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In vitro Cytotoxicity Profiling of Croscarmellose Sodium and Selected Plasticisers on A549 and Caco-2 Cancer Cell Lines

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Abstract

Human exposure to pharmaceutical excipients and plasticisers is ubiquitous, comparable *in vitro* cytotoxicity data with human-derived cell models are rare. The cytotoxic potential of croscarmellose sodium (CCS), trioctyltrimellitate (TOTM), and bis(2-ethylhexyl) phthalate (DEHP), all routinely used to modify disintegration, flexibility and processability in pharmaceutical formulations, was evaluated using the MTT metabolic viability assay as a surrogate measure of cellular integrity and proliferative capacity. The human cell lines used were the lung adenocarcinoma (A549) and the colorectal adenocarcinoma (Caco-2) cell lines as typical pulmonary and intestinal epithelial models, respectively, and the transformed embryonic kidney line (HEK293T) as a non-epithelial comparison. Cells were treated with doses ranging from 5 to 100 µM for 24-72 h. All three compounds reduced the metabolic activity in a dose-dependent manner in A549 and Caco-2 cells, although HEK293T cells were somewhat less sensitive. CCS caused a little viability decrease, as expected from its known low toxicity. In contrast, TOTM and DEHP generated more marked cytotoxic effects and A549 cells were more susceptible than Caco-2 cells. Differential cellular susceptibility was also highlighted by divergence in calculated IC_{50} values among models. These results taken together show that plasticisers are capable of producing quantifiable deleterious effects at doses typically seen. They give preliminary cytotoxicity data useful to toxicological screening and hazard identification. The results are specific to the setting of a regulatory risk assessment. Future work with mechanistic endpoints, physiologically relevant exposure doses, and *in vivo* validation will be important to enhance excipient safety evaluation and influence formulation design.

Keywords: *In vitro* Cytotoxicity, MTT Assay, Plasticisers (Phthalates and Trimellitates), Pharmaceutical Excipients, Toxicological Screening.

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1. Introduction

Pharmaceutical excipients and plasticisers are a broad category of chemicals that are purposefully added to consumer, pharmaceutical, medical, and food-contact goods to improve manufacturability, stability, and functional performance[1–4]. Disintegrants, binders, and fillers are examples of excipients that are essential to medication formulation and delivery because they support factors like patient acceptability, dissolving, and bioavailability[1,2]. Because of its hydration-swelling qualities and capacity to promote quick tablet disintegration and drug release, croscarmellose sodium (CCS), a cross-linked cellulose derivative, is frequently used as a super-disintegrant in solid oral dosage forms[5]. Although systematic cellular toxicity data are

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still comparatively scarce when compared to active pharmaceutical ingredients, CCS is widely considered safe for pharmaceutical usage based on known toxicological studies and is included in major pharmacopoeias[6]. On the other hand, to provide flexibility, durability, and processability, plasticisers such as phthalates and trimellitates are added to polymeric materials, such as polyvinyl chloride (PVC), which is used in blood bags, medical tubing, and food packaging[7]. Because of their ability to disrupt hormones and their documented links to reproductive, developmental, metabolic, and organ-specific toxicities in experimental and epidemiological investigations, phthalate plasticisers have come under increased scientific and regulatory attention[8]. Human exposure to plasticisers is common and can happen through cutaneous absorption, consumption of contaminated food, inhalation of polluted indoor air or dust, and migration from medical devices during clinical procedures, such as parenteral nutrition and transfusions[9].

There is a lack of comparative *in vitro* data analysing the effects of excipients and plasticisers on cellular metabolic activity and survivability across several human-derived cell models, despite widespread regulatory interest. Prior to further in-depth mechanistic, *in vivo*, or regulatory studies, cell-based assays offer a controlled platform for initial toxicological screening, enabling evaluation of dose-dependent cytotoxic responses, cellular susceptibility, and hazard identification[10–12]. Among these, the MTT test is frequently used to measure changes in mitochondrial metabolic activity as a stand-in for metabolic stress and cell viability[11]. MTT tests provide valuable first-tier cytotoxicity information pertinent to toxicological classification and comparative chemical assessment, even though they do not clarify molecular processes like apoptosis, necrosis, or oxidative stress[13].

Using an MTT-based metabolic viability assay, the current study sought to compare the *in vitro* cytotoxic effects of CCS, trioctyltrimellitate (TOTM), and bis(2-ethylhexyl) phthalate (DEHP) in three human-derived cell lines: lung adenocarcinoma (A549), colorectal adenocarcinoma (Caco-2), and transformed embryonic kidney (HEK293T). The goal was to produce initial toxicological data that described cellular susceptibility differences and concentration-dependent metabolic responses in representative epithelium and non-epithelial models. These results do not imply therapeutic, anticancer, or clinical applicability; rather, they are just meant for toxicological screening and hazard assessment[9,14].

2. Materials and Methods

Sigma-Aldrich (USA) provided trioctyltrimellitate and bis(2-ethylhexyl) phthalate, while Amazon provided croscarmellose sodium. Table 1 provides a summary of these compounds' molecular formulae and physicochemical characteristics.

