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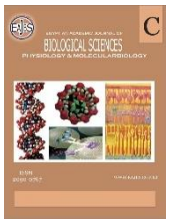

Potassium Benzoate Induced Cytotoxicity via Alteration of Gene Expression-Associated Apoptosis and Cell Cycle Genes in Tumor Cell Lines

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ABSTRACT

Certain food preservatives, including potassium benzoate, have been shown to possess carcinogenic properties. This study investigated the cytotoxic effects of potassium benzoate, a common food additive, on HepG2 liver cancer cells and THLE2 non-tumorigenic liver cells. Using the Neutral Red assay, we observed a concentration-dependent decrease in cell viability for both cell lines, with HepG2 cells showing higher sensitivity. The IC50 values were determined to be 72.50 µg/mL for HepG2 cells and 645.7 µg/mL for THLE2 cells, indicating potassium benzoate's potential cytotoxicity on both malignant and non-malignant cells. Flow cytometry analysis revealed that potassium benzoate treatment induced G2/M phase arrest in HepG2 cells, with a significant increase in the proportion of cells in the G2/M phase from 12% to 43%. Gene expression analysis using qRT-PCR demonstrated upregulation of p53, Bax, and p21, while Bcl-2, cyclin B1, and CDK1 were downregulated. These findings suggest that potassium benzoate has a high potential for cytotoxicity and genotoxicity when used as a food preservative. This study contributes to the growing body of evidence on the potential health impacts of food additives and underscores the need for further research into their long-term effects on human health.

INTRODUCTION

Food preservatives play a critical role in modern food production, protecting food against spoilage, and enhancement but they can have adverse effects on human cells (Patel and Ramani, 2017, Shaker *et al.*, 2022, Shankar *et al.*, 2024). Preservatives are substances that can inhibit, and/or stop the microorganisms' growth or any degradation they cause. The large dosages of preservatives have hazardous or toxic properties on human and animal bodies. A direct effect on cells and DNA could appear (Patel and Ramani (2017). According to several studies, excessive consumption of food preservatives has been shown to cause harmful effects including irritable bowel syndrome, diarrhea, nausea, vomiting, rhinitis, bronchospasms, urticaria, eczema, dermatitis, angioedema, hyperactivity, migraine, and other behavioral disorders. Moreover, toxic compounds and preservatives can cause attention deficit hyperactivity disorder (ADHD), asthma, heart difficulties, obesity, cancer, and other conditions (Tuormaa, 1994 & Sambu *et al.*, 2022).

In addition, certain types of food preservatives and coloring agents can contribute to the activation of inflammatory pathways. This effect is seen in a dose-dependent matter and may induce carcinogenesis or increase the likelihood of developing cancer Raposa *et al*. (2016).

Potassium Benzoates [E212] and sodium [E211] are the most commonly used preservatives, found in soft drinks, pickles, fruit juices, margarine, cider, fruit jam, syrup, and fruit butter (Emerald Kalama Chemical, 2004). Moreover, they are also utilized in baby goods and other health and personal-care products, as well as in agriculture where they are found in animal feed and poultry products. Potassium benzoate can be used to replace sodium benzoate when the preserving power of benzoic acid is desired but low sodium content is required to meet dietary guidelines. It is also often utilized in marmalades, low-sugar jams, jelly, and alcohol-free beer (New Seed Chemical, 2015).

Potassium benzoate significantly increased the number of chromosomal aberrations per cell at all studied concentrations from 62.5 to 1000 μg/mL and in all treatment groups for periods of 24 and 48 hours. The same study also found that it significantly increased the exchanges of sister chromatid per cell at all concentrations and in all treatment groups, caused delays in the cell cycle, also significantly increased the formation of micronuclei in nearly all the tested concentrations for a period of 48 h (Zengin *et al*., 2011).

Food preservatives can be dangerous if restrictions limiting their use are unintentionally or purposefully violated. Thus, policies on the quantity to be added to food should be enforced (Olofinnade *et al.,* 2021).

