The Effect of Flavonoid Galangin on the Cell Viability and Toxicity of MCF-7 Human Breast Cancer Cells Exposed to Polycyclic Aromatic Hydrocarbons (PAHs)

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Polycyclic aromatic hydrocarbons (PAHs) are chemical compounds ubiquitous in the environment; many of which are known human and animal carcinogen. The purpose of this study was to investigate MCF-7 epithelial breast cancer cells' viability and toxicity following a short-term in vitro exposure to a cocktail of PAHs and benzo[a]pyrene (B[a]P) separately in the presence and absence of varying concentrations of flavonoid galangin. It was predicted that presence of galangin will completely or partially block the damaging effects of PAHs. It was hypothesized that exposing the cells to 7 µM cocktail of PAHs or 10 µM B[a]P in the presence of 30 µM galangin would reduce the toxicity and increase the mitochondrial activity of the cells as compared to that in the absence of galangin. Experimentation was accomplished by exposing identical samples containing 75,000 MCF-7 cells each for 24 h to exposure medium containing 10 µM B[a]P alone, 7 µM cocktail of PAHs alone, various doses (5 µM, 30 µM, 100 µM) of galangin alone, mixture of 10 µM B[a]P and the various doses of galangin, and mixture of 7 µM cocktail of PAHs and the various doses of galangin. Viability of the sample cells were measured by MTT assays, whereas their toxicity were measured by LDH assays done on spent exposure media. The results are as follows, at significance level (α) = .05, 7 µM cocktail of PAHs in the presence of 100µM galangin was found to be highly toxic to the cells after 24 h of exposure. At α = .05, 7 µM cocktail of PAHs, 7 µM cocktail of PAHs concomitant with 5, 30 or 100 µM of galangin, 10 µM B[a]P concomitant with 100 µM of galangin, and 100 µM of galangin alone considerably reduced the viability of the cells.

Introduction

In today's world, with large share of the global economy centering on industries, manufacturing plants, and transportation, it is no surprise organic pollutants are pervasive in the environment. Among the pollutants are a dominant group of organic compounds composed of at least two fused Benzene rings, called polycyclic aromatic hydrocarbons (PAHs). Figure 1 displays sample representations of PAHs (A. Maigari & M. Maigari, 2015). They are formed mostly either from incomplete combustion of organic matter or high temperature and pressure processes, and are common byproducts of combustion processes as well (Menzie, 1992). Figure 2 provides the overall summary of the sources of PAHs in the environment (Abdel-Shafya & Mansour, 2016).

Of the at least 100 known PAHs (Zedeck, 1980), Benzo[a]pyrene (B[a]P), a five-ring PAH, is the most analyzed and has been classified as a probable carcinogen (see Figure 3). It is found in fossil fuels, crude oils, shale oils, and coal tars. The major sources of non-occupational exposure to B[a]P include tobacco products and grilled foods (HSDB, 2012). An average person in North America is estimated to intake 3.12mg/day – 96.2% by food, 1.6% air, 0.2% water, and soil 0.4% (Menzie, 1992).

PAHs usually penetrate tissues of your body that contain fat and tend to be stored mostly in your kidneys, liver, and mammary glands. PAHs are primarily metabolized by the enzymatic systems found in the tissues into many different metabolites, some of which are more harmful. Based on a study done on mice by Uno et al. in 2006, it was shown that B[a]P and other PAHs stimulate enzymes that biologically transform the former into carcinogenic and mutagenic metabolites which possibly can bind to DNA and proteins in humans. According to Agency for Toxic Substances and Disease Registry (ATSDR), several of the PAHs outlined in Figure 3 have caused tumors in laboratory animals from inhalation, consumption, and prolonged dermal exposure. Induction of cancer in the laboratory animals usually occurred at the site of administration of PAHs, although tumors can form at other locations as well. Cancer in humans from exposure to PAHs has generally occurred in lungs and skin following prolonged inhalation and dermal exposure, respectively. Studies have shown that pregnant mice exposed to high levels of B[a]P see decline in their reproductive capabilities, and their offspring show birth defects and decreased body weight (ATSDR, 1995).

Bioflavonoids are naturally occurring polyphenolic compounds found in fruits, vegetables, tea, and wine. Galangin (see figure 4), a member of the flavonol class of bioflavonoids , is known to be present in high concentrations in medicinal plants like alpinia officinarum (a common Asian spice) and helichrysum aureonitens (found in grasslands of South Africa). A gram of galangin is found in about 13.5mg of propolis, an anti-inflammatory composite gum produced by honeybees (Quadri et al., 2000). Galangin shows various pharmacological activities such as anti-mutagenic, anti-clastogenic, anti-oxidative, radical scavenging, metabolic enzyme modulating, and anti-cancer activity (Heo et al., 2001; Cushnie et al., 2007; Patel et al., 2012). They have also been shown to possess a variety of biological activities at non-toxic concentrations in organisms (Murray et al., 2006). Galangin is one of the active polyphenols found in some varieties of honey like Manuka (Patel & Cichello, 2013), which studies have reported as promising remedy in the treatment of various ailments like cardiovascular diseases, inflammation, and bacterial infection (Subramanian et al., 2017).

In pre-clinical drug screening process, potential beneficial substances are usually tested against mammalian cell lines to assess any cytotoxic effects the compounds exert on the body's own cells (Barnabe, 2017). A substance is said to be cytotoxic if it interferes with a cell's ability to attach, affects its rate of growth or causes it to die (Bacanli et al., 2017). The cytotoxic effects that a cell culture experiences are commonly expressed through cytotoxicity measures obtained through assays, which allow for the quantitative measurement of cell death during cell culture. Two commonly used assays for determining cytotoxicity in vitro are MTT assay and LDH assay, and they express the cytotoxicity in terms of cellular viability (through mitochondrial absorbance) and cellular toxicity (through units of cellular LDH enzyme expelled), respectively. Higher the cytotoxicity of the foreign compound, higher are the units of LDH enzyme expelled by the cells and lower are their mitochondrial absorbance. The rationale for measuring direct cytotoxicity is that mechanisms relating to cytotoxicity share some correlation with mechanisms relating to mutagenicity, carcinogenicity, and genotoxicity (Johnson et al., 2009).

Studies have shown that galangin exhibits anti-breast cancer properties in vitro in MCF-7 cells (So et al., 1997). In a study done by Ciolino and Yeh

in 1999, the authors determined that galangin was beneficial in preventing the bio-transformation of 7,12,-dimethylbenz(a)anthracene (DMBA), a PAH, into genotoxic metabolites that bind to the DNA in MCF-7 breast cancer cells. Cytotoxicity data of galangin and PAHs in the presence of galangin across different cell lines are far and few between. However, in a very recent study, Bacanli et al. (2017) assessed the cytotoxicity profile of galangin alone on healthy V79 - Chinese hamster fibroblast cell line, BT-474 – human mammary carcinoma cell line, and HeLa – the human cervix epithelial adenocarcinoma cell line Henrietta Lacks over 18, 24, 48 h incubation periods. For these cell lines, the authors concluded that cell viability declines with increasing concentrations of galangin and cell survival rate was much lower after 48h. Chien et al. (2015) found galangin to have some time- and dose-dependent cytotoxic effect on human liver cells (HepG2) and no effect on Chang liver cells.

This study investigated the viability (mitochondrial activity) and toxicity of MCF-7 epithelial breast cancer cells following a 24 h in vitro exposure to a cocktail of PAHs and B[a]P individually, in the presence or absence of various concentrations of galangin. A 7 µM cocktail of common PAHs used in this study was an environmentally and occupationally relevant concentration. Following the 24 h exposure, the resulting toxicity and viability of the exposed samples of cells were measured using MTT assay and LDH assay respectively, and their correlation with varying doses of galangin were examined. In light of the anti-oxidative, radical scavenging, metabolic enzyme modulating, and anti-cancer activity potential of galangin, the purpose of the study was to ascertain if galangin could completely or partially block the damaging effects of PAHs on MCF-7 cells. If it does combat the harmful effects of PAHs, then some varieties of honey like Manuka and India root (Asian spice from the ginger family), which have active concentrations of galangin (Patel & Cichello, 2013), could be potential form of shield against the PAHs. It was hypothesized that exposing the cells to 7 µM cocktail of PAHs or 10 µM B[a]P in the presence of 30 µM galangin would reduce the toxicity and increase the viability of the cells as compared to that in the absence of galangin.