Cisplatin (positive control) is a pharmaceutical-grade alkylating agent used to treat solid tumours and haematologic malignancies. Sigma-Aldrich (St. Louis, MO, USA) has been supplying trypsin-EDTA, Dulbecco's Modified Eagle Medium (DMEM), phosphate-buffered saline (PBS), and MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. Gibco (Thermo Fisher Scientific, USA) provided the foetal bovine serum (FBS). We bought 96-well plates and cell culture flasks (25 cm^2 and 75 cm^2) from Eppendorf (India).

2.1 Culture of Cells

The cells were kept in DMEM supplemented with 1% (v/v) penicillin–streptomycin and 10% (v/v) FBS. Cultures were kept in a humidified environment with 5% CO_2 at 37 °C. When cells reached 70–80% confluence, they were subcultured using 0.25% trypsin–EDTA and seeded into the proper culture containers for the experiments that followed.

2.2 MTT Cytotoxicity Test

The MTT method was used to measure cell viability. In short, cells were seeded at a density that would allow for

Table 1. Identification and chemical properties

Compound name (Plasticizers)	Molecular Formula	Molecular Weight	Physical State	References
Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$ or $C_6H_4(COOC_8H_{17})_2$	390.6 g/mol	Colourless to pale yellow oily liquid	[15]
TrioctylTrimellitate(Trioctyl benzene-1,2,4-tricarboxylate)	$C_{33}H_{54}O_6$	546.8 g/mol	Liquid	[16]
Croscarmellose	$C_8H_{16}O_8$	240.21 g/mol	White to off-white powder	[17]

logarithmic growth in 24 hours on 96-well plates. After adding test samples and cisplatin (positive control) at the appropriate amounts, the mixture was incubated for 24 to 48 hours. Following treatment, each well was added 20 µL of MTT solution (5 mg mL^{-1} in PBS), and the plates were further incubated for 3 to 4 hours. After removing the medium, dimethyl sulfoxide (DMSO) was used to dissolve the formazan crystals. A microplate reader was used to quantify absorbance at 570 nm. In comparison to untreated control cells, cell viability (%) was computed.

2.3 Cell Lines and Maintenance

Three cell lines were used in the assay: the non-malignant HEK293T cell line, the Caco-2 colon cancer cell line, and the A549 lung cancer cell line. Adenocarcinomic human alveolar basal epithelial cells, or A549 cells, were first created in 1972 by D. J. Giard and associates using explant culture of malignant lung tissue from a male Caucasian patient who was 58 years old[18]. They are frequently used as an *in vitro* model for studying lung cancer and developing anticancer medications. The type II pulmonary epithelial cells that give rise to A549 cells are in charge of supporting alveolar transport functions, such as the passage of water and electrolytes[19]. These cells grow adherently and form monolayers *in vitro*. They produce lecithin and have large concentrations of unsaturated fatty acids, both of which are necessary for preserving membrane phospholipids.

A549 cells are also frequently employed as a type II alveolar model in drug metabolism research and as a host for transfection investigations[20]. The emergence of multilamellar bodies during extended culture is a sign of differentiation readiness.

The cell line Caco-2 was created by immortalising human colorectal cancer cells. In culture, Caco-2 cells can self-differentiate into a variety of intestinal epithelial cells[21]. It was developed by Jorgen Fogh at the Sloan-Kettering Institute for Cancer Research[22]. Despite being derived from colon (large intestine) cancer, the cells undergo polarisation and differentiation during culture, giving rise to a phenotype that resembles the enterocytes lining the small intestine in both function and appearance[23]. Polarised Caco-2 cells display microvilli, tight junctions, and several additional enterocyte-specific transporters and enzymes, such as active transporters for amino acids, bile acids, carboxylic acids, peptidases, esterases, P-glycoprotein, etc[24].

Human embryonic kidney 293 cells, often known as HEK 293, HEK-293, or 293 cells, are an immortalised cell line that was first created from HEK cells taken from a female foetal kidney in the 1970s[25]. Because of its fast growth, genetic stability, and excellent transfection efficiency, this cell line has been used extensively in research for years[26]. HEK-derived cell lines are utilised in biotechnology to create therapeutic proteins and viral vectors for gene therapy applications, and to assess the safety of various chemical compounds[26,27] By transfecting the plasmid pRSV-1609, the tsA1609 allele of the SV40 big T antigen was expressed, creating the HEK 293T variant. Neomycin/G418 resistance was also given during this process; this allele is fully active at 33 °C, somewhat active at 37 °C, and inactive at 40 °C[27]. HEK 293T has extremely high DNA transfection efficiency, just like its parent line. Due to SV40 big T antigen expression, plasmids containing the SV40 origin of replication can replicate episomally in 293T cells, momentarily retaining high copy numbers and significantly raising the yields of recombinant proteins or retroviral particles[28]. HEK 293 cells are frequently utilised as hosts for heterologous gene expression because they are simple to cultivate and transfect. Transfecting one or more target genes and then analysing the resultant protein products are typical uses. Compatibility with other transfection techniques, such as the calcium phosphate procedure, which can yield efficiencies close to 100%, further increases their versatility. The National Centre for Cell Science (NCCS), Pune, provided the HEK 293T cell line used in this investigation. Cells were maintained at 37 °C in DMEM supplemented with 10% FBS and penicillin–streptomycin (0.5 U mL^{-1}) in a humidified environment with 5% CO_2 and 95% air.