Potassium benzoate has been seen to induce toxicity and teratogenicity in embryonic development. The possible teratogenic impacts of Potassium benzoate on embryonic eye development were investigated and were found to decrease cell proliferation (Afshar *et al.,* 2013). Potassium benzoate additives caused histopathologic effects in the eyes of mice fetuses; nevertheless, the mechanisms behind this action remain unknown. Intriguingly, sodium benzoate has beneficial effects in the treatment of schizophrenia (Jarskog, 2006), melanogenic properties, and has been demonstrated to have memory-protective activity in multiple sclerosis patients and those with Alzheimer's disease (Modi *et al.,* 2015).

The liver, as the primary organ responsible for detoxification, is particularly vulnerable to the effects of ingested substances. Therefore, studying the impact of food additives on liver cells is crucial for understanding their potential systemic effects. The HepG2 cell line, derived from human hepatocellular carcinoma, has been widely used as an in vitro model for hepatotoxicity studies (Waldherr *et al.,* 2018). Additionally, comparing the effects on cancerous cells (HepG2) with those on non-tumorigenic cells (THLE2) can provide insights into the differential cytotoxicity of these compounds.

Recent advancements in molecular biology techniques have enabled more detailed investigations into the mechanisms of cytotoxicity. Flow cytometry analysis allows for precise examination of cell cycle perturbations (Adan *et al.,* 2017 and Hussein *et al.,* 2021), while quantitative real-time PCR (qRT-PCR) enables the measurement of changes in gene expression levels of key regulators involved in cell cycle control and apoptosis (Neumann *et al.,* 2016).

The present study aims to elucidate the cytotoxic effects of potassium benzoate on HepG2 and THLE2 liver cells, focusing on cell viability, cell cycle distribution, and the expression of genes related to apoptosis and cell cycle regulation. By employing a multi-faceted approach, we seek to provide a comprehensive understanding of the cellular responses to this widely used food additive. Our findings may contribute to the ongoing assessment of food additive safety and inform future regulatory decisions.

MATERIALS AND METHODS

Cell Lines and Culture Conditions:

Human hepatocellular carcinoma cells (HepG2) and non-tumorigenic liver cells (THLE2) were obtained from the American Type Culture Collection (ATCC) CRL-10742™ and CRL-2706™, respectively (Manassas, Virginia USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cultures were maintained at 37°C in a humidified atmosphere with 5% CO_2.

Determination of Cytotoxicity Activity on Liver Cell line:

In vitro, a neutral red (NR) cytotoxicity test was done by the cell culture laboratory (Cairo University Research Park, Faculty of Agriculture, Cairo University).

NR-cytotoxicity assay was performed as described by Fotakis and Timbrell (2006). Cells were seeded in a microtiter plate (24-well) with 1X106 cells/well. Plates were incubated at 37ºC for 24 h in CO2 (5%) to accomplish monolayer confluence.

The effects of five various doses of potassium benzoate (10, 25, 50, 100, and 200 µg/mL) were added to the human hepatocellular carcinoma cancer cells (HepG2) and normal liver cells (THLE2) *in vitro*. This concentration range was chosen based on preliminary cytotoxicity screening and previous literature on food additive toxicity studies. These concentrations encompass levels below and above the typical concentrations found in food products, allowing for the assessment of dose-dependent effects. In addition, different concentrations of doxorubicin (3µg/ml) were also added as a reference chemo-therapeutic drug (positive control treatment for comparison). Medium without chemical treatment was used as a control. After treatment, the dye medium was eliminated, and the plates were cleaned with formol-calcium. Acetic acid-ethanol (500µL) was added, and plates were left for 15 min at ambient temperature for dye extraction. Spectrophotometric readings were performed using a SpectraMax 190 Microplate Reader with a 540 nm filter to determine the absorbance of the extracted dye. The experiment was performed in triplicate and repeated three times independently.