Methods

The MCF-7 cells were obtained from the ATC collection and had been stored frozen in liquid nitrogen. One vial of these cells was thawed and grown to confluence in a T-25 cm^2 tissue culture flask that had been especially treated for cell growth. Once the cells reached confluence, they were passaged again using a mixture of trypsin/EDTA and added to a T-75cm^2 tissue culture flask. The cells were grown in 100ml of medium that contained 300 mg of glucose, 1% of non-essential amino acids, vitamins, minerals, 1% antibiotic/anti-mycotic, 10% fetal bovine serum, and 1mg of insulin. When the cells reached confluence they were passaged, counted, and 75K cells/well were added to fourteen duplicate exposure wells of a sterile 96-well tissue-culture plate. A total of twenty-eight wells in the 96-well plate were used.

The MCF-7 cells in each exposure well were incubated for 48 h in a basal exposure medium containing 300 mg of glucose, 0.5% fetal bovine serum, 1% antibiotic/anti-mycotic, 1% non-essential amino acids, and 1mg of insulin. Then they were exposed for 24 h to special additives that were added to the basal exposure medium. The additives were 10 µM B[a]P alone, 7 µM cocktail of PAHs alone, various doses - 5, 30, and 100 µM - of galangin individually, and 10 µM B[a]P and 7 µM cocktail of PAHs individually concomitant with various doses of galangin. Of the remaining sets, one set of duplicates were left alone with just basal exposure medium (referred to as control), to one set 1% DMSO were added, and to another set 2% Acetonitrile were added. After 24 h, MTT assays were performed on the attached cells in the wells, while LDH assays were performed on the spent exposure media.

The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay is a colorimetric procedure to determine if a cell's mitochondria is able to metabolize and reduce the reagent diphenyl tetrazolium bromide. Healthy cells will reduce the reagent, a yellow tetrazole, to purple formazan in the mitochondria over a two hour time-period. The cells are then exposed for 10 minutes to an acidic alcohol solution, DMSO, during which different intensities of purple become visible. The purple formazan is detected using spectrophotometer at 570nm wavelength (with 680nm wavelength as reference), and the reading obtained is the absorbance.

The lactate dehydrogenase (LDH) assay is a colorimetric procedure that measures intra-cellular stress based upon color changes during the assay. Bio-chemical stress will induce cells to produce LDH, a soluble yet stable cytoplasmic enzyme, which leaks across the cell membrane, as the latter is compromised or damaged, into the exposure medium. For the assay a substrate was made from a medium containing phosphate buffered saline, NAD, sodium pyruvate, and tetrazolium. When the exposure medium (100µl) is assayed, a biochemical chain reaction occurs. NAD is reduced to NADH, which causes LDH to become catalyzed into pyruvate. The catalyst diaphorase uses the NADH to reduce tetrazolium salt to a red formazan product that is by a spectrophotometer at 490nm wavelength (with 680nm wavelength as reference) and the reading obtained is the absorbance. The absorbance is changed to units of LDH enzyme by using the absorbance from a sample containing specific units of LDH enzyme. In this study the absorbance were translated @ 0.011384 units of LDH enzyme per unit of absorbance. Samples containing high levels of LDH are dark red in color.

Statistical analyses were performed using Prism 3.02 software (Graphpad Inc., San Diego, CA). Differences between group mean values were determined by a one-factor analysis of variance (ANOVA), followed by Tukey post-hoc analysis for pair wise comparison of means.

Experimental Design

Independent variable: Exposure basal medium with varying additives as outlined below

	Trial 1		Trial 2		Trial 3		Trial 4		Average	
Exposure Medium with	Toxicity	Viability	Toxicity	Viability	Toxicity	Viability	Toxicity	Viability	Toxicity	Viability
Ctrl										
5µM Gal										
30µM Gal										
100µM Gal										
10µM BaP										
10µM BaP+5µM Gal										
10µM BaP+30µM Gal										
10µM BaP+100µM Gal										
7µM PAH										
7µM PAH+5µM Gal										
7µM PAH+30µM Gal										
7µM PAH+100µM Gal										

Dependent variables: Toxicity expressed in units of LDH enzyme from LDH assay, cell viability expressed as absorbance from MTT assay. MTT Absorbance expressed is net of blanks.

Constants: Exposure media base, temperature at which exposure occurs, exposure time, cell count in each exposure well, spectrophotometer measurement wavelength and reference wavelength set for each assay, time-period for which cells in each exposure well were seeded.

Notes: Ctrl stands for control exposure medium; Gal stands for galangin; 2% Aceto stands for 2% acetonitrile; µM stands for micro molar; 1% DMSO and 2% Acetonitrile are purely vehicle control; Absorbance and the units of LDH enzyme for each treatment are averaged across the duplicates.

Results

Table 1 displays the units of LDH enzyme in the distinct spent exposure media after the 24 h exposure period, for each trial of the experiment. Column chart in Figure 5 plots the mean LDH enzyme units in each spent exposure medium over the four trials of the experiment. In the chart, MCF-7 cell samples exposed to only 7 µM PAH cocktail or co-exposed to 7 µM PAH cocktail and 5, 30 or 100 µM of galangin released discernably higher units of LDH enzyme compared to the control sample. Also visible was the decline in the number of units of LDH enzyme released when a cell sample was co-exposed to 7 µM PAH cocktail and 5 µM galangin as compared to the cell sample exposed to 7 µM PAH cocktail alone. Higher units of LDH enzyme in the exposure medium signify higher intra-cellular stress or toxicity induced in the exposed cells by the additives in the basal exposure medium. There were no discernable differences in the units of LDH enzyme released by the rest of the samples compared to the control.

Table 2 shows the ANOVA summary table for the ANOVA run on the mean units of LDH enzyme across the fourteen distinct spent exposure media outlined in Table 1. The ANOVA test was run with H_0: $\mu 1 = \mu 2 = \ldots = \mu 14$, and H_a: At least one mean is different, at $\alpha = 0.05$. The table indicates that at least one sample spent exposure medium had a statistically significant different mean number of units of LDH enzyme, as $p (< 0.0001) < \alpha = 0.05$.

Readout 1 (in the appendix) displays confidence intervals of Tukey pairwise comparison of mean units of LDH enzyme in the spent exposure media. The pairwise comparisons were run to find spent exposure media which have mean units of LDH enzyme that are statistically significantly different, as the ANOVA test only tells overall that some spent exposure media have mean units of LDH enzyme that are statistically significantly different. Contrary to what was gleaned from the chart in figure 5, the Tukey pairwise comparisons only confirm statistically significantly the higher toxicity of samples of cells co-exposed to 7 µM PAH cocktail and 100 µM galangin. Also, the decline in the mean number of units of LDH enzyme released to the exposure medium by the cell samples co-exposed to 7 µM PAH cocktail and 5 µM of galangin as compared to that released by the cell samples exposed to 7 µM PAH cocktail alone was not statistically significant.

Table 3 displays the absorbance from MTT assay done on the cells attached in the exposure wells for each trial of the experiment. Figure 6 displays the mean absorbance of exposed MCF-7 cells in each sample across all the trials. Higher absorbance indicates higher mitochondrial activity (viability) of the cells in the sample, while lower absorbance indicates lower mitochondrial activity. One can see from the chart that cell samples exposed to 7 µM PAH cocktail alone or concomitant with doses of 5, 30 or 100 µM of galangin exhibited very low absorbance compared to the sample exposed to the control medium. Likewise, samples exposed to 100 µM of galangin or 10 µM B[a]P concomitant with 100 µM galangin also exhibited considerably reduced absorbance. The other exposed samples barring the sample exposed to 30 µM galangin also exhibited lower absorbance compared to the control sample.