2.4 Principle of the Assay Conducted

The MTT Assay is a colourimetric test that measures how much succinate dehydrogenase in mitochondria reduces yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). The experiment depends on the quantity of cells present and the presumption that non-viable cells or their metabolites do not decrease tetrazolium. Dark purple formazan crystals are formed when MTT penetrates cells, passes through the mitochondria, and becomes insoluble. The formazan reagent is released and quantified spectrophotometrically at 570 nm after the cells have been dissolved with DMSO.

2.5 Experimental Procedure

Cell viability was measured in triplicate using the MTT Assay at five different concentrations for each of the three

chemical substances that were chosen (5, 10, 25, 50, and 100 µM). Additionally, an untreated control was kept. Trypsinised cells were used in the trypan blue experiment to count the number of living cells in the cell solution. A hemocytometer was used to count the cells, which were then put in a 96-well plate culture medium at a density of 5.0 x 10^3 cells/well in 100 µL media and incubated for the entire night at 37°C. Following incubation, 100 µL of new media with different quantities of chemical compounds was added to the wells of the microtiter plate in lieu of the old media. The solution was changed out with new media after 48 hours. Each well received around 0.5 mg of MTT solution per millilitre, and the plates were incubated at 37°C for three hours. At the end of the incubation time, precipitates are formed by the cells with metabolically active mitochondria, reducing the MTT salt to chromophore formazan crystals. A microplate reader was used to measure the optical density (OD) of solubilised crystals in dimethyl sulfoxide (DMSO) at 570 nm in order to compute the percentage growth inhibition.

$$\% \ Inhibition = \frac{100(Control - Treatment)}{Control}$$

The linear regression equation y = mx + c, where y = 50 and m and c values were obtained from the viability graph, was used to calculate the IC_{50} value.

3. Results and Discussion

Four test samples were assessed for their cytotoxic activity against A549 (human lung adenocarcinoma) and Caco-2 (human colorectal adenocarcinoma) cell lines, utilising IC_{50} values as potency markers. The IC_{50} values are summarised in Table 2.

Cisplatin had the maximum cytotoxicity against A549 cells, with an IC_{50} of 13.62 ± 0.127 µM, followed by bis(2-ethylhexyl)phthalate (77.38 ± 0.829 µM). Croscarmellose Sodium had the lowest IC_{50} (48.14 ± 0.692 µM) against Caco-2 cells, whereas Trioctyltrimellitate was the least potent against both cell lines, especially Caco-2 (104.79 ± 2.264 µM).

The IC_{50} results show that the examined substances have different cytotoxic potentials against A549 and Caco-2 cell lines. Cisplatin, employed as a positive control, exhibited high cytotoxicity against A549 cells, as predicted, indicating its therapeutic significance in lung cancer treatment. The greater IC_{50} against Caco-2 cells (66.42 ± 0.738 µM) indicates lower sensitivity of colorectal cancer cells under the testing circumstances. Among the three non-reference compounds, bis(2-ethylhexyl)phthalate had the most lethal impact on both cell lines, particularly A549 cells. This finding shows that bis(2-ethylhexyl) phthalate may disrupt cellular mechanisms that are more important in lung cancer cells than in colorectal cancer cells. Trioctyltrimellitate had poor cytotoxicity against both cell lines, with IC_{50} values surpassing 90 µM, suggesting low antiproliferative activity. Interestingly, croscarmellose sodium had moderate activity, with Caco-2 cells exhibiting much more cytotoxicity than A549 cells. Cell-specific absorption processes, metabolic profiles, and other biochemical interactions might all have an impact on this variable sensitivity. The overall trend indicates that Caco-2 cells are less susceptible to the examined compounds than A549 cells, with the exception of croscarmellose sodium. In summary, the findings show that cytotoxic responses vary among cancer cell lines, emphasising the necessity of cell-type-specific assessments. Bis(2-ethylhexyl) phthalate showed the most promising activity of the test materials, whereas trioctyltrimellitate had little cytotoxic potential in this investigation.