Cell Cycle By Propidium Iodide (PI) Via Flow Cytometry:

HepG2 cells were digested with 500 µL warm Trypsin-EDTA and 500 µL warm PBS-EDTA (0.25% phosphate-buffered saline-EDTA) and kept for 10 minutes at 37°C. The pellet was rinsed twice with warm PBS and resuspended in 500 µL PBS before spinning and supernatant removal. To fix the cells, add 150 µL PBS and 350 µL ice-cold 70% ethanol. Mix them using a pipette, vortex them multiple times, and keep them at 4°C for 1 hour. To eliminate the ethanol, the mixture was spun up for 10 minutes at 350 rpm before the supernatant was carefully eliminated. The mixture was washed twice with warm PBS. The cell pellet was immersed again in 500 µL of warm PBS before centrifugation and supernatant removal. The resulting pellet was resuspended in 100 µL of PBS and kept at 4°C for up to four days. Cells have been coated with 100 µL PI solution and 50 µL RNase A solution (100 µg/mL) and kept in the dark for 30-60 minutes. The dyed cells were examined using an Attune flow cytometer (Applied Bio-systems, USA). The results indicate the average of three separate trials. The experiment was performed and repeated three times independently.

Expression Levels of Apoptosis and Cell Cycle Regulatory Genes:

The expression level of mRNA of apoptotic-related (*p53*, *Bax*, and *Bcl-2*) genes and cell cycle regulatory genes (*p21, CycB1*, and *CDK1*) were examined using two-step quantitative RT-PCR. The IC_{50} concentration (72.5 µg/ml) was used to treat the cell in this study. The total cellular RNA was isolated from the treated and untreated cells using the Tri-Pure Isolation Reagent kit following the directions provided by the company (Roche, USA).

RNA (5 µg) was reverse transcribed into cDNA using Quantiscript reverse

transcriptase (Qiagen, #205310). The cDNA was employed as a template to measure the relative expression of mRNA of the target genes in HepG2 cells, using β-actin as the standard gene. The extracted cDNA was amplified with 2X Maxima SYBR Green/ROX qPCR Master Mix according to the company's procedure (Thermo Scientific, USA, #K0221) and gene-specific primers are listed in Table (1). The experiment was performed and repeated three times independently. The relative change in gene expression was reported as a fold change utilizing the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001).

Table 1: Forward and reverse primers sequence for genes under investigation

Primer	Sequence		Reference
p53	F- 5'-CCCAGGTCCAGATGAAG-3'	R- 5'-CAGACGGAAACCGTAGC-3'	**Al-Senosy *et al.*, 2018**
Bcl-2	F-5'-GGATGCCTTTGTGGAACTGT-3'	R-5'-AGCCTGCAGCTTTGTTTCAT-3'	**Al-Senosy *et al.*, 2018**
Bax	F-5'-TTTGCTTCAGGGTTTCATC-3'	R-5'-CAGTTGAAGTTGCCGTCAGA-3'	**Al-Senosy *et al.*, 2018**
P21	F-5'-ATGAAATTCACCCCCTTTCC -3'	R-5'-CCCTAGGCTGTGCTCACTTC -3'	**Komissarova and Rossman 2010**
CycB1	F-5'- CTCCTGTCTGGTGGGAGGA -3'	R-5'-CTGATCCAGAATAACACCTGA-3'	**El-Attar *et al.*, 2019**
CDK1	F-5'- TTTTCAGAGCTTTGGGCACT -3'	R-5'- CCATTTTGCCAGAAATTCGT-3'	**El-Attar *et al.*, 2019**
β-actin	F-5'- CACCAACTGGGACGACAT -3'	R-5'- ACAGCCTGGATAGCAACG -3'	**Al Senosy *et al.*, 2018**

Statistical Analysis:

The variation between the groups was determined using a one-way analysis of variance (ANOVA) with GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA). Means were compared using Tukey's honestly significant difference test. The data was given as mean ± standard error (SEM), and the level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Assessment of *In Vitro* Cytotoxicity on Human Cell Lines Using NR Cytotoxicity Assay:

The Neutral Red assay revealed a concentration-dependent decrease in cell viability for both HepG2 and THLE2 cell lines. However, increased cytotoxicity was higher in the HepG2 cell line than in THLE2 cells. According to data analysis, potassium benzoate was not harmful to HepG2 or THLE2 cells at 10 µg/ml concentration. Potassium benzoate significantly lowered the viability of HepG2 cancer cells at 25 µg/ml dosage, which exhibited a 31% reduction in viability while THLE2 cells showed minimal effects. Higher doses were required to alter the viability of the THLE-2 cell line. Additionally, the analysis of data revealed that potassium benzoate reduced cell viability in both normal and tumor cells, indicating a cytotoxic impact; the dose-response curve for cell viability is shown in Figure (1). Doxorubicin and potassium benzoate had IC50s of 26.82 and 72.50 µg/mL, respectively, against hepatoma cell line cells (HepG2), whereas THLE2 had an IC50 of 645.7 µg/mL after potassium benzoate treatment. These findings suggest that potassium benzoate may have a cytotoxic impact on both malignant and non-malignant cells but HepG2 cells are significantly more sensitive to potassium benzoate.

The NR assay is a quick, cost-effective, semi-automated assay that may be employed with many cell types in culture to provide quantitative results for ranking test compounds based on their potency. Babich and Borenfreund (1990) used this test to assess the cytotoxicity of butylated hydroxyl toluene and butylated hydroxyl anisole on keratinocytes, human dermal fibroblasts, melanocytes, and melanoma tumor cells.

As previously stated, potassium benzoate gave the greatest cytotoxic impact of

the investigated food additives. Clastogenic, cytotoxic, and mutagenic effects of the mentioned preservatives have been proven *in vitro* using tests on human lymphocytes, and these agents may be carcinogenic as well (Türkoğlu, 2007). The use of cytotoxicity assays for food additive safety estimation is predicted to be helpful for evaluating the total possible cytotoxicity caused by the use of different additives, as well as the synergistic and antagonistic effects they may have in the presence of different toxins. The cytotoxicity assay is faster and simpler than animal tests for detecting synergistic effects within the various combinations of food additives (Yamashoji and Isshiki, 2001).

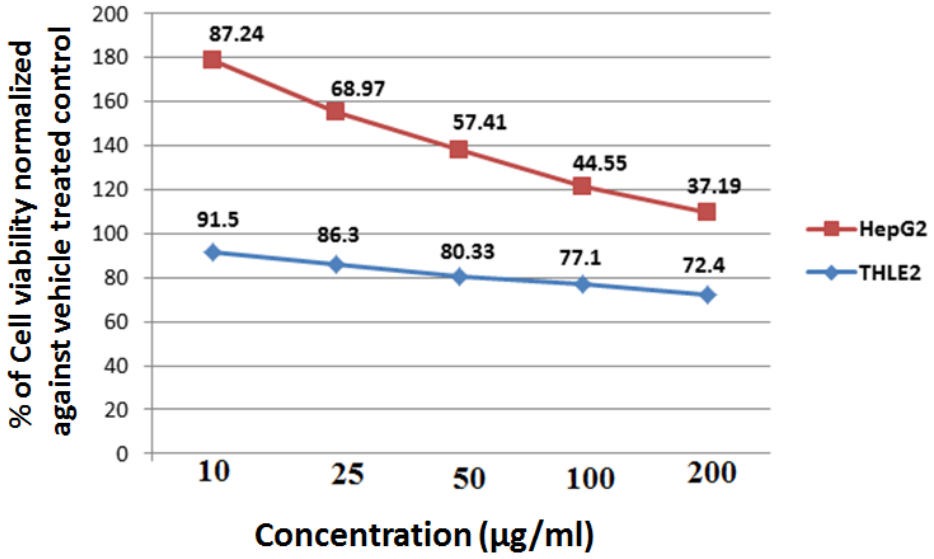

Fig. 1. Cytotoxicity of Potassium benzoate at different concentrations to HepG2 and THLE2 cells. The viability was quantitated by *in vitro* neutral red cytotoxic assay. The results are presented as the mean ± SD of three replicates.

