastoma cells. Because no information was available about the effects of PG on neuroblastoma cancer type, we anticipated that the findings of this study may offer new, potentially useful information for other studies.

Author Contributions: Concept – A.H., B.C.; Design-A.H., A.T., B.C.; Supervision-A.H., M.G., Resources –M.S.; Data Collection and/or Processing-E.S., F.Y.; Analysis and/or Interpretation-A.H., M,G., Literature Search-B.C.; Writing Manuscript-B.C., M.G.; Critical Review – B.C.,,M.K.

Conflict of Interest: The author(s) have declared no conflict of interest.

Financial Disclosure: The author(s) did not receive any financial support for this study.

References

Ahn, H., Im, E., Lee, D. Y., Lee, H.-J., Jung, J. H., & Kim, S.-H. (2019). Antitumor effect of Pyrogallol via miR-134 mediated S phase arrest and inhibition of PI3K/AKT/Skp2/cMyc signaling in hepatocellular carcinoma. *International journal of molecular sciences, 20*(16), 3985.

Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., & Supuran, C. T. (2021). Natural products in drug discovery: Advances and opportunities. *Nature Reviews Drug Discovery*, *20*(3), 200-216.

Bardaweel, S. K., Gul, M., Alzweiri, M., Ishaqat, A., ALSalamat, H. A., & Bashatwah, R. M. (2018). Reactive oxygen species: The dual role in physiological and pathological conditions of the human body. *The Eurasian journal of medicine*, *50*(3), 193.

Bhoopathi, P., Mannangatti, P., Emdad, L., Das, S. K., & Fisher, P. B. (2021). The quest to develop an effective therapy for neuroblastoma. *Journal of Cellular Physiology*, *236*(11), 7775-7791.

Blagosklonny, M. (2005). Carcinogenesis, cancer therapy and chemoprevention. *Cell Death & Differentiation*, *12*(6), 592-602.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, *72*(1-2), 248-254.

Briguglio, G., Costa, C., Pollicino, M., Giambò, F., Catania, S., & Fenga, C. (2020). Polyphenols in cancer prevention: New insights. *International Journal of Functional Nutrition*, *1*(2), 1-1.

Brodeur, G. M. (2018). Spontaneous regression of neuroblastoma. *Cell and tissue research*, *372*(2), 277-286.

Ferah Okkay, I., Okkay, U., Cicek, B., Yilmaz, A., Yesilyurt, F., Mendil, A. S., & Hacimuftuoglu, A. (2021). Neuroprotective effect of bromelain in 6-hydroxydopamine induced in vitro model of Parkinson's disease. *Molecular Biology Reports*, *48*(12), 7711-7717.

Glasauer, A., & Chandel, N. S. (2014). Targeting antioxidants for cancer therapy. *Biochemical pharmacology*, *92*(1), 90-101.

Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, *249*(22), 7130-7139.

Han, Y. H., Kim, S. Z., Kim, S. H., & Park, W. H. (2008). Pyrogallol inhibits the growth of human lung cancer Calu-6 cells via arresting the cell cycle arrest. *Toxicology in Vitro*, *22*(6), 1605-1609.

Hayes, J. D., Dinkova-Kostova, A. T., & Tew, K. D. (2020). Oxidative stress in cancer. *Cancer cell*.

Kim, S. W., Han, Y. W., Lee, S. T., Jeong, H. J., Kim, S. H., Kim, I. H., Kim, S. Z. (2008). A superoxide anion generator, pyrogallol, inhibits the growth of HeLa cells via cell cycle arrest and apoptosis. *Molecular Carcinogenesis: Published in cooperation with the University of Texas MD Anderson Cancer Center*, *47*(2), 114-125.

Koo, B.-S., Lee, W.-C., Chung, K.-H., Ko, J.-H., & Kim, C.-H. (2004). A water extract of

Curcuma longa L.(Zingiberaceae) rescues PC12 cell death caused by pyrogallol or hypoxia/reoxygenation and attenuates hydrogen peroxide induced injury in PC12 cells. *Life sciences, 75*(19), 2363-2375.