Croscarmellose sodium's cytotoxicity was tested against lung carcinoma (A549), colon carcinoma (Caco-2), and non-cancerous human embryonic kidney (HEK293T) cell lines using the MTT assay at concentrations ranging from 5-100 µM. Percentage growth inhibition results were determined compared to untreated controls (0%)

Table 2. IC_{50} values (µM) of test samples against A549 and Caco-2 cell lines

Sr. No.	Sample Name	IC_{50} (µM)		
		A549	Caco-2	HEK293T
1	Croscarmellose Sodium	81.26 ± 1.218	48.14 ± 0.692	-
2	Trioctyltrimellitate	91.75 ± 1.748	104.79 ± 2.264	-
3	Bis(2-ethylhexyl) phthalate	77.38 ± 0.829	53.72 ± 0.672	-
4	Cisplatin	13.62 ± 0.127	66.42 ± 0.738	-

to create a baseline metabolic activity for each cell line. In both cancer cell lines, exposure to croscarmellose sodium clearly reduced cell viability in a concentration-dependent manner. Inhibition of A549 cells rose from 7.39 ± 0.87% at 5 µM to 13.34 ± 0.59% at 10 µM, suggesting that antiproliferative action began at low dosages. At intermediate and higher dosages, there was a noticeable rise in inhibition, which reached 26.96 ± 0.66% at 25 µM, 41.16 ± 0.41% at 50 µM, and 55.37 ± 0.44% at 100 µM. Caco-2 cells showed a similar dose-dependent pattern, but with much greater sensitivity. Caco-2 inhibition (27.90 ± 1.76%) was more than twice that of A549 at 10 µM; at 25 µM and 50 µM, inhibition values rose to 44.35 ± 1.05% and 62.45 ± 0.84%, respectively (Figure 1).

Caco-2 cells are more sensitive to croscarmellose sodium than A549 at all tested concentrations, as seen by the 74.47 ± 0.92% inhibition at the highest tested concentration, 100 µM. On the other hand, HEK293T cells showed very little cytotoxicity from croscarmellose sodium. At 5 µM (2.34 ± 0.16%), 10 µM (4.67 ± 0.30%), and 25 µM (7.39 ± 0.55%), inhibition levels stayed quite low. HEK293T inhibition (11.72 ± 0.81%) was significantly less than that shown in cancer cell lines, even at the maximum dose (100 µM). This discrepancy suggests that croscarmellose sodium selectively targets cancerous cells, indicating a level of selective cytotoxicity (Table 3).

When combined, these results show that croscarmellose sodium has low toxicity to non-malignant cells while exhibiting dose-dependent antiproliferative effects with increased action towards colon cancer cells compared to lung carcinoma. Since differential inhibition across malignant and non-malignant cell models is a desirable feature in anticancer screening procedures, the observed selectivity is noteworthy. The therapeutic potential and biological mechanism of action of croscarmellose sodium would be further clarified by future research evaluating mechanistic pathways (such as apoptosis induction, ROS production, and mitochondrial damage) and calculating IC_{50} values.

In comparison to croscarmellose sodium, TOTM showed less inhibitory efficacy. Caco-2 inhibition varied from 2.85 ± 0.96% to 46.47 ± 1.47% across the same dose range, whereas inhibition in A549 cells ranged from 1.76 ± 0.82% at 5 µM to 38.19 ± 0.66% at 100 µM. Even while Caco-2 cells were more responsive once more, the level of inhibition was still rather low. These findings imply that TOTM may have modest cytostatic effects rather than acting through highly cytotoxic pathways (Figure 2).

Additionally, HEK293T cells showed little sensitivity to TOTM, with inhibition values at the maximum dose being less than 20%. Notably, HEK293T inhibition (6.29-11.79%) during low-dose exposure (5-25 µM) was either somewhat greater or comparable to that seen

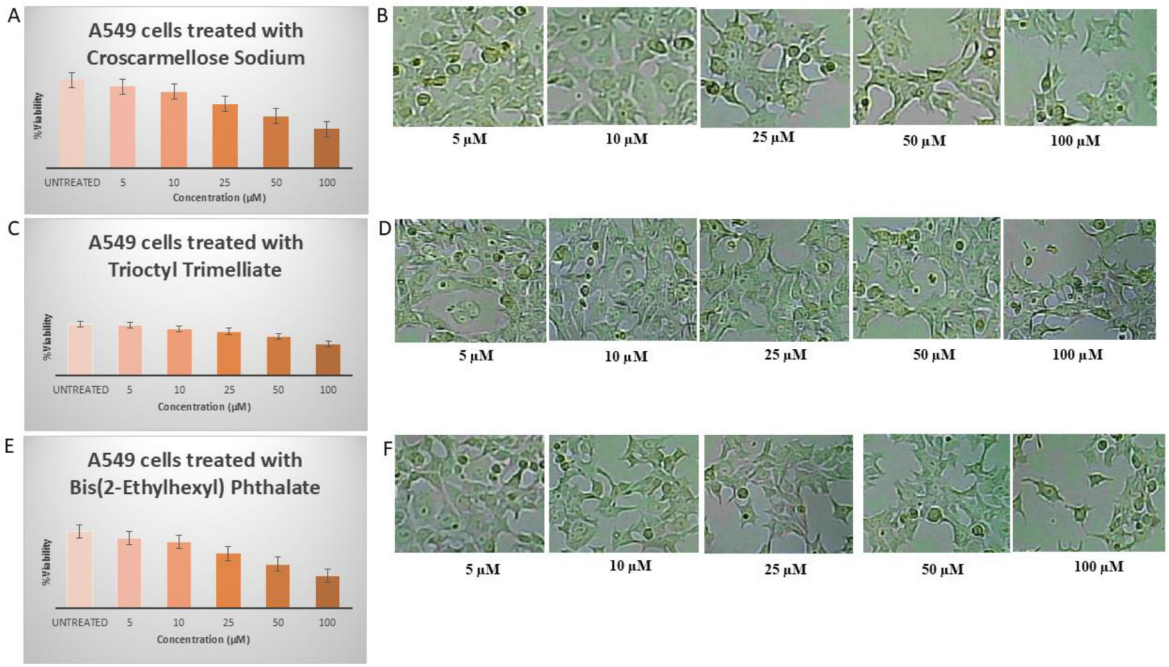


Figure 1. Differential cytotoxic effects of croscarmellose sodium, trioctyl trimellitate, and bis(2-ethylhexyl) phthalate on A549 cells.