Some investigations have found that benzoates have genotoxic effects. Chromosome abnormalities have been seen in human lymphocytes, Chinese hamster cells, and mouse bone marrow cells subjected to sorbate and/or benzoate (Piper and Piper (2017), Pongsavee (2015), Mamur *et al.*, 2010). Benzoic acid has been associated with sister chromatid exchange, chromosomal abnormalities, and micronucleus prevalence in human cells. (Al-Tai, 2014). Furthermore, the genotoxic impacts of sodium benzoate on human cells were reduced at various dosages utilizing sister chromatid exchange and chromosomal aberration tests (Patel and Ramani, 2017).

Park *et al*. (2011) found that sodium benzoate reduced the viability of rat cortical neuron cells. These findings align with the research results of Yilmaz and Karabay (2018), who discovered that sodium benzoate has substantial cytotoxic effects on both colon cancer (HCT116) cells and fibroblast (L929) cells, however, the induced cytotoxicity was greater in HCT116 than L929.

Using the MTT test, Wei *et al.* (2016) discovered that borax reduced the viability of Hep-G2 cells, inhibiting their proliferation. Furthermore, Canturk *et al.* (2016) employed the NR cytotoxicity test to determine the cytotoxicity of boric on healthy human lymphocytes and (HL-60) acute leukemia cell line.

Comparable results were found by El-Hefny *et al.*, (2020), who demonstrated that benzoic acid inhibits cell proliferation while also promoting apoptosis using MTT.

Effect of Potassium Benzoate on Cell Cycle Arrest Via Flow Cytometry.

Concerning the reduction rate of liver tumor (HepG2) cell viability, it was important to evaluate the cytotoxic impact of potassium

benzoate on cell cycle arrest via flow cytometry to investigate cell cycle distribution. Flow cytometry analysis confirmed that a potassium benzoate concentration of 72.50 µg/mL resulted in a significant increase in G2/M phase arrest, with the percentage of cells in this phase rising from 21% ± 1.56 in the control to 43% ± 2.18 in the treated group. Conversely, the percentage of cells in the G0/G1 phase significantly decrease from 60% ± 1.4 to 37% ± 1.83. These findings are reported in Table (2) and Figure (2). Mamur *et al*. (2010) showed that potassium sorbate can induce cell cycle arrest. Other studies have shown that preservatives and colorants can have mutagenesis and cytotoxic effects, as well as disrupt the cell cycle (Türkoğlu, 2007; Poul *et al*., 2009). Many cytotoxic chemicals cause mitotic cell death (apoptosis), which coincides with G2-M arrest (Sleiman and Stewart, 2000). Chung *et al*. (2017) discovered that sinularin caused G2/M arrest by upregulating the expression of G2/M phase genes such as p53 and p21. Additionally, sinularin promoted apoptosis by lowering the expression of the anti-apoptotic Bcl-2 gene while raising the Bax expression.

Table 2. Effect of Potassium benzoate on the arrest of the cell cycle. HepG2 and THLE2 were treated with Potassium benzoate at 72.50 µg/mL to check the distribution of the cell cycle. Cells were incubated in the presence of food additive and absence of control for 24 h followed by flow cytometry analysis.

Phase	Control	Cells treated with Potassium Benzoate
G_0/G_1	60% ± 1.40	37% ± 1.83*
S	19% ± 1.01	20% ± 1.05
G_2/M	21% ± 1.56	43% ± 2.18*

∗ Statistical significance was determined using one-way ANOVA and considered significant at ($p < 0.05$) between treated and control cells