Moloney, J. N., & Cotter, T. G. (2018). *ROS signalling in the biology of cancer.* Paper presented at the Seminars in cell & developmental biology.

Park, W. H., Han, Y. H., Kim, S. H., & Kim, S. Z. (2007a). Pyrogallol, ROS generator inhibits As4. 1 juxtaglomerular cells via cell cycle arrest of G2 phase and apoptosis. *Toxicology, 235*(1-2), 130-139.

Park, W. H., Han, Y. W., Kim, S. H., & Kim, S. Z. (2007b). A superoxide anion generator, pyrogallol induces apoptosis in As4. 1 cells through the depletion of intracellular GSH content. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 619*(1-2), 81-92.

Qureshi, S. S., Bhagat, M. G., Kembhavi, S. A., Chinnaswamy, G., Vora, T., Prasad, M., Shah, S. (2018). A cross-sectional study of the distribution of pediatric solid tumors at an Indian tertiary cancer center. *Indian journal of cancer, 55*(1), 55.

Saeki, K., Hayakawa, S., Isemura, M., & Miyase, T. (2000). Importance of a pyrogallol-type structure in catechin compounds for apoptosis-inducing activity. *Phytochemistry, 53*(3), 391-394.

Sawada, M., Nakashima, S., Kiyono, T., Nakagawa, M., Yamada, J., Yamakawa, H., Nozawa, Y. (2001). p53 regulates ceramide formation by neutral sphingomyelinase through reactive oxygen species in human glioma cells. *Oncogene, 20*(11), 1368-1378.

Schieber, M., & Chandel, N. S. (2014). ROS function in redox signaling and oxidative stress. *Current biology, 24*(10), R453-R462.

Schumacker, P. T. (2006). Reactive oxygen species in cancer cells: live by the sword, die by the sword. *Cancer cell, 10*(3), 175-176.

Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. *Journal of functional foods, 18*, 820-897.

Siegel, S., & Siegel, B. (1958). Autoxidation of pyrogallol: general characteristics and inhibition by catalase. *Nature, 181*(4616), 1153-1154.

Sun, L.-r., Zhou, W., Zhang, H.-m., Guo, Q.-s., Yang, W., Li, B.-j.,Cui, R.-j. (2019). Modulation of multiple signaling pathways of the plant-derived natural products in cancer. *Frontiers in oncology, 9*, 1153.

Tang, J. Y., Huang, H. W., Wang, H. R., Chan, Y. C., Haung, J. W., Shu, C. W., Chang, H. W. (2018). 4β-Hydroxywithanolide E selectively induces oxidative DNA

damage for selective killing of oral cancer cells. *Environmental toxicology*, *33*(3), 295-304.

Trachootham, D., Zhou, Y., Zhang, H., Demizu, Y., Chen, Z., Pelicano, H., Liu, J. (2006). Selective killing of oncogenically transformed cells through a ROS-mediated mechanism by β-phenylethyl isothiocyanate. *Cancer cell*, *10*(3), 241-252.

Wendel, A. (1981). [44] Glutathione peroxidase. In *Methods in enzymology* (Vol. 77, pp. 325-333): Elsevier.

Wheeler, C. R., Salzman, J. A., Elsayed, N. M., Omaye, S. T., & Korte Jr, D. W. (1990). Automated assays for superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activity. *Analytical biochemistry*, *184*(2), 193-199.

Yang, C.-J., Wang, C.-S., Hung, J.-Y., Huang, H.-W., Chia, Y.-C., Wang, P.-H., Huang, M.-S. (2009). Pyrogallol induces G2-M arrest in human lung cancer cells and inhibits tumor growth in an animal model. *Lung Cancer*, *66*(2), 162-168.

Zafar, A., Wang, W., Liu, G., Wang, X., Xian, W., McKeon, F., Zhang, R. (2021). Molecular targeting therapies for neuroblastoma: Progress and challenges. *Medicinal Research Reviews*, *41*(2), 961-1021.