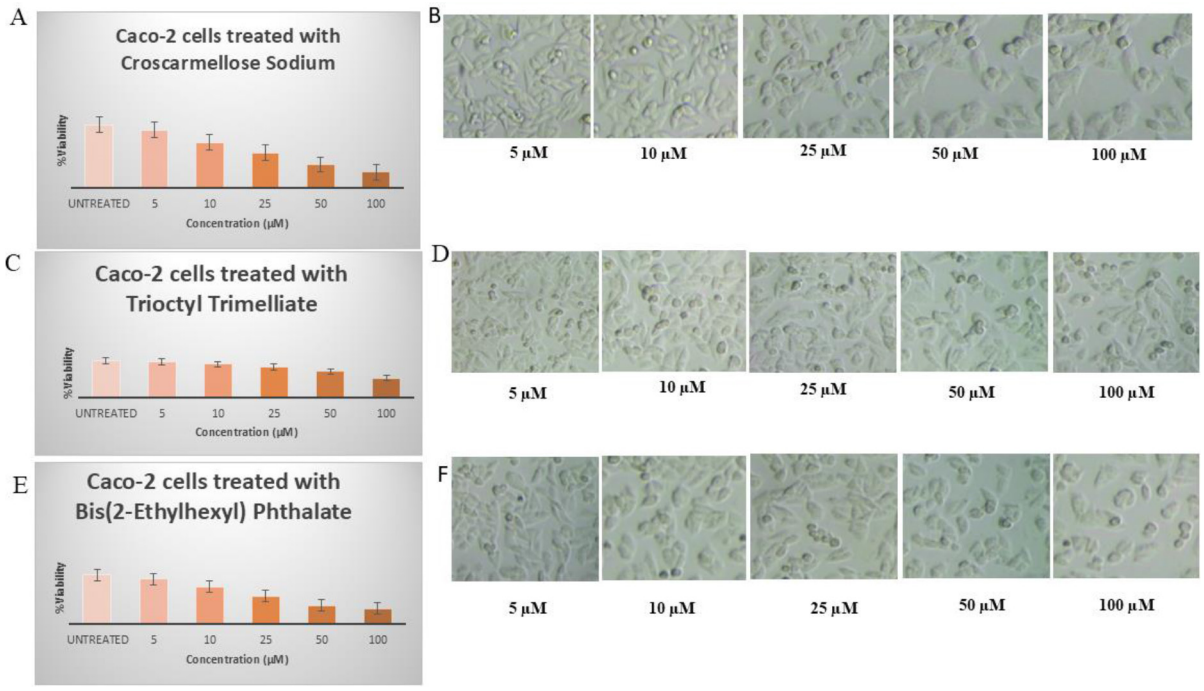


Figure 2. Cytotoxic effects of croscarmellose sodium, trioctyl trimellitate, and bis(2-ethylhexyl) phthalate on Caco-2 cells.