Table 4 shows the ANOVA summary table for the ANOVA run on the mean absorbance of MCF-7 cell samples exposed to different exposure media outlined in Table 3 for 24 h. The ANOVA test was run with H_0: $\mu 1 = \mu 2 = \ldots = \mu 14$, and H_a: At least one mean is different, at $\alpha = 0.05$. The table indicates that at least one sample had a statistically significant different mean absorbance, as $p (< 0.0001) < \alpha = 0.05$.

Readout 2 (in the appendix) displays confidence intervals of pairwise Tukey comparison of mean absorbance in the exposed cell samples. The pairwise comparisons were run to find samples whose mean absorbance are statistically significantly different, as the ANOVA test only reveals overall that some sample mean absorbance are statistically significantly different. Tukey comparison confirms the takeaways from Figure 6 that cell samples exposed to 7 µM PAH cocktail alone, 7 µM PAH cocktail concomitant with doses of 5, 30 or 100 µM of galangin, 100 µM of galangin and 10 µM B[a]P concomitant with 100 µM galangin exhibited very low absorbance compared to the sample exposed to the control medium.

Discussion

The purpose of this study was to investigate MCF-7 epithelial breast cancer cells' viability and toxicity following a short-term in vitro exposure to a cocktail of PAHs and B[a]P individually in the absence or presence of varying concentrations of galangin. It was predicted that presence of flavonoid galangin will completely or partially block the damaging effects of PAHs. It was hypothesized that exposing the cells to 7 µM cocktail of PAHs or 10 µM B[a]P in the presence of 30 µM galangin would reduce the toxicity and increase the viability (mitochondrial activity) of the cells as compared to that in the absence of galangin. 7 µM cocktail of PAHs in the presence or absence of 30 µM galangin did not show statistically significantly different mean units of LDH enzyme expelled to the exposure medium or mean MTT absorbance (see readout 1 and readout 2 in the appendix) compared to the control sample. The same reasoning applied to 10 µM B[a]P in the presence or absence of galangin as well. Therefore, the hypothesis was not supported by the results from the study.

The cytotoxicity of the MCF-7 cell samples in terms of toxicity expressed via mean units of LDH enzyme in the spent exposure media (as gathered from Table 1, Figure 5, and Readout 1) revealed that 7 µM PAH cocktail in the presence of 100 µM galangin was statistically significantly (at $\alpha < 0.05$) very toxic compared to all other samples, barring the samples exposed to 7 µM PAH cocktail in the presence of nil or 30 µM galangin. A higher number of units of LDH enzyme in the spent exposure medium reflect the higher degree to which the exposure medium has induced toxicity in the MCF-7 cells by compromising their cell membranes. Based on readout 1 in the appendix, the mean units of LDH enzyme released (to the exposure medium) by the sample of cells co-exposed to 7 µM PAH cocktail and 100 µM galangin was statistically no different from that released by the samples co-exposed to 7 µM PAH cocktail and nil or 30 µM galangin. However, unlike the mean units of the enzyme released by the sample co-exposed to 7 µM PAH cocktail and 100 µM galangin, the mean units of the enzyme released by the samples co-exposed to 7 µM PAH cocktail and nil or 30 µM galangin were statistically no different from the units released by the rest of the samples, including the control. Therefore, it can be substantiated that 7 µM PAH cocktail in the presence of 100 µM galangin was the only toxic medium, in terms of LDH enzyme units released by the cell samples to the exposure media.

The cytotoxicity of the MCF-7 cell samples in terms of viability expressed via mean MTT absorbance (as gathered from Table 3, Figure 6, and Readout 2) reveal that samples exposed to 7 µM PAH cocktail in the presence of 0, 5, 30 or 100 µM galangin, 10 µM B[a]P in the presence of 100

μM of galangin, and 100 μM galangin show statistically significant ($\alpha < 0.05$) reduction in absorbance compared to the control sample. Lower absorbance means lower viability (cellular metabolism or mitochondrial activity) of the cells, while higher absorbance means higher viability. The percentage of viable cells in these samples is nothing but the ratio of absorbance (after netting out the blanks) in a sample with respect to the control sample expressed in percent. Accordingly, the viability of the MCF-7 cell samples exposed to 7 μM PAH cocktail, 7 μM PAH cocktail in the presence of 5, 30 or 100 μM galangin, 10 μM B[a]P in the presence of 100 μM galangin, and 100 μM galangin were 44%, 18%, 11%, 4%, 33%, and 26% (all rounded to whole) respectively.

The comparison of results from LDH and MTT assays established that 7 μM PAH cocktail in the presence of 100 μM galangin was highly cytotoxic to the MCF-7 cells by completely breaching the integrity of their cell membranes, and by nearly shutting down their mitochondrial activity respectively. It was ascertained that 7 μM PAH cocktail, 7 μM PAH cocktail in the presence of 5 or 30 μM galangin, 10 μM B[a]P in the presence of 100 μM galangin, and 100 μM galangin are highly cytotoxic by significantly reducing the mitochondrial activity of the cells, but they do not express themselves as cytotoxic by breaching the integrity of the cell membranes. It was established from the study that at lower levels, like at 5 μM and 30 μM, there was no statistical evidence that galangin raises or lowers the cytotoxicity of the cells expressed in either manner. However, 100 μM of galangin reduces the viability of the cells with no significant impact on their cell membrane integrity. Both the assays presented statistical evidence that 10 μM B[a]P was not cytotoxic, and the presence of 5 μM and 30 μM galangin did not alter the cytotoxicity it exerts. Findings from LDH assays revealed that 7 μM PAH cocktail did not affect the integrity of cell membrane negatively, and that the presence of 5 μM and 30 μM of galangin did not alter the toxicity it exerts. However, MTT assays revealed that 7 μM PAH cocktail reduced the viability of the MCF-7 cells as compared to that induced by the control sample medium, but there is no statistical evidence that galangin in lower doses, 5 μM and 30 μM, alters the cell viability that the cocktail induces. These assays provided convincing statistical evidence that presence of 100 μM galangin significantly reduced the viability of the cells even further than that induced separately by 7 μM PAH cocktail and 10 μM B[a]P. There was substantial evidence in the study that the presence of 100 μM galangin made 7 μM PAH cocktail to be toxic, causing significant amounts of LDH enzyme to be released into the exposure medium. However, 10 μM B[a]P in the presence of 100 μM galangin did not show similar effect.

There are some characteristic conclusions from this study, and there were few studies to which they could be compared to. The finding that galangin at 24 h exposure is cytotoxic in terms of viability of MCF-7 cells at 100 μM and non-cytotoxic in terms of viability and cell membrane integrity at doses 5 μM and 30 μM were in line with the observations put forth by the study done by Bacanli et al. (2017). The authors in that study concluded using numerous different measurement methods that galangin has no cytotoxic effects on different cell lines under 100 μM concentrations. The finding from this study that 10 μM B[a]P is non-cytotoxic to MCF-7 cells after 24 h exposure departs from the suggestion by Zhu et al. (2014) that B[a]P is directly toxic to humans at 5 μM, but more in line with the conclusive evidence from Uno et al. (2006) that it is the B[a]P metabolites that are toxic to humans and not the B[a]P itself . Table 5 summarizes the results of the study.

There are a number of sources of error in this study. Most importantly, due to lack of experience, errors could have crept in measurements of additives using the pipette, when making the exposure media. Great care was taken to prevent experimentation errors; however human error is almost certainly present in a minor degree.

The dose- and time- dependent effects of galangin co-exposed with PAH cocktail or B[a]P on MCF-7 cells could not be established in our study as the doses of galangin were limited just to 5, 30, and 100 μM and the time-duration of the exposures were set to 24 h. To establish a dose- and time-dependent profile of galangin, future studies should be done with doses of galangin ranging from 0 to 100 μM in increments of 2.5 μM and with time-duration of the exposures ranging from 24 h through 168 h (a week) in increments of 24 h. These studies could also help establish conclusive evidence of time-dependent cytotoxic effects of 10 μM B[a]P on the cells. In addition, the study could possibly be expanded by varying the levels of B[a]P and PAH cocktail as well as by pre-exposing the cells to galangin before exposing them to the PAHs. Further research could include doing the same study with the various PAHs constituting the PAH cocktail used in the study.