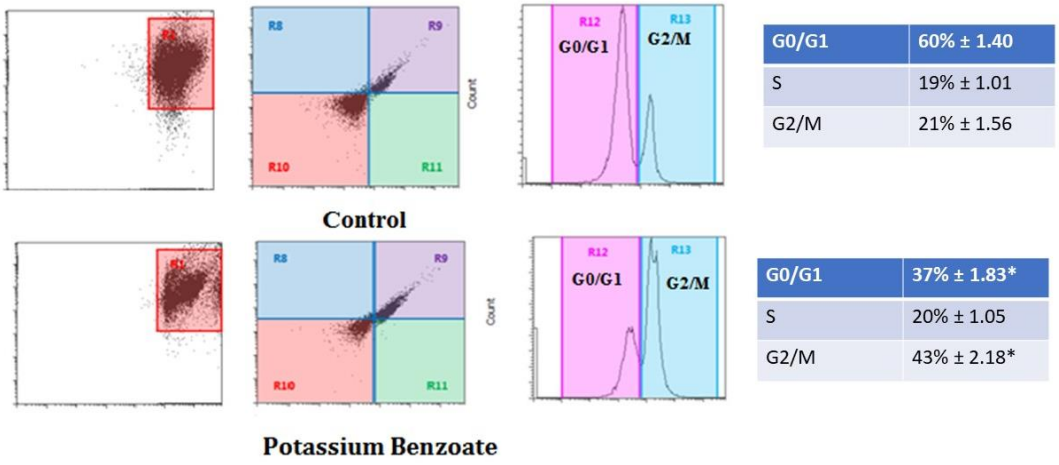

Fig 2. Impact of Potassium Benzoate on the cell cycle arrest. Liver cancer cells (HepG2) were treated with Potassium Benzoate at 72.50µg/mL to examine the cell cycle distribution followed by flow cytometry analysis. The data are presented as means ± SEM.
For three replicates. * Significantly at $P < .05$.

Expression Level Assessment of Apoptosis Regulatory Genes:

The effect of apoptosis in potassium benzoate-induced cytotoxicity of liver cancer (HepG2) cell lines was investigated. Real-time PCR (qRT-PCR) was carried out to evaluate apoptosis-related gene expression levels in HepG2 cells, including p53, Bax, and Bcl-2. The IC50 dosage of potassium benzoate (72.5µg/ml) was used as a cell

treatment in this investigation. Figure (3) revealed that p53 and Bax expression levels were significantly elevated in these cells than in the control, whereas Bcl-2 expression was reduced.

In addition to these genes, the expression levels of cell cycle regulatory genes, along with *p21*, *cyclin B1*, and *CDK1* in HepG2 cells were also determined. Figure (3) shows that the expression level of *p21* was up-regulated in comparison to the untreated control HepG2 cells, whereas the expression levels of *CDK1* and *cyclin B1* were downregulated at a dose of potassium benzoate equivalent to their IC50 for 24 h. The results indicated that potassium benzoate was able to trigger G2/M arrest and that this mechanism involved both transcriptional suppression and apoptosis induction.