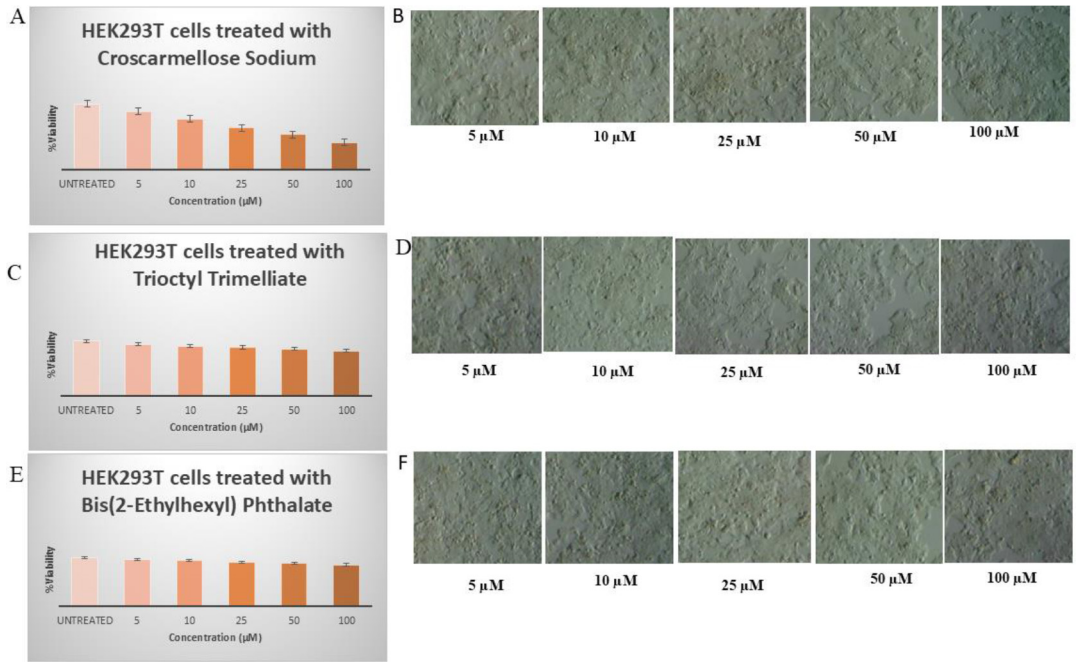


Figure 3. Comparative cytotoxicity of croscarmellose sodium, trioctyl trimellitate, and bis(2-ethylhexyl) phthalate on HEK293T cells.

in cancer cells at equivalent levels. In comparison to the other compounds examined, this response pattern suggests that TOTM has limited potency and relatively low selectivity (Table 3).

Similar to croscarmellose sodium, DEHP exhibited a dose-dependent inhibitory pattern against A549; however, at higher doses, it was significantly more active. Inhibition levels in A549 cells rose from 8.79 ± 1.20% at 5

Table 3. The percentage cell growth inhibition of A549, Caco-2 and HEK293T by croscarmellose sodium, trioctyl trimellitate and bis(2-ethylhexyl) phthalate *in vitro*

Concentration (µM)	Percent inhibition		
	Lung Cancer Cell Line A549	Colon Cancer Cell Line Caco-2	Non-malignant Cell Line HEK293T
Croscarmellose Sodium			
Untreated	0.00 ± 0.00(0)	0.00 ± 0.00(0)	0.00 ± 0.00(0)
5	7.39 ± 0.87(7.39)	8.28 ± 0.79(8.28)	2.34 ± 0.16(2.34)
10	13.34 ± 0.59(13.34)	27.90 ± 1.76(27.9)	4.67 ± 0.30(4.67)
25	26.96 ± 0.66(26.96)	44.35 ± 1.05(44.35)	7.39 ± 0.55(7.39)
50	41.16 ± 0.41(41.16)	62.45 ± 0.84(62.45)	9.38 ± 0.39(9.38)
100	55.37 ± 0.44(55.37)	74.47 ± 0.92(74.47)	11.72 ± 0.81(11.72)
Trioctyl Trimellitate			
Untreated	0.00 ± 0.00(0)	0.00 ± 0.00(0)	0.00 ± 0.00(0)
5	1.76 ± 0.82(1.76)	2.85 ± 0.96(2.85)	6.29 ± 0.38(6.29)
10	8.34 ± 0.44(8.34)	9.60 ± 0.64(9.6)	9.31 ± 0.80(9.31)
25	13.53 ± 0.18(13.53)	16.87 ± 1.03(16.87)	11.79 ± 0.77(11.79)
50	23.09 ± 0.48(23.09)	28.64 ± 0.24(28.64)	14.71 ± 0.12(14.71)
100	38.19 ± 0.66(38.19)	46.47 ± 1.47(46.47)	18.59 ± 0.34(18.59)
Bis(2-Ethylhexyl) Phthalate			
Untreated	0.00 ± 0.00(0)	0.00 ± 0.00 (0)	0.00 ± 0.00(0)
5	8.79 ± 1.20(8.79)	8.12 ± 0.64(8.12)	3.26 ± 0.29(3.26)
10	13.80 ± 0.48(13.8)	24.36 ± 0.86(24.36)	5.43 ± 0.41(5.43)
25	28.81 ± 1.05 (28.81)	43.30 ± 0.85 (43.3)	9.52 ± 0.23(9.52)
50	42.52 ± 1.01 (42.52)	61.61 ± 0.71 (61.61)	11.79 ± 0.55 (11.79)
100	57.53 ± 0.62 (57.53)	67.93 ± 1.3 (67.93)	14.12 ± 0.69 (14.12)

µM to 57.53 ± 0.62% at 100 µM. Once more, Caco-2 cells showed increased sensitivity, with inhibitions of 24.36 ± 0.86% and 67.93 ± 1.30% at 10 and 100 µM, respectively. These findings confirm that Caco-2 cells are consistently more vulnerable than A549 cells to metabolic inhibition caused by small molecules (Figure 1-2).

Crucially, HEK293T cells demonstrated little inhibition at all DEHP dosages (3.26-14.12%), suggesting that non-malignant cells' mitochondrial metabolic function is not significantly disrupted. The selectivity profile for croscarmellose sodium is somewhat similar to this one (Table 3).