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Figure 1. Representative PAHs (A. Maigari & M. Maigari, 2015)

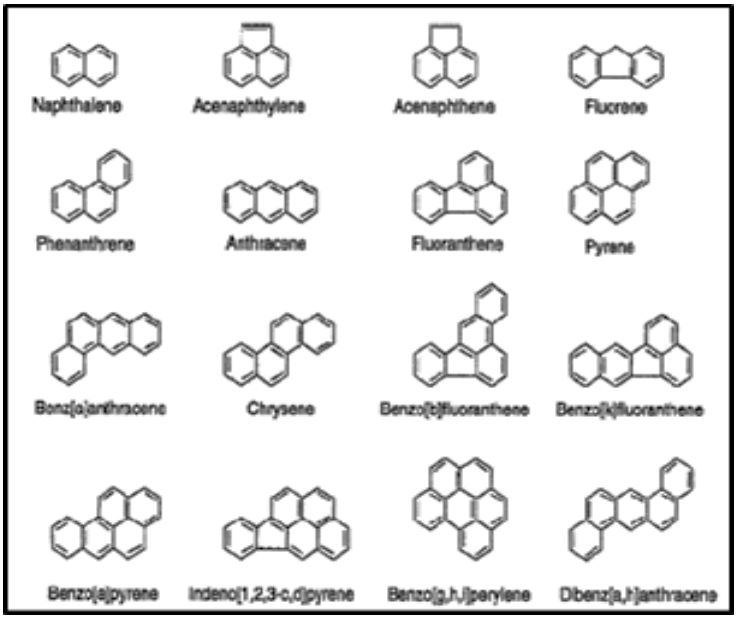

Figure 2. Sources of PAH (Abdel-Shafy & M. Maigari, 2015)

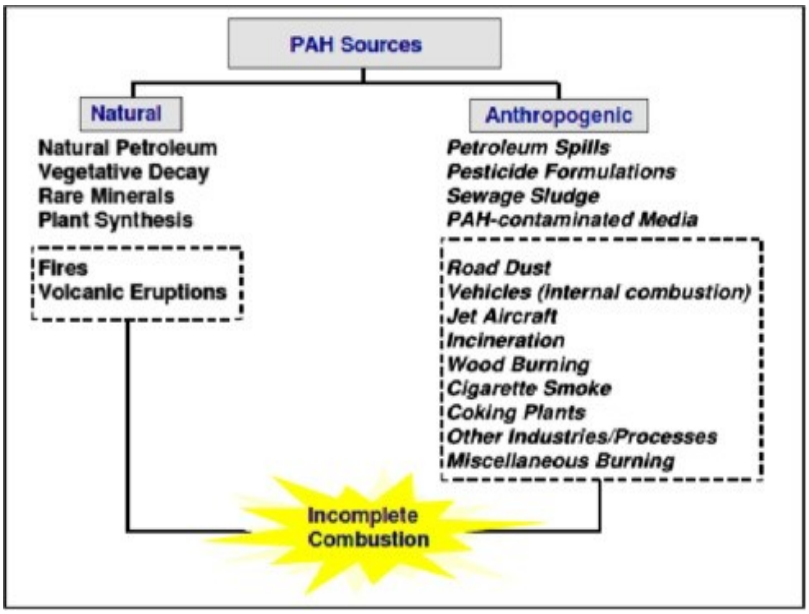

Figure 3. Carcinogenicity of several individual PAHs (Lee & Vu, 2010)
DHHS – Departmental human and health services, USA;
EPA – Environmental protection agency, USA;
IARC – International agency for research on cancer, France;

	EPA	IARC	DHHS
Acenaphthene			
Acenaphthylene	*Not classifiable*		
Anthanthrene	*Not classifiable*	*Not classifiable*	
Benz(a)anthracene	*Probably Carcinogen*	*Probably Carcinogen*	*Animal Carcinogen*
Benzo(a)pyrene	*Probably Carcinogen*	*Probably Carcinogen*	*Animal Carcinogen*
Benzo(b)fluoranthene	*Probably Carcinogen*	*Probably Carcinogen*	*Animal Carcinogen*
Benzo(e)pyrene		*Not classifiable*	
Benzo(ghi)perylene	*Not classifiable*	*Not classifiable*	
Benzo(j)fluoranthene	*Not included*	*Probably Carcinogen*	*Animal Carcinogen*
Benzo(k)fluoranthene	*Probably Carcinogen*	*Probably Carcinogen*	
Chrysene	*Probably Carcinogen*	*Not classifiable*	
Dibenz(ah)anthracene	*Probably Carcinogen*		Animal Carcinogen
Fluoranthene	*Not classifiable*	*Not classifiable*	
Fluorene	*Not classifiable*	*Not classifiable*	
Ideno(1,2,3-cd)pyrene	*Probably Carcinogen*	*Probably Carcinogen*	Animal Carcinogen
Phenanthrene	*Not classifiable*		
Pyrene	*Not classifiable*	*Not classifiable*	

Figure 4. Chemical structure of galangin

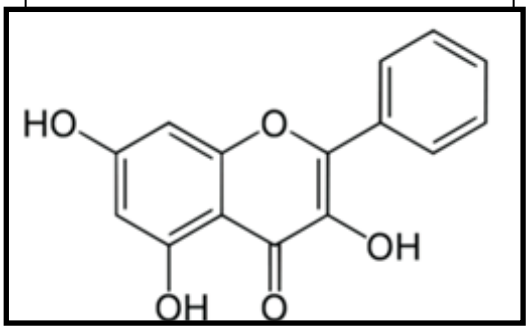

Table 1. Units of LDH enzyme from LDH assay done on the spent exposure medium

Doses Added to the Basal Exposure Medium	Units of LDH Enzyme				
	Trail 1	Trial 2	Trial 3	Trial 4	Mean
Ctrl	0.00234	0.00243	0.00246	0.00294	0.00254
1% DMSO	0.00197	0.00266	0.00221	0.00242	0.00232
2% Aceto	0.00309	0.00408	0.00279	0.00305	0.00325
5µM Gal	0.00255	0.00251	0.00234	0.00315	0.00264
30µM Gal	0.00193	0.00200	0.00160	0.00190	0.00186
100µM Gal	0.00094	0.00252	0.00216	0.00250	0.00203
10µM BaP	0.00247	0.00250	0.00288	0.00120	0.00226
10µM BaP+5µM Gal	0.00217	0.00249	0.00155	0.00248	0.00217
10µM BaP+30µM Gal	0.00118	0.00195	0.00483	0.00236	0.00258
10µM BaP+100µM Gal	0.00319	0.00211	0.00513	0.00233	0.00319
7µM PAH	0.00645	0.00570	0.01186	0.00407	0.00702
7µM PAH+5µM Gal	0.00675	0.00656	0.00704	0.00318	0.00588
7µM PAH+30µM Gal	0.02229	0.00548	0.01483	0.00245	0.01127
7µM PAH+100µM Gal	0.02626	0.02056	0.01539	0.00290	0.01628

Table 1 displays the units of LDH enzyme in the spent exposure medium, categorized by additives to the basal exposure medium in the exposure wells, for all the trials of the experiment. Higher units indicate higher toxicity, while lower units indicate lower toxicity. The control exposure medium has no additive and therefore, is purely a basal exposure medium.

Table 2. ANOVA summary table for ANOVA run on mean units of LDH enzyme

One-way analysis of variance					
P value	P<0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	14				
F	5.009				
R squared	0.6079				
ANOVA Table	**SS**	**df**	**MS**	**F(DFn,DFd)**	**P value**
Treatment (between columns)	0.0009397	13	0.000072290	F(13,42) = 5.009	P<0.0001
Residual (within columns)	0.0006061	42	0.000014430		
Total	0.001546	55			

Table 2 displays the ANOVA summary table for ANOVA test run on the mean units of LDH enzyme across 14 distinct spent exposure media outlined in Table 1. It can be seen that at least one spent exposure medium had a statistically significant different mean units of LDH enzyme at $\alpha = 0.05$, $F(13,42) = 5.009$, $p < 0.0001$.