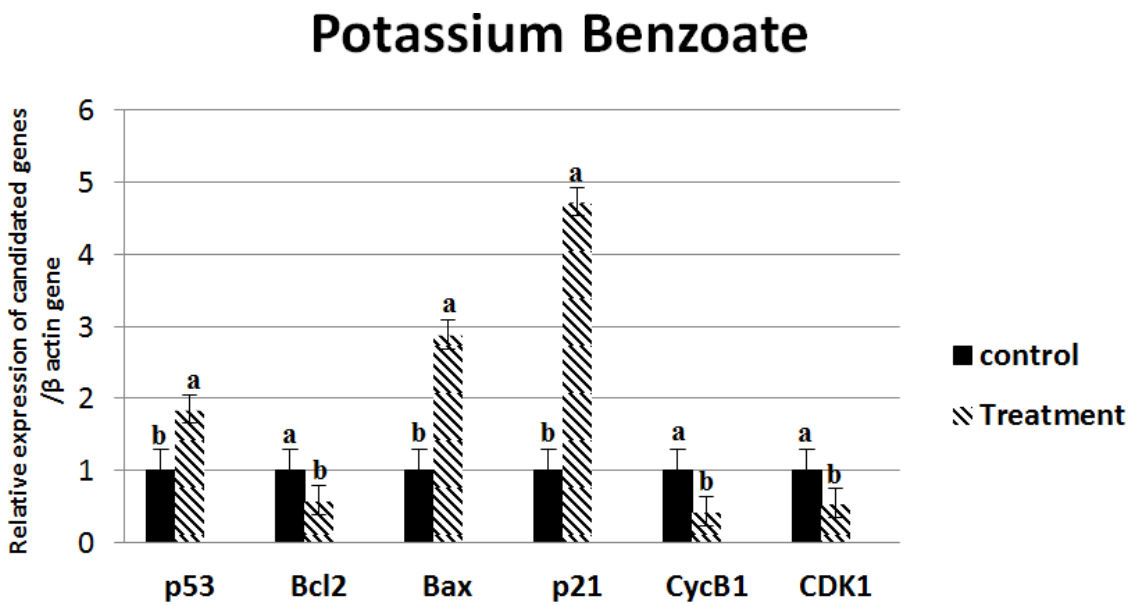

Fig. 3. Effects of Potassium Benzoate on cell cycle and apoptosis-related genes after exposure HepG2 cells, mRNA expression of *p53*, *Bcl-2*, and *Bax* as apoptosis-related genes. qRT-PCR was used to examine the cell cycle regulatory genes (p21, CycB1, and CDK1). The findings were calibrated to β-actin. Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test. Columns containing various letters show significant differences at $P < 0.05$.

Impact of sodium benzoate on cell viability in liver cell line normal THLE2 and cancer HepG2, looking at *P53* activity and the activity of *Bcl*-2 and *Bax* genes, which are important key regulators of apoptosis. The findings showed that sodium benzoate exposure inhibited cell viability by inducing apoptosis. The cytotoxic activity of sodium benzoate on HepG2 was higher than that seen on THLE2 cells (non-tumorigenic) at the same concentrations (Aledwany *et al*., 2018). These findings align with and support our experimental observations.

Changes in gene expression are one of the early reactions to chemical exposure. Assessment of gene expression variations associated with chemical exposure is critical for establishing knowledge of the toxicology mechanisms begun by specific chemical exposures (Aardema and MacGregor, 2005). Alteration of p53 gene expression-dependent apoptosis is one of the most common indicators of genotoxicity; any chemical that may increase p53 expression is considered a genotoxin (Strasser *et al*., 1994 & Kirsch-Volders *et al*., 2003).

Several studies have found that p53 is crucial in maintaining the equilibrium between proliferation and apoptosis (Polager and Ginsberg, 2009). This gene is essential

for the G2 checkpoint since it suppresses the G2/M transition. Furthermore, p53 balances the proapoptotic (Bax) gene and the antiapoptotic (Bcl-2) gene via regulating transcriptional activity (Leu *et al*., 2004). Interestingly, Raposa *et al*., (2016) detected beneficial effects of sodium benzoate exposure in the treatment of schizophrenia; these effects could be due to the activation of the *MAPK8* gene, leading to the downregulation of apoptotic activity (Jarskog, 2006).

Sodium benzoate was reported to induce the expression of caspase-3 in HCT116 colon cancer cells at doses ranging from 12.5 to 50 mM. It also enhanced Bim gene expression, but there was little change in the Bcl-xl expression level; this might support the stimulation of apoptotic signaling by this chemical (Yilmaz and Karabay 2018).

Zhao *et al.*, (2017) investigated the impact of specific matter PM (2.5 micron) on alterations in cell cycle-associated gene expression in vitro and in vivo. Increased expression of p53 and CDK2 indicates an attempt to repair cellular damage and maintain homeostasis through metaphase/anaphase arrest during early genotoxic exposure. Dysregulation of this cycle may become a sign of carcinogenesis. After long-term exposure to PM (2.5 microns), p21 expression increased dramatically. p21 encodes a strong CDK inhibitor that attaches and prevents the activity of cyclin-CDK2 or CDK4 complexes, acting as a G1-phase cell cycle regulator. p21 is often misregulated in human malignancies, although its expression pattern varies depending on the cellular context and circumstances; it can function as either a tumor suppressor or an oncogene. This gene expression is tightly regulated by p53, and this protein facilitates p53-dependent G1 phase arrest in response to a range of stress stimuli (Abbas and Dutta, 2009; Pan *et al.*, 2014).

p21 prevents CDK1 kinase activity that follows genotoxic tensions, causing growth arrest in the G2 phase of the cell cycle (Abbas and Dutta 2009, Akter *et al.*, 2021). This suggested that CDK1 may be a key target in carcinogenesis in some tissues.