The three chemicals' potencies and selectivities differed significantly, according to a comparative investigation. In both cancer cell lines, croscarmellose sodium and DEHP had the most inhibitory effects overall. Caco-2 cells were especially sensitive (croscarmellose sodium ≈ DEHP > TOTM), whereas A549 cells showed a somewhat diminished but comparable trend (DEHP ≈ croscarmellose sodium > TOTM). TOTM, on the other hand, showed relatively low activity at all concentrations. Crucially, toxicity against non-malignant HEK293T cells remained minimal, particularly for DEHP and croscarmellose sodium, exhibiting favourable selectivity profiles and implying that these substances do not significantly reduce basal metabolic activity in non-malignant cells. While TOTM's low inhibitory profile is consistent with its behaviour as a low-toxicity excipient rather than a cytotoxic compounds, Caco-2 cells' increased susceptibility may be due to metabolic or membrane-related changes from A549 cells. When taken as a whole, our results show dose-dependent inhibition with compound-specific efficacy and selectivity, which encourages further testing of DEHP and croscarmellose sodium in mechanistic and IC_{50}-based investigations.

4. Conclusion

The study's overall findings show that, while trioctyltrimellitate shows relatively little cytotoxic activity under the tested conditions, croscarmellose sodium and bis(2-ethylhexyl) phthalate exhibit dose-dependent inhibitory effects against A549 and Caco-2 carcinoma cell lines. Interestingly, non-malignant HEK293T cells showed very little inhibition from all three substances, with croscarmellose sodium and bis(2-ethylhexyl) phthalate showing the highest level of selectivity. These results show that certain plasticisers and excipients can alter the viability of cancer cells, indicating possible pharmacological or toxicological significance.

There are still significant information gaps, though. The molecular mechanisms underpinning growth suppression, long-term exposure effects, apoptosis induction, oxidative stress contributions, and metabolic system disruptions were not evaluated in this work. Furthermore, it is yet unclear how these discoveries relate to biology in more physiologically relevant systems (such as *in vivo* settings or 3D tumour models). To more precisely describe the safety profile and biological consequences of these compounds, these gaps must be filled through mechanistic and translational research.

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6. Data Availability Statement

The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request. Supporting raw data, including IC_{50} values and cell viability measurements, can be provided for academic or reproducibility purposes.

7. References

1. "What is cancer? was originally published by the National Cancer Institute. 2021. https://www.cancer.gov/about-cancer/understanding
2. Anand P, Kunnumakara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, *et al.* Cancer is a preventable disease that requires major lifestyle changes. Pharm Res. 2008; 25(9):2097–2116. https://doi.org/10.1007/s11095-008-9661-9 PMid: 18626751 PMCid: PMC2515569.
3. Colditz GA, Wei EK. Preventability of cancer: the relative contributions of biologic and social and physical environmental determinants of cancer mortality. Annu Rev Public Health. 2012; 33(1):137–156. https://doi.org/10.1146/annurev-publhealth-031811-124627 PMid: 22224878 PMCid: PMC3631776.
4. NIH. Environmental carcinogens and cancer risk. 2023. https://www.cancer.gov/about-cancer/causes-prevention/risk/substances/carcinogens
5. Huang WX, Desai M, Tang Q, Yang R, Vivilecchia RV, Joshi Y. Elimination of metformin–croscarmellose sodium interaction by competition. Int J Pharm. 2006; 311(1–2):33–39. https://doi.org/10.1016/j.ijpharm.2005.12.017 PMid: 16426778.
6. Ataman Chemicals. https://www.atamanchemicals.com/croscarmellose-sodium_u32306/.CROSCARMELLOSE SODIUM
7. Lee YM, Lim Y, Kang S, Kim S. Migration of phthalate and non-phthalate plasticisers from polyvinyl chloride (PVC) materials. Sci Total Environ. 2025; 1002:180559. https://doi.org/10.1016/j.scitotenv.2025.180559 PMid: 41005168.
8. Moghazy M, Papathanasiou M, Tzoupis H, Papavasileiou KD, Xing C, Lauschke VM, *et al.* A systematic literature review of reproductive toxicological studies on phthalates. Int J Mol Sci. 2025; 26(18):8761. https://doi.org/10.3390/ijms26188761 PMid: 41009332 PMCid: PMC12469734.
9. Behrensdorf-Nicol HA, Wild E, Bonifas U, Klimek J, Hanschmann KM, Krämer B, *et al. In vitro* potency determination of botulinum neurotoxin serotype A based on its receptor-binding and proteolytic characteristics. Toxicol In Vitro. 2018; 53:80–88. https://doi.org/10.1016/j.tiv.2018.07.008 PMid: 30016653.
10. Dos Santos RS, Medina-Gali RM, Babiloni-Chust I,Marroqui L, Nadal A. In vitro assays to identify metabolism-disrupting chemicals with diabetogenic activity in a humanpancreatic β-cell model. Int J Mol Sci. 2022; 23(9):5040. https://doi.org/10.3390/ijms23095040
11. Michelangeli F, Mohammed NA, Jones B, Tairu M, Al-Mousa F. Cytotoxicity by endocrine disruptors through effects on ER Ca^{2+} transporters, aberrations in Ca^{2+} signalling pathways and ER stress. FEBS Open Bio. 2024;