Figure 5. Column chart of mean units of LDH enzyme

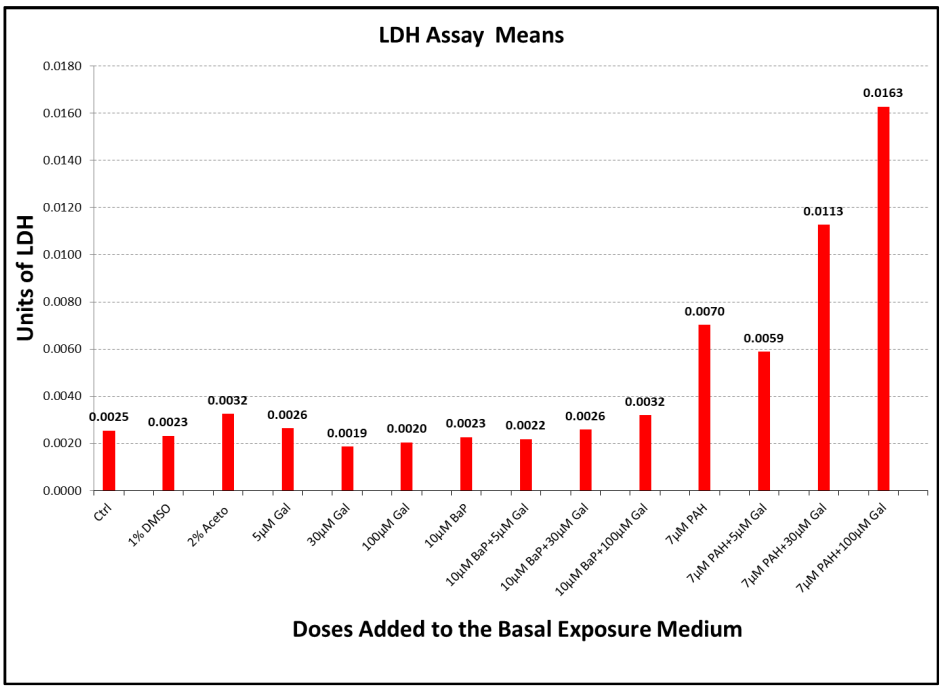

Figure 5 displays the mean units of LDH enzyme in the spent exposure medium, categorized by additives to the basal exposure medium in the exposure wells, across all the trials of the experiment. Higher units indicate higher toxicity, while lower units indicate lower toxicity. The control exposure medium has no additive and therefore, is purely a basal exposure medium.

Table 3. Absorbance of exposed MCF-7 cell samples

Doses Added to the Basal Exposure Medium	Absorbance				
	Trial 1	Trial 2	Trial 3	Trial 4	Mean
Ctrl	2.1440	1.9395	1.6870	1.8820	1.9131
1% DMSO	1.4535	1.6865	1.5205	1.9150	1.6439
2% Acet	1.8305	2.0270	1.3725	1.9940	1.8060
5µM Gal	1.7390	1.8145	1.6280	2.1835	1.8413
30µM Gal	1.6695	2.1045	1.6815	2.4845	1.9850
100µM Gal	0.3680	0.4180	0.5260	0.6860	0.4995
10µM BAP	1.1930	1.3185	1.4235	1.7220	1.4143
10µM BAP + 5µM	1.3875	1.4715	1.6595	1.8650	1.5959
10µM BAP + 30µM	1.6290	1.4290	0.7115	1.7420	1.3779
10µM BAP + 100µM	0.8995	0.4205	0.5730	0.6345	0.6319
7µM PAH	1.8355	0.3945	0.5905	0.5640	0.8461
7µM PAH + 5µM	0.3050	0.3205	0.3610	0.3715	0.3395
7µM PAH + 30µM	0.4035	0.1505	0.1855	0.1155	0.2138
7µM PAH + 100µM	0.2680	0.0000	0.0000	0.0020	0.0675

Table 3 displays the absorbance in each sample of cells, categorized by additives to the basal exposure medium in the exposure wells, for all the trials of the experiment. Higher absorbance indicate higher mitochondrial activity (viability), while lower absorbance indicate lower mitochondrial activity. The control exposure medium has no additive and therefore, is purely a basal exposure medium.

Figure 6. Column chart of mean absorbance of MCF-7 cell samples

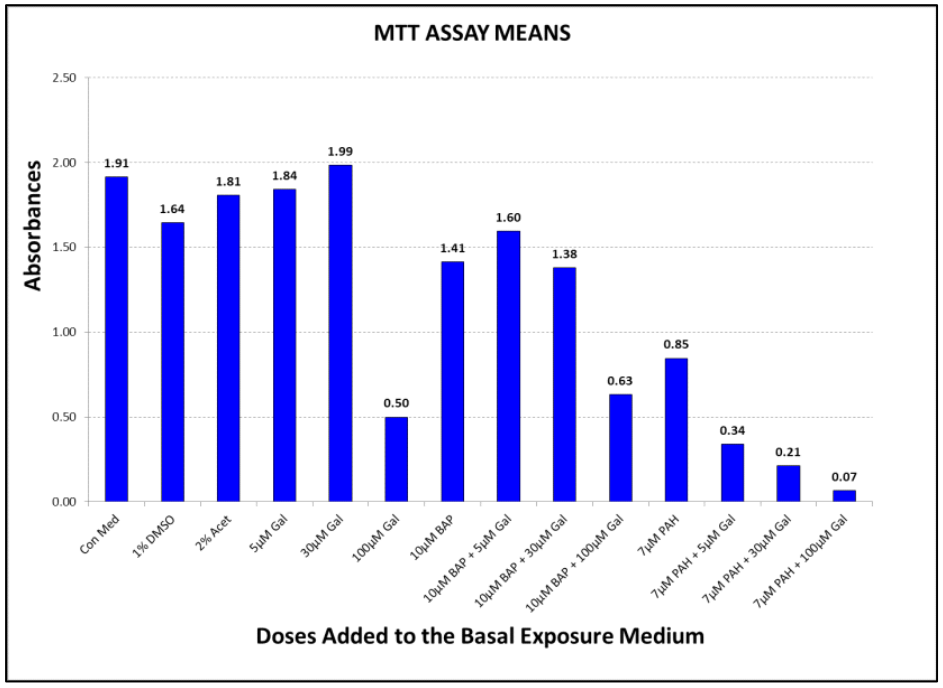

Figure 6 displays the mean absorbance in each sample of cells, categorized by additives to the basal exposure medium in the exposure wells, across all the trials of the experiment. Higher absorbance indicates higher mitochondrial activity (cell viability), while lower units indicate lower mitochondrial activity. The control exposure medium has no additive and therefore, is purely a basal exposure medium.

Table 4. ANOVA summary table for repeated measures ANOVA on absorbance

One-way analysis of variance					
P value	P<0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	14				
F	21.92				
R squared	0.8715				
ANOVA Table	**SS**	**df**	**MS**	**F(DFn,DFd)**	**P value**
Treatment (between columns)	24.96	13	1.92	F(13,42) = 21.92	P<0.0001
Residual (within columns)	3.679	42	0.08761		
Total	28.64	55			

Table 4 shows the ANOVA summary table for the ANOVA test run on the mean absorbance of the samples exposed to different exposure media outlined in Table 3. It can be seen that at least one sample had a statistically significant different mean absorbance at $\alpha = 0.05$, $F(13,42) = 21.92$, $p < 0.0001$.