The results of this study agree with those found by Zengin *et al*. (2011) who discovered that potassium benzoate has mutagenic and cytotoxic activity on human *in vitro* lymphocytes. It was observed that concentrations from 62.5 to 1000 µg/mL of potassium benzoate reduced the mitotic index, and elevated the chromosomal aberration frequency, sister chromatid exchange, and micronuclei in human lymphocytes.

Yilmaz and Karabay (2018) evaluated the effect of sodium benzoate on cell viability in colon cancer (HCT116) cell lines, looking at caspase-3 activity and the activity of NFkB, Bcl-xl, Bim genes, which are important key regulators of apoptosis. The findings demonstrated that sodium benzoate treatment inhibited cell viability by inducing apoptosis. The cytotoxic activity of sodium benzoate on HCT116 was higher than that seen on L929 fibroblast cells (non-tumorigenic) at the same concentrations.

Our findings are consistent with those of El-Hefny *et al.*, (2020), who found that treatment with benzoic acid increased the gene expression of the tumor suppressor (p53) and pro-apoptotic (Bax) while decreasing the gene expression of the apoptosis regulator (Bcl-2) in HepG2 cells.

Finally, our findings support the hypothesis that potassium benzoate induces cytotoxicity in liver cancer cells, particularly through mechanisms involving apoptosis and cell cycle regulation. The significant reduction in cell viability, especially in HepG2 cells, suggests that potassium benzoate may pose health risks associated with its consumption as a food preservative.

The observed G2/M phase arrest aligns with previous studies indicating that various food additives can disrupt normal cell cycle progression, leading to increased apoptosis. The upregulation of pro-apoptotic genes, such as p53 and Bax, and the downregulation of anti-apoptotic Bcl-2 support the notion that potassium benzoate promotes apoptotic pathways in HepG2 cells.

These results highlight the potential genotoxic effects of potassium benzoate and underscore the need for further research to elucidate the long-term consequences of its consumption on human health. Regulatory policies should consider these findings to ensure food safety and protect public health.

Several limitations should be considered. Firstly, the in vitro nature of our experiments may not fully represent the complex in vivo environment, including metabolic processes and tissue interactions. Secondly, our focus on acute exposure effects does not address potential long-term, low-dose exposure scenarios that are more relevant to human consumption patterns. Additionally, the use of only two cell lines limits the generalizability of our findings to other cell types or tissues. Future studies should address these limitations by incorporating in vivo models, examining chronic low-dose exposures, and expanding the range of cell types investigated.

Conclusions

This study evaluated the cytotoxicity of potassium benzoate, a food preservative commonly used in industry, by *in vitro* NR cytotoxic assays on human liver cancer and normal liver cell lines. Cell cycle arrest and genes associated with cell cycle regulation and apoptosis in the HepG2 cell line were investigated as well. Our findings demonstrated that potassium benzoate increased cytotoxicity by decreasing the viability of both liver normal and cancer cells. Cell cycle inhibition at the G2/M phase was evident. Furthermore, induction of apoptosis via increased *p53* and *Bax* expression combined with decreased *Bcl-2* gene expression correlated with the induction of the apoptosis pathway. Moreover, the mRNA expression of cycle-associated genes were altered. This provides evidence that potassium benzoate can act as a mutagen with the potential to induce cancer.

Declarations:

Ethical Approval: Not applicable

Conflict of interests: The authors declare no conflicts of interest.

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