14(9):1384–1396. https://doi.org/10.1002/2211-5463.13880 PMid: 39138623 PMCid: PMC11492318.
12. Strand D, Nylander E, Höglund A, Lundgren B, Martin JW, Karlsson O. Screening persistent organic pollutants for effects on testosterone and estrogen synthesis at human-relevant concentrations using H295R cells in 96-well plates. Cell Biol Toxicol. 2024; 40(1):69. https://doi.org/10.1007/s10565-024-09902-4 PMid: 39136868 PMCid: PMC11322491.
13. Ziemba B. Advances in cytotoxicity testing: from *in vitro* assays to *in silico* models. Int J Mol Sci. 2025; 26(22):11202. https://doi.org/10.3390/ijms262211202 PMid: 41303685 PMCid: PMC12653452.
14. Kambia K, Dine T, Gressier B, Dupin-Spriet T, Luyckx M, Brunet C. Evaluation of the direct toxicity of trioctyltrimellitate (TOTM), di(2-ethylhexyl) phthalate (DEHP) and their hydrolysis products on isolated rat hepatocytes. Int J Artif Organs. 2004; 27(11):971–978.
15. Bis(2-ethylhexyl) phthalate, $C_{24}H_{38}O_4$, CID 8343. PubChem. 2025. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Bis_2-ethylhexyl_-phthalate
16. Tris(2-ethylhexyl) trimellitate, $C_{33}H_{54}O_6$, CID 18725. PubChem. 2025. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Tris_2-ethylhexyl_-trimellitate
17. Carmellose (JP17/NF/INN),C8H16O8, CID 24748. PubChem. 2025. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Carmellose-_JP17_NF_INN
18. Giard DJ, Aaronson SA, Todaro GJ, Arnstein P, Kersey JH, Dosik H, *et al. In vitro* cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. J Natl Cancer Inst. 1973; 51(5):1417–1423. https://doi.org/10.1093/jnci/51.5.1417 PMid: 4357758 PMCid: PMC9092969.
19. Foster KA, Oster CG, Mayer MM, Avery ML, Audus KL. Characterisation of the A549 cell line as a Type II pulmonary epithelial cell model for drug metabolism. Exp Cell Res. 1998; 243(2):359–366. https://doi.org/10.1006/excr.1998.4172 PMid: 9743595.
20. Fehrenbach H. Alveolar epithelial type II cell: defender of the alveolus revisited. Respir Res. 2001; 2(1):33–46. https://doi.org/10.1186/rr36 PMid: 11686863 PMCid: PMC59567.
21. Borchardt RT, Hidalgo, I. J., Raub, T. J., and Borchardt, R. T.: Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability, Gastroenterology. 96, 736-749, 1989 - the backstory. AAPS J. 2011; 13(3):323–327. https://doi.org/10.1208/s12248-011-9283-8 PMid: 21607810 PMCid: PMC3160153.
22. Fogh J, Fogh JM, Orfeo T. One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice 23. J Natl Cancer Inst. 1977; 59(1):221–226. https://doi.org/10.1093/jnci/59.1.221 PMid: 327080.
23. Sambuy Y, De Angelis I, Ranaldi G, Scarino ML, Stammati A, Zucco F. The Caco-2 cell line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics. Cell Biol Toxicol. 2005; 21(1):1–26. https://doi.org/10.1007/s10565-005-0085-6 PMid: 15868485.
24. Hubatsch I, Ragnarsson EGE, Artursson P. Determination of drug permeability and prediction of drug absorption in Caco-2 monolayers. Nat Protoc. 2007; 2(9):2111–2119. https://doi.org/10.1038/nprot.2007.303 PMid: 17853866.
25. Russell WC, Graham FL, Smiley J, Nairn R. Characteristics of a human cell line transformed by DNA from human adenovirus Type 5. J Gen Virol. 1977; 36(1):59–72. https://doi.org/10.1099/0022-1317-36-1-59 PMid: 886304 PMCid: PMC10833543.
26. Thomas P, Smart TG. HEK293 cell line: a vehicle for the expression of recombinant proteins. J Pharmacol Toxicol Methods. 2005; 51(3):187–200. https://doi.org/10.1016/j.vascn.2004.08.014 PMid: 15862464.
27. DuBridge RB, Tang P, Hsia HC, Leong PM, Miller JH, Calos MP. Analysis of mutation in human cells by using an Epstein-Barr virus shuttle system. Mol Cell Biol. 1987; 7(1):379–387. https://doi.org/10.1128/mcb.7.1.379-387.1987 PMid: 3031469 PMCid: PMC365079.
28. Pear WS, Nolan GP, Scott ML, Baltimore D. Production of high-titer helper-free retroviruses by transient transfection. Proceedings of the National Academy of Sciences. 1993; 90(18):8392–8396. https://doi.org/10.1073/pnas.90.18.8392 PMid: 7690960 PMCid: PMC47362.

Author Contribution

Prof. P. Ramachandra Reddy was responsible for conceptualization, methodology, manuscript review and editing, and funding acquisition. Manuscript preparation, methodology, data collection, and data analysis were performed by U. R. Sri Veena. Manuscript preparation, reviewing, and editing were done by Somedutta Maity and Ramesh Koduru. All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the research work.

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