Table 5. Summary of the cytotoxic effects on MCF-7 cells

Exposure Medium with	Cytotoxicity	
	Increase in Toxicity*	Decrease in Viability**
Ctrl		
5µM Gal	*no*	*no*
30µM Gal	*no*	*no*
100µM Gal	*no*	*yes*
10µM BAP	*no*	*no*
10µM BAP + 5µM Gal	*no*	*no*
10µM BAP + 30µM Gal	*no*	*no*
10µM BAP + 100µM Gal	*no*	*yes*
7µM PAH	*no*	*yes*
7µM PAH + 5µM Gal	*no*	*yes*
7µM PAH + 30µM Gal	*no*	*yes*
7µM PAH + 100µM Gal	*yes*	*yes*

** Toxicity (cell membrane integrity) expressed through statistically significant LDH enzyme units in the exposure medium.*
*** Viability (cellular metabolism or mitochondrial activity) expressed through statistically significant MTT absorbance in the cells*

Appendix

Readout 1- Tukey pairwise comparisons of mean LDH units across different cell samples at 95% confidence

Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
Cntrl vs 1% DMSO	0.0002277	0.1199	P > 0.05	-0.009334 to 0.009789
Cntrl vs 2% Aceto	-0.0007060	0.3717	P > 0.05	-0.01027 to 0.008855
Cntrl vs 5uM Gal	-0.0000939	0.0495	P > 0.05	-0.009655 to 0.009468
Cntrl vs 30uM Gal	0.0006859	0.3611	P > 0.05	-0.008876 to 0.01025
Cntrl vs 100uM Gal	0.0005137	0.2705	P > 0.05	-0.009048 to 0.01008
Cntrl vs 10uM BaP	0.0002818	0.1483	P > 0.05	-0.009280 to 0.009843
Cntrl vs 10uM BaP +5uM Gal	0.0003686	0.1940	P > 0.05	-0.009193 to 0.009930
Cntrl vs 10uM BaP +30uM Gal	-0.0000370	0.0195	P > 0.05	-0.009598 to 0.009524
Cntrl vs 10uM BaP +100uM Gal	-0.0006461	0.3401	P > 0.05	-0.01021 to 0.008915
Cntrl vs 7uM PAH	-0.0044770	2.3570	P > 0.05	-0.01404 to 0.005085
Cntrl vs 7uM PAH + 5uM Gal	-0.0033380	1.7580	P > 0.05	-0.01290 to 0.006223
Cntrl vs 7uM PAH + 30uM Gal	-0.0087220	4.5920	P > 0.05	-0.01828 to 0.0008392
Cntrl vs 7uM PAH +100uM Gal	-0.0137400	7.2310	P < 0.001	-0.02330 to -0.004174
1% DMSO vs 2% Aceto	-0.0009337	0.4915	P > 0.05	-0.01050 to 0.008628
1% DMSO vs 5uM Gal	-0.0003216	0.1693	P > 0.05	-0.009883 to 0.009240
1% DMSO vs 30uM Gal	0.0004582	0.2412	P > 0.05	-0.009103 to 0.01002
1% DMSO vs 100uM Gal	0.0002860	0.1506	P > 0.05	-0.009275 to 0.009847
1% DMSO vs 10uM BaP	0.0000541	0.0285	P > 0.05	-0.009507 to 0.009616
1% DMSO vs 10uM BaP +5uM Gal	0.0001409	0.0742	P > 0.05	-0.009421 to 0.009702
1% DMSO vs 10uM BaP +30uM Gal	-0.0002647	0.1393	P > 0.05	-0.009826 to 0.009297
1% DMSO vs 10uM BaP +100uM Gal	-0.0008737	0.4600	P > 0.05	-0.01044 to 0.008688
1% DMSO vs 7uM PAH	-0.0047050	2.4770	P > 0.05	-0.01427 to 0.004857
1% DMSO vs 7uM PAH + 5uM Gal	-0.0035660	1.8770	P > 0.05	-0.01313 to 0.005995
1% DMSO vs 7uM PAH + 30uM Gal	-0.0089500	4.7120	P > 0.05	-0.01851 to 0.0006115
1% DMSO vs 7uM PAH +100uM Gal	-0.0139600	7.3510	P < 0.001	-0.02352 to -0.004401
2% Aceto vs 5uM Gal	0.0006121	0.3222	P > 0.05	-0.008949 to 0.01017
2% Aceto vs 30uM Gal	0.0013920	0.7328	P > 0.05	-0.008170 to 0.01095
2% Aceto vs 100uM Gal	0.0012200	0.6421	P > 0.05	-0.008342 to 0.01078
2% Aceto vs 10uM BaP	0.0009877	0.5200	P > 0.05	-0.008574 to 0.01055
2% Aceto vs 10uM BaP +5uM Gal	0.0010750	0.5657	P > 0.05	-0.008487 to 0.01064
2% Aceto vs 10uM BaP +30uM Gal	0.0006690	0.3522	P > 0.05	-0.008892 to 0.01023
2% Aceto vs 10uM BaP +100uM Gal	0.0000599	0.0315	P > 0.05	-0.009502 to 0.009621
2% Aceto vs 7uM PAH	-0.0037710	1.9850	P > 0.05	-0.01333 to 0.005791
2% Aceto vs 7uM PAH + 5uM Gal	-0.0026320	1.3860	P > 0.05	-0.01219 to 0.006929
2% Aceto vs 7uM PAH + 30uM Gal	-0.0080160	4.2200	P > 0.05	-0.01758 to 0.001545
2% Aceto vs 7uM PAH +100uM Gal	-0.0130300	6.8590	P < 0.01	-0.02259 to -0.003468
5uM Gal vs 30uM Gal	0.0007798	0.4106	P > 0.05	-0.008782 to 0.01034
5uM Gal vs 100uM Gal	0.0006076	0.3199	P > 0.05	-0.008954 to 0.01017
5uM Gal vs 10uM BaP	0.0003757	0.1978	P > 0.05	-0.009186 to 0.009937
5uM Gal vs 10uM BaP +5uM Gal	0.0004625	0.2435	P > 0.05	-0.009099 to 0.01002
5uM Gal vs 10uM BaP +30uM Gal	0.0000569	0.0300	P > 0.05	-0.009505 to 0.009618
5uM Gal vs 10uM BaP +100uM Gal	-0.0005521	0.2907	P > 0.05	-0.01011 to 0.009009
5uM Gal vs 7uM PAH	-0.0043830	2.3070	P > 0.05	-0.01394 to 0.005178
5uM Gal vs 7uM PAH + 5uM Gal	-0.0032450	1.7080	P > 0.05	-0.01281 to 0.006317
5uM Gal vs 7uM PAH + 30uM Gal	-0.0086280	4.5430	P > 0.05	-0.01819 to 0.0009331
5uM Gal vs 7uM PAH +100uM Gal	-0.0136400	7.1820	P < 0.001	-0.02320 to -0.004080
30uM Gal vs 100uM Gal	-0.0001722	0.0907	P > 0.05	-0.009734 to 0.009389
30uM Gal vs 10uM BaP	-0.0004041	0.2128	P > 0.05	-0.009966 to 0.009157
30uM Gal vs 10uM BaP +5uM Gal	-0.0003173	0.1671	P > 0.05	-0.009879 to 0.009244
30uM Gal vs 10uM BaP +30uM Gal	-0.0007229	0.3806	P > 0.05	-0.01028 to 0.008839
30uM Gal vs 10uM BaP +100uM Gal	-0.0013320	0.7012	P > 0.05	-0.01089 to 0.008229
30uM Gal vs 7uM PAH	-0.0051630	2.7180	P > 0.05	-0.01472 to 0.004399
30uM Gal vs 7uM PAH + 5uM Gal	-0.0040240	2.1190	P > 0.05	-0.01359 to 0.005537
30uM Gal vs 7uM PAH + 30uM Gal	-0.0094080	4.9530	P > 0.05	-0.01897 to 0.0001533
30uM Gal vs 7uM PAH +100uM Gal	-0.0144200	7.5920	P < 0.001	-0.02398 to -0.004860
100uM Gal vs 10uM BaP	-0.0002320	0.1221	P > 0.05	-0.009793 to 0.009329
100uM Gal vs 10uM BaP +5uM Gal	-0.0001451	0.0764	P > 0.05	-0.009707 to 0.009416
100uM Gal vs 10uM BaP +30uM Gal	-0.0005507	0.2899	P > 0.05	-0.01011 to 0.009011
100uM Gal vs 10uM BaP +100uM Gal	-0.0011600	0.6106	P > 0.05	-0.01072 to 0.008402
100uM Gal vs 7uM PAH	-0.0049910	2.6270	P > 0.05	-0.01455 to 0.004571
100uM Gal vs 7uM PAH + 5uM Gal	-0.0038520	2.0280	P > 0.05	-0.01341 to 0.005709
100uM Gal vs 7uM PAH + 30uM Gal	-0.0092360	4.8620	P > 0.05	-0.01880 to 0.0003255
100uM Gal vs 7uM PAH +100uM Gal	-0.0142500	7.5020	P < 0.001	-0.02381 to -0.004687
10uM BaP vs 10uM BaP +5uM Gal	0.0000868	0.0457	P > 0.05	-0.009475 to 0.009648
10uM BaP vs 10uM BaP +30uM Gal	-0.0003188	0.1678	P > 0.05	-0.009880 to 0.009243
10uM BaP vs 10uM BaP +100uM Gal	-0.0009278	0.4885	P > 0.05	-0.01049 to 0.008634
10uM BaP vs 7uM PAH	-0.0047590	2.5050	P > 0.05	-0.01432 to 0.004803
10uM BaP vs 7uM PAH + 5uM Gal	-0.0036200	1.9060	P > 0.05	-0.01318 to 0.005941
10uM BaP vs 7uM PAH + 30uM Gal	-0.0090040	4.7400	P > 0.05	-0.01857 to 0.0005574
10uM BaP vs 7uM PAH +100uM Gal	-0.0140200	7.3790	P < 0.001	-0.02358 to -0.004456
10uM BaP +5uM Gal vs 10uM BaP +30uM Gal	-0.0004056	0.2135	P > 0.05	-0.009967 to 0.009156
10uM BaP +5uM Gal vs 10uM BaP +100uM Gal	-0.0010150	0.5342	P > 0.05	-0.01058 to 0.008547
10uM BaP +5uM Gal vs 7uM PAH	-0.0048450	2.5510	P > 0.05	-0.01441 to 0.004716
10uM BaP +5uM Gal vs 7uM PAH + 5uM Gal	-0.0037070	1.9520	P > 0.05	-0.01327 to 0.005854
10uM BaP +5uM Gal vs 7uM PAH + 30uM Gal	-0.0090910	4.7860	P > 0.05	-0.01865 to 0.0004706
10uM BaP +5uM Gal vs 7uM PAH +100uM Gal	-0.0141000	7.4250	P < 0.001	-0.02367 to -0.004542
10uM BaP +30uM Gal vs 10uM BaP +100uM Gal	-0.0006091	0.3207	P > 0.05	-0.01017 to 0.008952
10uM BaP +30uM Gal vs 7uM PAH	-0.0044400	2.3370	P > 0.05	-0.01400 to 0.005122
10uM BaP +30uM Gal vs 7uM PAH + 5uM Gal	-0.0033010	1.7380	P > 0.05	-0.01286 to 0.006260
10uM BaP +30uM Gal vs 7uM PAH + 30uM Gal	-0.0086850	4.5730	P > 0.05	-0.01825 to 0.0008762
10uM BaP +30uM Gal vs 7uM PAH +100uM Gal	-0.0137000	7.2120	P < 0.001	-0.02326 to -0.004137
10uM BaP +100uM Gal vs 7uM PAH	-0.0038310	2.0170	P > 0.05	-0.01339 to 0.005731
10uM BaP +100uM Gal vs 7uM PAH + 5uM Gal	-0.0026920	1.4170	P > 0.05	-0.01225 to 0.006869
10uM BaP +100uM Gal vs 7uM PAH + 30uM Gal	-0.0080760	4.2520	P > 0.05	-0.01764 to 0.001485
10uM BaP +100uM Gal vs 7uM PAH +100uM Gal	-0.0130900	6.8910	P < 0.01	-0.02265 to -0.003528
7uM PAH vs 7uM PAH + 5uM Gal	0.0011380	0.5993	P > 0.05	-0.008423 to 0.01070
7uM PAH vs 7uM PAH + 30uM Gal	-0.0042450	2.2350	P > 0.05	-0.01381 to 0.005316
7uM PAH vs 7uM PAH +100uM Gal	-0.0092580	4.8740	P > 0.05	-0.01882 to 0.0003031
7uM PAH + 5uM Gal vs 7uM PAH + 30uM Gal	-0.0053840	2.8340	P > 0.05	-0.01495 to 0.004178
7uM PAH + 5uM Gal vs 7uM PAH +100uM Gal	-0.0104000	5.4740	P < 0.05	-0.01996 to -0.0008153
7uM PAH + 30uM Gal vs 7uM PAH +100uM Gal	-0.0050130	2.6390	P > 0.05	-0.01457 to 0.004548

Readout 2- Tukey pairwise comparisons of mean absorbance across different cell samples at 95% confidence

Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
Cntrl vs 1% DMSO	0.2693	1.819	P > 0.05	-0.4757 to 1.014
Cntrl vs 2% Aceto	0.1071	0.7239	P > 0.05	-0.6378 to 0.8521
Cntrl vs 5uM Gal	0.07188	0.4857	P > 0.05	-0.6731 to 0.8168
Cntrl vs 30uM Gal	-0.07187	0.4857	P > 0.05	-0.8168 to 0.6731
Cntrl vs 100uM Gal	1.414	9.552	P < 0.001	0.6687 to 2.159
Cntrl vs 10uM BaP	0.4989	3.371	P > 0.05	-0.2461 to 1.244
Cntrl vs 10uM BaP +5uM Gal	0.3173	2.144	P > 0.05	-0.4277 to 1.062
Cntrl vs 10uM BaP +30uM Gal	0.5353	3.617	P > 0.05	-0.2097 to 1.280
Cntrl vs 10uM BaP +100uM Gal	1.281	8.658	P < 0.001	0.5363 to 2.026
Cntrl vs 7uM PAH	1.067	7.210	P < 0.001	0.3220 to 1.812
Cntrl vs 7uM PAH + 5uM Gal	1.574	10.630	P < 0.001	0.8287 to 2.319
Cntrl vs 7uM PAH + 30uM Gal	1.699	11.480	P < 0.001	0.9544 to 2.444
Cntrl vs 7uM PAH +100uM Gal	1.846	12.470	P < 0.001	1.101 to 2.591
1% DMSO vs 2% Aceto	-0.1621	1.096	P > 0.05	-0.9071 to 0.5828
1% DMSO vs 5uM Gal	-0.1974	1.334	P > 0.05	-0.9423 to 0.5476
1% DMSO vs 30uM Gal	-0.3411	2.305	P > 0.05	-1.086 to 0.4038
1% DMSO vs 100uM Gal	1.144	7.733	P < 0.001	0.3994 to 1.889
1% DMSO vs 10uM BaP	0.2296	1.552	P > 0.05	-0.5153 to 0.9746
1% DMSO vs 10uM BaP +5uM Gal	0.048	0.3243	P > 0.05	-0.6970 to 0.7930
1% DMSO vs 10uM BaP +30uM Gal	0.266	1.797	P > 0.05	-0.4790 to 1.011
1% DMSO vs 10uM BaP +100uM Gal	1.012	6.838	P < 0.01	0.2670 to 1.757
1% DMSO vs 7uM PAH	0.798	5.390	P < 0.05	0.05279 to 1.543
1% DMSO vs 7uM PAH + 5uM Gal	1.304	8.814	P < 0.001	0.5594 to 2.049
1% DMSO vs 7uM PAH + 30uM Gal	1.430	9.664	P < 0.001	0.6852 to 2.175
1% DMSO vs 7uM PAH +100uM Gal	1.576	10.650	P < 0.001	0.8314 to 2.321
2% Aceto vs 5uM Gal	-0.03525	0.2382	P > 0.05	-0.7802 to 0.7097
2% Aceto vs 30uM Gal	-0.179	1.21	P > 0.05	-0.9240 to 0.5660
2% Aceto vs 100uM Gal	1.307	8.828	P < 0.001	0.5615 to 2.051
2% Aceto vs 10uM BaP	0.3918	2.647	P > 0.05	-0.3532 to 1.137
2% Aceto vs 10uM BaP +5uM Gal	0.2101	1.42	P > 0.05	-0.5348 to 0.9551
2% Aceto vs 10uM BaP +30uM Gal	0.4281	2.893	P > 0.05	-0.3168 to 1.173
2% Aceto vs 10uM BaP +100uM Gal	1.174	7.934	P < 0.001	0.4292 to 1.919
2% Aceto vs 7uM PAH	0.960	6.486	P < 0.01	0.2149 to 1.705
2% Aceto vs 7uM PAH + 5uM Gal	1.467	9.909	P < 0.001	0.7215 to 2.211
2% Aceto vs 7uM PAH + 30uM Gal	1.592	10.760	P < 0.001	0.8473 to 2.337
2% Aceto vs 7uM PAH +100uM Gal	1.739	11.750	P < 0.001	0.9935 to 2.483
5uM Gal vs 30uM Gal	-0.1438	0.9713	P > 0.05	-0.8887 to 0.6012
5uM Gal vs 100uM Gal	1.342	9.066	P < 0.001	0.5968 to 2.087
5uM Gal vs 10uM BaP	0.427	2.885	P > 0.05	-0.3180 to 1.172
5uM Gal vs 10uM BaP +5uM Gal	0.2454	1.658	P > 0.05	-0.4996 to 0.9903
5uM Gal vs 10uM BaP +30uM Gal	0.4634	3.131	P > 0.05	-0.2816 to 1.208
5uM Gal vs 10uM BaP +100uM Gal	1.209	8.172	P < 0.001	0.4644 to 1.954
5uM Gal vs 7uM PAH	0.995	6.724	P < 0.01	0.2502 to 1.740
5uM Gal vs 7uM PAH + 5uM Gal	1.502	10.150	P < 0.001	0.7568 to 2.247
5uM Gal vs 7uM PAH + 30uM Gal	1.628	11.000	P < 0.001	0.8825 to 2.372
5uM Gal vs 7uM PAH +100uM Gal	1.774	11.990	P < 0.001	1.029 to 2.519
30uM Gal vs 100uM Gal	1.486	10.040	P < 0.001	0.7405 to 2.230
30uM Gal vs 10uM BaP	0.5708	3.857	P > 0.05	-0.1742 to 1.316
30uM Gal vs 10uM BaP +5uM Gal	0.3891	2.629	P > 0.05	-0.3558 to 1.134
30uM Gal vs 10uM BaP +30uM Gal	0.6071	4.102	P > 0.05	-0.1378 to 1.352
30uM Gal vs 10uM BaP +100uM Gal	1.353	9.143	P < 0.001	0.6082 to 2.098
30uM Gal vs 7uM PAH	1.139	7.696	P < 0.001	0.3939 to 1.884
30uM Gal vs 7uM PAH + 5uM Gal	1.646	11.120	P < 0.001	0.9005 to 2.390
30uM Gal vs 7uM PAH + 30uM Gal	1.771	11.970	P < 0.001	1.026 to 2.516
30uM Gal vs 7uM PAH +100uM Gal	1.918	12.960	P < 0.001	1.173 to 2.662
100uM Gal vs 10uM BaP	-0.915	6.181	P < 0.01	-1.660 to -0.1698
100uM Gal vs 10uM BaP +5uM Gal	-1.096	7.408	P < 0.001	-1.841 to -0.3514
100uM Gal vs 10uM BaP +30uM Gal	-0.878	5.935	P < 0.01	-1.623 to -0.1334
100uM Gal vs 10uM BaP +100uM Gal	-0.1324	0.8945	P > 0.05	-0.8773 to 0.6126
100uM Gal vs 7uM PAH	-0.3466	2.342	P > 0.05	-1.092 to 0.3983
100uM Gal vs 7uM PAH + 5uM Gal	0.16	1.081	P > 0.05	-0.5850 to 0.9050
100uM Gal vs 7uM PAH + 30uM Gal	0.2858	1.931	P > 0.05	-0.4592 to 1.031
100uM Gal vs 7uM PAH +100uM Gal	0.432	2.919	P > 0.05	-0.3130 to 1.177
10uM BaP vs 10uM BaP +5uM Gal	-0.1816	1.227	P > 0.05	-0.9266 to 0.5633
10uM BaP vs 10uM BaP +30uM Gal	0.03638	0.2458	P > 0.05	-0.7086 to 0.7813
10uM BaP vs 10uM BaP +100uM Gal	0.782	5.287	P < 0.05	0.03741 to 1.527
10uM BaP vs 7uM PAH	0.5681	3.839	P > 0.05	-0.1768 to 1.313
10uM BaP vs 7uM PAH + 5uM Gal	1.075	7.262	P < 0.001	0.3298 to 1.820
10uM BaP vs 7uM PAH + 30uM Gal	1.201	8.112	P < 0.001	0.4555 to 1.945
10uM BaP vs 7uM PAH +100uM Gal	1.347	9.100	P < 0.001	0.6018 to 2.092
10uM BaP +5uM Gal vs 10uM BaP +30uM Gal	0.218	1.473	P > 0.05	-0.5270 to 0.9630
10uM BaP +5uM Gal vs 10uM BaP +100uM Gal	0.964	6.514	P < 0.01	0.2190 to 1.709
10uM BaP +5uM Gal vs 7uM PAH	0.750	5.066	P < 0.05	0.004788 to 1.495
10uM BaP +5uM Gal vs 7uM PAH + 5uM Gal	1.256	8.489	P < 0.001	0.5114 to 2.001
10uM BaP +5uM Gal vs 7uM PAH + 30uM Gal	1.382	9.339	P < 0.001	0.6372 to 2.127
10uM BaP +5uM Gal vs 7uM PAH +100uM Gal	1.528	10.330	P < 0.001	0.7834 to 2.273
10uM BaP +30uM Gal vs 10uM BaP +100uM Gal	0.746	5.041	P < 0.05	0.001038 to 1.491
10uM BaP +30uM Gal vs 7uM PAH	0.5318	3.593	P > 0.05	-0.2132 to 1.277
10uM BaP +30uM Gal vs 7uM PAH + 5uM Gal	1.038	7.016	P < 0.001	0.2934 to 1.783
10uM BaP +30uM Gal vs 7uM PAH + 30uM Gal	1.164	7.866	P < 0.001	0.4192 to 1.909
10uM BaP +30uM Gal vs 7uM PAH +100uM Gal	1.310	8.854	P < 0.001	0.5654 to 2.055
10uM BaP +100uM Gal vs 7uM PAH	-0.2143	1.448	P > 0.05	-0.9592 to 0.5307
10uM BaP +100uM Gal vs 7uM PAH + 5uM Gal	0.2924	1.976	P > 0.05	-0.4526 to 1.037
10uM BaP +100uM Gal vs 7uM PAH + 30uM Gal	0.4181	2.825	P > 0.05	-0.3268 to 1.163
10uM BaP +100uM Gal vs 7uM PAH +100uM Gal	0.5644	3.814	P > 0.05	-0.1806 to 1.309
7uM PAH vs 7uM PAH + 5uM Gal	0.5066	3.423	P > 0.05	-0.2383 to 1.252
7uM PAH vs 7uM PAH + 30uM Gal	0.6324	4.273	P > 0.05	-0.1126 to 1.377
7uM PAH vs 7uM PAH +100uM Gal	0.779	5.261	P < 0.05	0.03366 to 1.524
7uM PAH + 5uM Gal vs 7uM PAH + 30uM Gal	0.1258	0.8497	P > 0.05	-0.6192 to 0.8707
7uM PAH + 5uM Gal vs 7uM PAH +100uM Gal	0.272	1.838	P > 0.05	-0.4730 to 1.017
7uM PAH + 30uM Gal vs 7uM PAH +100uM Gal	0.1463	0.9882	P > 0.05	-0.5987 to 0.8912